

Trait physiology and crop modelling to link phenotypic complexity to underlying genetic systems.

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Abstract

New tools derived from advances in molecular biology have not been widely adopted in plant breeding because of the inability to connect information at gene level to the phenotype in a manner that is useful for selection. We explore whether a crop growth and development modelling framework can link phenotype complexity to underlying genetic systems in a way that strengthens molecular breeding strategies. We use gene-to-phenotype simulation studies on sorghum to consider the value to marker-assisted selection of intrinsically stable QTLs that might be generated by physiological dissection of complex traits. The consequences on grain yield of genetic variation in four key adaptive traits – phenology, osmotic adjustment, transpiration efficiency, and staygreen – were simulated for a diverse set of environments by placing the known extent of genetic variation in the context of the physiological determinants framework of a crop growth and development model. It was assumed that the three to five genes associated with each trait, had two alleles per locus acting in an additive manner. The effects on average simulated yield, generated by differing combinations of positive alleles for the traits incorporated, varied with environment type. The full matrix of simulated phenotypes, which consisted of 547 location-season combinations and 4235 genotypic expression states, was analysed for genetic and environmental effects. The analysis was conducted in stages with gradually increased understanding of gene-to-phenotype relationships, which would arise from physiological dissection and modelling. It was found that environmental characterisation and physiological knowledge helped to explain and unravel gene and environment context dependencies. We simulated a marker-assisted selection (MAS) breeding strategy based on the analyses of gene effects. When marker scores were allocated based on the contribution of gene effects to yield in a single environment, there was a wide divergence in rate of yield gain over all environments with breeding cycle depending on the environment chosen for the QTL analysis. It was suggested that knowledge resulting from trait physiology and modelling would overcome this dependency by identifying stable QTLs. The improved predictive power would increase the utility of the QTLs in MAS. Developing and implementing this gene-to-phenotype capability in crop improvement requires enhanced attention to phenotyping, ecophysiological modelling, and validation studies to test the stability of candidate QTLs.

Media summary

Virtual plant technologies are used to show that physiological understanding and crop modelling can provide a bridge between gene and whole plant levels of biological organisation that will allow more effective use of molecular genetics in plant breeding

Key words

Virtual plants, gene-to-phenotype modelling, complex traits, trait physiology, molecular breeding

Introduction

The enhanced ability to undertake genome scale molecular biology and accumulate associated data has not been matched by an improved ability to design and engineer improved plants. Molecular biology has delivered commercial successes related to enabling crop plants to resist pests and tolerate herbicides. These achievements have been made via single gene transformations that scale well from molecular expression to plant level response (Somerville and Somerville, 1999). While there is increasing interest in molecular breeding for more complex crop growth and development traits, successful approaches based on single genes cannot be readily adapted to more complex traits. The challenge remains to manipulate more complex growth and development traits associated with crop adaptation and yield.

The lack of general adoption in plant breeding of new tools derived from advances in molecular biology is not associated with lack of investment or endeavour. Rather, it seems to be related to the inability to connect information at gene level to the expressed phenotype in a manner that is useful for selection and plant breeding (Miflin, 2000). Enhanced capabilities in genotyping have not been matched by enhanced capabilities in phenotyping. The situation is particularly complicated for complex growth and development traits, as they are associated with genes interacting in networks so that gene-to-phenotype relationships are not straightforward, as shown in rice by Li et al (2001) and Luo et al (2001). The functional consequences for the organism, which arise from the interplay of the environment with these gene networks, operate at a higher level of biological organisation. Hence, gene-gene and gene-environment interactions have major influences on the expressed phenotype. Such context dependencies provide a major limitation for molecular breeding.

Integrating across biological levels of organisation using a gene-to-phenotype modelling approach may present a way forward, but it also presents a major challenge (Cooper et al, 2002). The notion of a virtual or *in silico* plant suitable for this purpose has occupied the thinking of molecular biologists (Minorsky, 2003) and whole plant physiologists (Hammer et al, 2002; Tardieu, 2003). The former suggests a path integrating from the gene and gene function level up to the organism phenotype (i.e., “bottom-up”), while the latter begins with the phenotype and uses physiological dissection to drive toward the molecular genomic level (i.e., “top-down”). We have argued elsewhere (Hammer et al, 2004) for informed dialectic across levels of biological organisation to enhance progress in systems biology and in developing the form of *in silico* plant most useful to crop improvement. We suggest that the “bottom-up” approach is likely to suffer from an inability to deal with complexity generated by gene and environment context dependencies, especially for complex growth and development traits. Although some recent gene network models [e.g., galactose metabolism in yeast (Peccoud et al, 2004); transition to flowering in *Arabidopsis* (Welch et al., 2003)] show promise for this approach, they are based on extensive underpinning research that has characterised relevant networks and pathways and they still relate only to components of whole organism function.

Crop models with generic approaches to underlying physiological processes (e.g., Wang et al., 2002) underpin the “top-down” approach to gene-to-phenotype modelling (Hammer et al., 2002). Crop simulation models have captured much of the understanding of plant growth and development processes generated over nearly 40 years of plant systems research (Sinclair and Seligman, 1996). They provide a dynamic framework for the physiological dissection of complex growth and development traits. However, initial attempts (White and Hoogenboom, 1996; Yin et al., 2003) to use crop models for this purpose by optimising a range of model coefficients so that the model best fitted observed phenotypic variation among genotypes, identified some key issues. The modest predictive capabilities found highlighted the need to better understand the physiological basis of the genetic variation involved via studies with controlled genetic backgrounds before seeking predictive capability across diverse material. The studies also showed the reliance of the approach on the validity with which the crop model architecture and associated coefficients captured and integrated the physiological basis of the genetic variation. More recent studies by Tardieu (2003) using an ecophysiological model of plant water use overcame both limitations. The parameters of control equations in the simple, but physiologically robust, component model were conclusively linked to genetic variation by their ability to predict the behaviour of transformed plants. The parameters thus represented coordinated genotypic responses that quantified a “meta-mechanism” at a higher level of biological organisation. In a similar manner, Reymond et al (2002) combined QTL analysis with an ecophysiological model of the response of maize leaf elongation rate to temperature and water deficit by conducting the QTL analysis on the model parameters. They were able to validate the QTLs by successfully predicting the elongation rates of new lines in the mapping population using their QTL profile to determine relevant parameters for use in the component model for leaf elongation. It remains to be seen whether this demonstrated gene-to-phenotype capability of modelling at organ or component scale can be successfully applied at the organism scale. It seems clear that crop models that better capture plant function and control will be required for this task (Hammer et al., 2002).

In a previous study using gene-to-phenotype modelling at crop scale for sorghum (Chapman et al., 2002; Chapman et al., 2003), we focussed on how gene-gene and gene-environment interactions that arise from the underlying determinants of complex crop growth and development traits would challenge molecular

breeding. We incorporated available knowledge on physiology and genetics of key traits in a crop scale model that had been adapted to simulate the interactions among physiological processes for any combination (i.e., hypothetical genotype) as realistically as possible while still maintaining full crop model functionality. We linked the simulated phenotypes for a broad range of water-limited production environments in Australia to the QU-GENE breeding system simulation platform (Podlich and Cooper, 1998) to explore effects of selection as a basis for discussion. The concept centred on improving the likelihood of favourably manipulating the phenotype, rather than predicting directly and precisely the effect of genetic variation on organism function. This approach incorporated four steps:

- the classification of environmental challenges in the production region
- the genetic basis and physiological mode of action of adaptive traits
- simulation of the gene/trait value in the total population of environments, and
- simulation of breeding strategy options

Here we explore the question of whether using a crop growth and development modelling framework can link phenotypic complexity to underlying genetic systems in a way that enhances the power of molecular breeding strategies. We extend the previous gene-to-phenotype simulation studies on sorghum to gain further insight in relation to this question by considering the value to marker assisted selection of intrinsically stable QTLs that might be generated by physiological dissection of complex traits. We discuss the key issues associated with developing and implementing this gene-to-phenotype capability in crop improvement.

Linking genetic variation in adaptive traits to physiological determinants

A robust physiological determinants framework for crop growth and development provides a means to analytically dissect phenotypic variation to aid understanding of underlying causes, while simultaneously providing a means to predict emergent phenotypic consequences by integrating effects of variation in component factors and processes. Crop modelling based around the continued improvement of initial framework concepts (e.g., Charles-Edwards, 1982; Tanner and Sinclair, 1983; Passioura, 1983; Monteith, 1986; Monteith, 1988; Sinclair and Horie, 1989) has now evolved to a level where the simultaneous pursuit of explanation and prediction at the whole crop level is possible (Hammer et al., 2002). The yield of a determinate grain crop is determined by the number of grain set and the size they are able to achieve. Both factors are strongly determined by rate of crop growth, but at differing stages of development – the former around flowering and the latter after flowering. Grain size is also influenced by the retranslocation of pre-anthesis assimilate. The timing of flowering is controlled by interplay of genetics and environment, particularly temperature and photoperiod. The rate of biomass accumulation relates to ability to capture light or water and the efficiency with which either can be used. Both depend on the nature of canopy development, which is influenced by crop development (via maximum leaf number), management (via density), temperature and genetics (e.g., tillering, branching, architecture). Capture of water depends on the nature of the soil profile, its rate of exploration by roots, and their ability to extract water. The efficiency of water use is affected by the aerial environment via the influence of vapour pressure deficit on water flux. The efficiency of radiation use is affected by leaf nitrogen status, which in turn is related to the availability of nitrogen to the crop. The interplay of the developmental timetable for organ growth with the availability of assimilate, water and nitrogen determine crop water and nitrogen status and growth and development patterns. The balances between demand for, and supply of, these factors among organs underpin organism controls of allocation and growth. Appropriately specifying and quantifying the response and control equations are critical in effective crop modelling. This is akin to modelling plant hormone action without modelling the hormones (de Wit and Penning de Vries, 1983). The specifications provide both the basis to identify and quantify key differences among lines in field studies, and the ability to predict emergent consequences of putative genetic variation on the phenotype.

In this study, we focussed on the physiology and genetics of four key adaptive traits for sorghum – phenology, osmotic adjustment, transpiration efficiency, staygreen - and placed the known genetic variation in the context of the physiological determinants framework of our crop models that had evolved progressively as insights improved (Chapman et al., 1993; Hammer, 1998; Hammer et al., 2001; Wang et al., 2003).

Phenology

Differences in the rate of development among sorghum genotypes are known to relate to differing responses to temperature and photoperiod (Hammer et al., 1989; Major et al., 1990). Genetic variation in phenology can be predicted by quantifying these responses with photo-thermal models (Craufurd et al., 1999). Differences in duration prior to floral initiation will generate differences in number of leaves produced with consequent effects on canopy leaf area development (Muchow and Carberry, 1990; Hammer et al., 1993) and thus, patterns of water use through the crop cycle.

A major drawback of this photo-thermal framework is that it does not account properly for some of the effects of temperature-photoperiod interactions on phenology that have been observed for sorghum. For example, the photo-thermal model predicts that thermal time to anthesis and final leaf number of a hybrid are independent of temperature under a given photoperiod, yet temperature effects, independent of photoperiod, have been reported (Caddel and Weibel, 1971; Major et al., 1990; Morgan et al., 2002). Consistent with this, phenology is accelerated under natural asynchrony between thermoperiod and photoperiod (ie temperature increases after transition from dark to light), but slowed under unnatural asynchrony (Morgan et al., 1987; Ellis et al., 1997).

There is some potential to capture these interactions using a gene network model, as illustrated by Welch et al (2003). They could generate genotype-temperature interactions for timing of transition to flowering in *Arabidopsis thaliana* using a gene network model. Transition to flowering in *Arabidopsis* is determined by a number of converging pathways, including a facultative long-day and a photoperiod-independent autonomous pathway, which primarily responds to temperature (Blázquez, 2000). Photoperiod-temperature interactions arise as a consequence of genes affecting both of these pathways (eg those affecting light receptors). Capturing these molecular interactions dynamically at the phenotypic level, however, requires appropriate quantitative knowledge to scale upwards from the molecular level.

In this study we used the photo-thermal models implemented in crop models for many species (Wang et al., 2002) and varied the thermal time required to reach floral initiation to simulate genetic differences. Hart et al (2000) found 3 major QTLs for maturity in sorghum, so we assumed three genes with two alleles per locus, acting in a simple additive manner, gave rise to the genetic variation, which ranged from 90 to 140 °Cd. This range was consistent with the range of maturity and leaf number known to occur in locally adapted sorghum hybrids (Hammer et al., 1989). Seven expression states spread uniformly over this range were associated with the number of positive alleles present over the three loci (ie 0 to 6; see Chapman et al (2003) for details).

Osmotic adjustment.

The accumulation of osmolyte compounds, usually called osmotic adjustment (OA), results in a decrease of cell osmotic potential and thus in maintenance of water absorption and cell turgor pressure, which might contribute to sustaining physiological processes and improving yield in water-limited environments (Ludlow and Muchow, 1990). A recent study of the physiological mode of action of OA in high and low OA sorghum lines (Snell, 2004) found that, under specific water-limited conditions, high OA lines had a greater ability to set grain and retranslocate carbohydrate from the stem to grain during grain filling. This effect may have resulted from enhanced maintenance of metabolic activity during grain set and filling. This result was consistent with earlier findings of Ludlow et al (1990). However, it is known that the specific environmental circumstances conferring any advantage occur infrequently and are low-yielding situations, so that the overall value of this trait is low (Hammer et al., 1999; Snell, 2004). While this finding is consistent with recent questioning of value of OA to crop yield under drought (Serraj and Sinclair, 2002), it remains relevant to this study because of these genotype-environment interactions.

In this study we generated genetic variation in OA by increasing the potential to set grain and the ability to retranslocate stem biomass to grain under specific moisture limitation conditions. The increase in grain number was generated by reducing the amount of crop biomass growth required between floral initiation and flowering to produce an individual grain from 0.00083 to 0.00075 g/grain. Enhanced remobilisation of assimilate from stem during grain filling was generated by increasing the fraction of stem biomass at flowering that was potentially available for retranslocation from 20 to 36%. Both mechanisms were only invoked under circumstances when the crop demand for moisture could not be met by the supply ability of the soil-root system. While this range was consistent with results of field studies (Snell, 2004), there

remains some uncertainty concerning the degree of water limitation required to initiate the changes. Based on findings of Basnayake et al (1995) we assumed two genes with two alleles per locus, acting in a simple additive manner, gave rise to the genetic variation. Five expression states spread uniformly over this range were associated with the number of positive alleles present over the two loci (i.e., 0 to 4; see Chapman et al (2003) for details).

Transpiration efficiency.

The amount of biomass produced by sorghum plants per unit of water transpired, that is transpiration efficiency (TE), is known to vary among genotypes (Donatelli et al., 1992; Henderson et al., 1997; Hammer et al., 1997). It has also been shown that these genetic differences are maintained under water limitation (Mortlock and Hammer, 1999). TE is inversely proportional to vapour pressure deficit of the atmosphere and once normalised for this effect the TE coefficient for sorghum is accepted as 9Pa (Tanner and Sinclair, 1983). In this study we generated genetic variation in TE by allowing the standard TE coefficient for sorghum to vary from 8 to 10Pa. This range was consistent with the variation observed in experimental studies (Hammer et al., 1997; Mortlock and Hammer, 1999). No QTLs or genes are known for transpiration efficiency in sorghum. We assumed five genes were involved with the expectation that it may be complex as it represents an integrated measure at whole plant level. That is, genetic variation in TE could be related to a number of underlying causal factors. We assumed that two alleles per locus, acting in a simple additive manner, gave rise to the genetic variation. Eleven expression states spread uniformly over the range in TE coefficient were associated with the number of positive alleles present over the five loci (i.e., 0 to 10; see Chapman et al (2003) for details).

Staygreen

Staygreen is the ability of leaves to retain their integrity (ie greenness) during the grain-filling period. Genetic variation in staygreen has been related to improved yield under water-limited conditions in sorghum (Borrell et al., 2000b). Three mechanisms of functional expression of stay-green have been identified (Borrell et al., 2000a; Thomas and Howarth, 2000): delayed onset of senescence, reduced rate of senescence, and increased LAI at anthesis. In sorghum, the expression of stay-green during grain filling can be viewed as a consequence of the balance between demand for nitrogen (N) by the grains and supply of N through soil N-uptake and translocation from vegetative plant parts, including stems and leaves. Leaf-N translocation occurs if grain-N demand cannot be met through soil-N uptake and stem-N translocation (van Oosterom et al., 2005b).

The onset of leaf senescence can be delayed by increased soil-N uptake during grain filling, as observed under terminal drought stress by Borrell and Hammer (2000). Such increased N-uptake could be associated with either increased water uptake (i.e., transpiration, T) or increased transpiration efficiency (TE) (AK Borrell, pers. comm.) as at least one of these is required to explain enhanced biomass accumulation and yield of staygreen types. Another mechanism to delay onset of leaf senescence is through increased availability of stem-N-for translocation, although this mechanism might compromise the leaf-N status if increased stem-N is not matched by increased total N-uptake.

Increased specific leaf nitrogen (SLN) or leaf area at anthesis can also reduce the rate of leaf senescence. Once leaf-N translocation starts, the amount of leaf area that needs to senesce in order to meet the N demand declines with improving leaf-N status (van Oosterom et al., 2005b). Genotypic variation in maximum SLN under optimum conditions has been observed for sorghum, and was associated with differences in leaf size (van Oosterom et al., 2005a). Increased leaf area at anthesis can be achieved through increased partitioning of dry matter and N to the leaves (Borrell and Hammer, 2000), although there are also indications that increased LAI of staygreen hybrids is due to inability of other hybrids to compensate for their smaller leaf area on the main shoot through tillering, particularly if resource availability is limited (van Oosterom et al., 2005a).

This framework can explain genotypic differences and effects of genotype-environment interaction on the expression of stay-green. Phenotypic expression becomes an emergent consequence of the interplay of differences in underlying traits like leaf size, leaf SLN, dry matter partitioning, N uptake and possibly transpiration or transpiration efficiency.

In this study we varied the target SLN of new leaf over the range 1.35 to 1.65 gN/m² leaf area to generate genetic variation in staygreen. This range was consistent with expectations from experimental studies (Borrell and Hammer, 2000). Tao et al (1999) identified 5 QTLs associated with stay-green in local sorghum germplasm, so we assumed five genes with two alleles per locus, acting in a simple additive manner, gave rise to the genetic variation. Eleven expression states spread uniformly over this range were associated with the number of positive alleles present over the five loci (i.e., 0 to 10; see Chapman et al (2003) for details). While the physiological framework presented above suggests that variation in staygreen is associated with other underlying drivers, they were not included in this initial study. The interaction with variation in transpiration efficiency will be incorporated via the separate treatment of that trait. It is possible that combinations of the 5 known QTLs for staygreen relate to differing underlying mechanisms, but insufficient is known at this stage to proceed further in this regard.

Classifying production environments and simulating phenotypes

Attributes of production environments and phenotypes arising from specific trait combinations were generated by simulation. We conducted the simulation studies using the sorghum module of the APSIM modelling platform (Keating et al., 2003), which utilises the generic framework outlined by Wang et al (2003). The sorghum module has undergone extensive development to enhance its capacity to realistically simulate the interactions among physiological processes (Hammer et al., 2001). Recent improvements have adopted concepts of ‘emergent’ properties (Hammer 1998) in seeking more realistic simulation of genetic variation in traits via underlying physiological functionality.

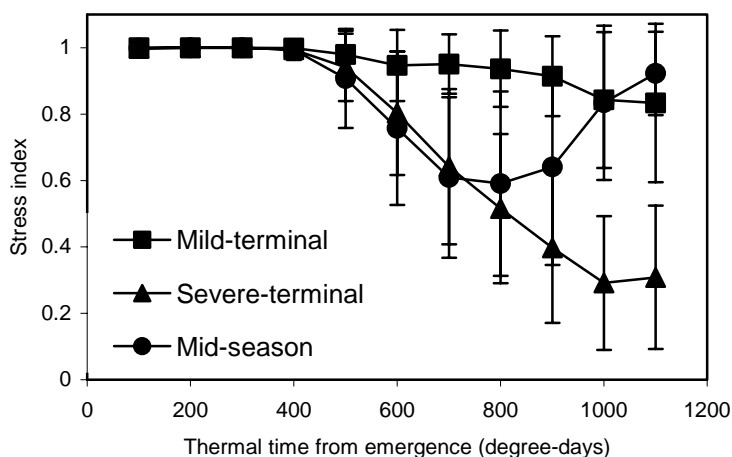


Figure 1. Patterns of water limitation throughout the growing season associated with the three environment types identified from the simulation and cluster analysis of sorghum production environments in NE Australia (after Chapman et al., 2002). The stress index is the ratio of water supply to crop demand. The lines show the mean stress index values for the location-season combinations making up each group and the vertical bars show the standard deviation. Mean anthesis date for the reference genotype used was 732 degree-days.

Environment types were characterised by quantifying the degree of crop water limitation simulated throughout the crop cycle. A reference genotype was simulated for a large sample (547) of location-season combinations and the resultant seasonal patterns of water limitation were clustered into like types (Chapman et al., 2002). Three distinct patterns were identified – mild terminal stress (MTS), severe terminal stress (STS), and mid-season stress (MSS) (Fig. 1). The MTS environment type occurred in 37% of location-season combinations and represented situations where little or no water limitation was experienced until well after anthesis. In contrast, the STS type (35% occurrence) reflected early onset of water limitation that became increasingly severe as the crop cycle progressed. The MSS type (28% occurrence) was associated with early onset of water limitation that was relieved during the grain-filling period.

The effects on average simulated yield, generated by differing combinations of positive alleles for the traits incorporated, varied with environment type (Fig. 2). The yield outcomes for any specific allele combination represent the emergent consequence of the perturbation of the functional plant-environment dynamics contained in the model that is associated with the changes in specifications of response and control equations. As expected, the average yield was greater in MTS environments. It was notable, however, that while combining all positive alleles for phenology (late flowering) resulted in higher average yield in MTS environments, the opposite occurred in STS environments (Fig. 2). Hence, a clear genotype-environment interaction was generated. While this interaction is perhaps well known, it nonetheless highlights the point of the potential for considerable confounding in the absence of the environment classification. The physiological basis of the interaction relates to the additional number of leaves and hence, greater canopy leaf area, generated with later maturity. This causes greater demand for water, which is detrimental in STS environments where water becomes limiting early in the growth cycle (Fig. 1). In contrast, in MTS environments, where water is not so limiting, the additional canopy leaf area is able to generate increased biomass accumulation and yield via enhanced light capture. There was also an interaction for the OA trait. Accumulating all positive alleles for OA generated some effect on yield in STS environments, but there was little effect in MTS environments. This reflects the need for specific environmental conditions for effective expression of this trait.

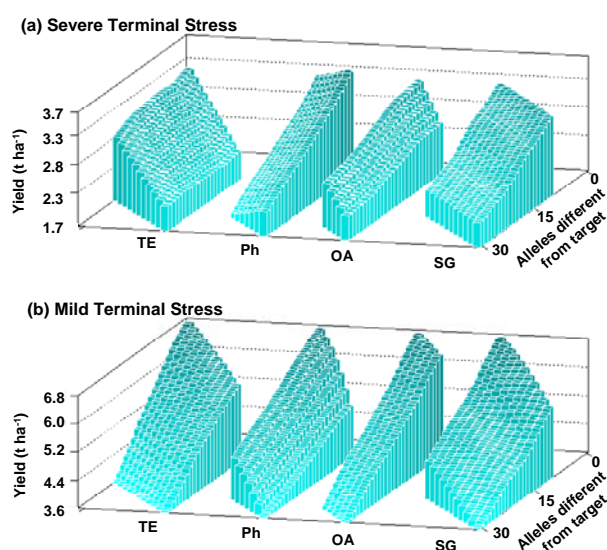


Figure 2. Average simulated yield in all (a) severe terminal stress and (b) mild terminal stress environments for all genotype combinations. The target genotype is defined as that genotype containing all the positive alleles for all traits. For each trait, the columns (from front to back of the figure) depict average yields associated with a specific number of positive alleles for that trait (11 columns for TE, 7 for phenology, 5 for osmotic adjustment, 11 for stay-green) and decreasing degree of difference from the target genotype due to combinations of positive alleles from the other three traits. For each trait the column to the left relates to genotypes with the maximum number of positive alleles. The column to the right for each group has no positive alleles for that trait.

The value of combinations of traits varied with environment type (Fig. 2). In STS environments, positive alleles for TE were associated with higher yield on their own, whereas, in MTS environments, their value only became evident when combined with positive alleles for other traits. This result reflects the over-riding value of more efficient use of water in generating biomass in water-limited environments. This trait delays the onset of water limitation as the available water resource is diminished less rapidly. Although not directly linked to the SG trait in this study, the TE trait is also likely to generate a staygreen phenotype by delaying the onset of senescence. This mechanism is of less importance in environments where water limitation is not as prevalent (MTS). Conversely, positive alleles for SG had little value in STS environments until combined with other traits but had greater individual effect in MTS environments. This reflects the mechanism based on N dynamics used to underpin this trait in this study. The additional N in the SG type generates more value in situations where water is not often limiting and the enhanced accumulation of biomass, associated with delayed senescence and higher RUE, can progress unimpeded. This result suggests that some other physiological mechanism (e.g., TE) is more likely responsible for causing staygreen in water-limited environments.

Increased understanding of G-P relationships

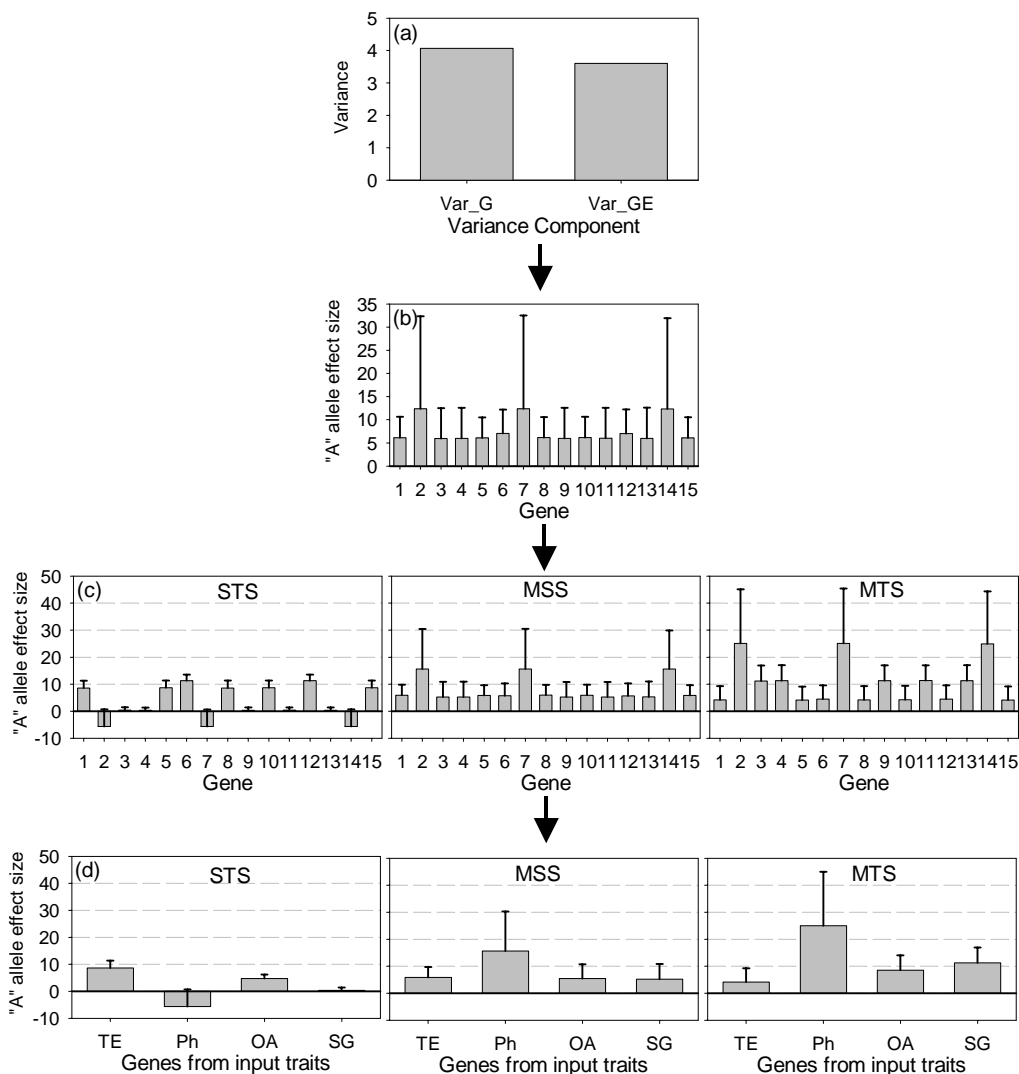


Figure 3. Analysis of genetic and environmental effects with increasing understanding of gene-to-phenotype relationships. The top panel shows variance components analysis from conventional phenotypic analysis on yield for the whole data set. The second panel extends this to analysis of gene effects, similar to QTL analysis. The bars represent average effects across all environments and the line indicates the standard deviation of effect size. The third panel extends this to analysis of gene effects by environment type, which must be defined by simulation. The lower panel extends this by grouping gene effects by underpinning physiological traits, which requires enhanced knowledge of trait physiology and genetics. In all cases, gene effects are defined in relation to the positive allele. A negative allele effect indicates that the opposite allele is defined as favourable.

The simulated average phenotypic effects among environment types mask the large variability among individual environments (547 location-season combinations) and genotypes (4235 expression states). In reality, only a sample of both is usually available and the environment and gene context dependencies present in any sample hinder interpretation and progress in crop improvement. In this study, we have generated the full set of combinations (individual environments x expression states) and use this to gain a more realistic appraisal of dealing with complex traits in variable environments.

Analysing genetic effects

To determine consequences of increasing physiological understanding on insight into genetic effects the complete data matrix of simulated phenotypes (4235 expression states, derived from allelic variation at 15 loci, for each of 547 environments, derived from location-season combinations) was subjected to a range of analyses (Fig. 3). In the first instance, a conventional quantitative genetics variance components analysis on yield (Comstock and Moll, 1963; Cooper and DeLacy, 1994) indicated near equal amounts of G and GxE interaction effects (top panel, Fig. 3). This reflects a common outcome faced in plant

breeding programs when phenotypic evaluation is all that is available to guide selection. In this case though, the additive effects incorporated in relating allelic variation to variables influencing response and control equations in the crop model had generated the significant GxE interaction. Next, an analysis of gene effects on yield for the 15 genes involved indicated the degree to which each gene influenced the phenotypic variation for yield (second panel, Fig. 3). In this analysis the average effect size of the positive allele was calculated for each of the 15 loci across the entire data set. It indicated what might be found from a QTL analysis with good statistical power (i.e., adequate population size and marker density). The result showed that the genes had varying levels of effect on the phenotypic variation. This reflects the situation where molecular information can assist in identifying key genomic regions but their basis and associations are unknown. Next, the typing of environments, based on using the crop model as a virtual entry in each trial, was incorporated in the analysis of gene effects. It was immediately clear that average gene effects varied substantially among environments (third panel, Fig. 3). In fact, genes with strong positive effects overall and in MTS and MSS environments, had negative average effects in STS environments. The environment typing was able to better resolve effects of particular genes within a general type of environment. That is, it started to unravel the GxE interaction in a way that would be useful to effective use of molecular information. Finally, the association of genes with their broad physiological basis was incorporated in the analysis (bottom panel, Fig. 3). The average effects varied substantially among traits in the different environments. The grouping of the genes based on their physiological linkage provides an even stronger basis to understand and utilise the GxE interaction. It becomes clear that in this case all negative effects in STS environments are associated with phenology, whereas major positive effects in that environment type are associated with TE. Such additional information would underpin a focus on specific molecular information (e.g., QTLs) in particular environments. It also provides a means to target relevant phenotyping in specific environment types.

While environmental characterisation and physiological knowledge help to explain and unravel gene and environment context dependencies, the analysis of average gene effects on yield across all environments masks some of the key effects of environmental variability. Although there is greater variability of gene effects on yield among environment types than within them, the gene effects still vary considerably within an environment type (Fig. 4). For many of the trait–environment type combinations, gene effects associated with individual environments can change sign. For phenology, the average gene effect is negative in STS and positive in both MSS and MTS environment types (Fig. 3). However, for individual environments within these groupings, gene effects can be positive in some STS environments and negative in some MSS and MTS environments. This inconsistency can cause major problems in the detection of QTLs and their effective use in molecular breeding.

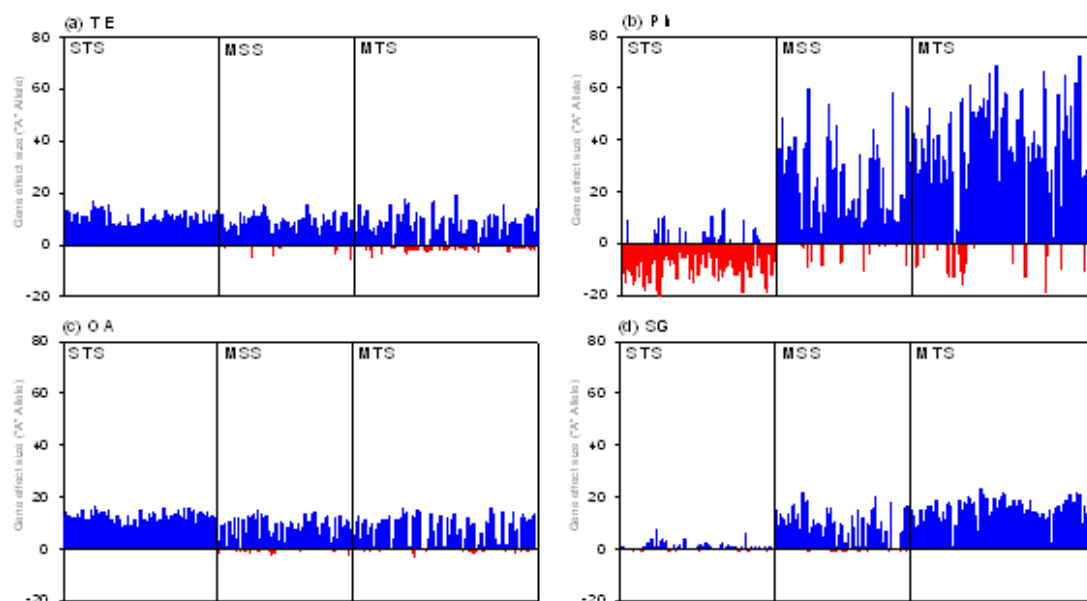


Figure 4. Analysis of gene effects for individual environments in the three environment types (STS, MSS, MTS) for genes associated with (a) TE, (b) Phenology, (c) OA, and (d) SG. Blue bars represent positive effects (of the positive allele) and red bars represent negative effects.

Investigating the power of breeding strategies

We simulated a marker-assisted selection (MAS) breeding strategy based on the analyses of gene effects presented above. The effects of all the genes were estimated at the start of the breeding process, and it was assumed that markers close to the genes were available. Marker scores were allocated based on the contribution of gene effects to yield in a single environment. That is, a QTL analysis was conducted using yield outcomes in each of the environments in the total population of environments (TPE, Fig. 4), and MAS was then conducted based on that QTL analysis. Hence, the number of scenarios simulated was the same as the number of environments used in the study. MAS was implemented in a manner such that an equal amount of weighting was given to the genotypic and phenotypic information in the random environments sampled from the TPE in each cycle of testing and selection in the breeding process.

There was a wide divergence in rate of yield gain in the TPE with breeding cycle depending on the environment chosen for the QTL analysis (Fig. 5). This result reflected the fact that particular genes only demonstrated their greater value in particular environments as noted above (Figs. 3 and 4). Hence, selection of environments for definition of QTLs assumes considerable importance as QTL-by-environment interaction can undermine the value of MAS. In particular, the yield gains associated with QTL analyses in STS environments were generally the lowest. In these environments, the favoured set of alleles for phenology were opposite to those required for yield advance in the TPE. That is, for those situations, the QTL analysis would result in selection pressure for the wrong alleles and lead to a sub-optimal response to selection in the TPE. While the use of a single environment for QTL definition might be considered extreme, and the phenology-by-environment interaction as not unexpected, the example highlights what might occur in situations where the underlying context is not known.

The knowledge associated with trait physiology and modelling clarifies the QTL-by-environment issues that confound this outcome with MAS. Such information enhances confidence in QTLs either by improved awareness of the importance of the environment type used in their definition (i.e., environment context dependency) or by improved awareness of association to specific traits (i.e., gene context dependency). Use of this knowledge in breeding should result in a greater chance of operating at the top end of the range for yield gain resulting from MAS (i.e., towards the black line in Fig.5). Potentially more rapid advance could arise by weighting the QTLs in relation to the importance of the associated trait in specific test environments. These potential advances require physiological studies and modelling to aid in detecting relevant and intrinsically stable QTLs via trait dissection studies, to derive relevant indices that would be useful in phenotyping, and to classify and assess relative importance of environment types.

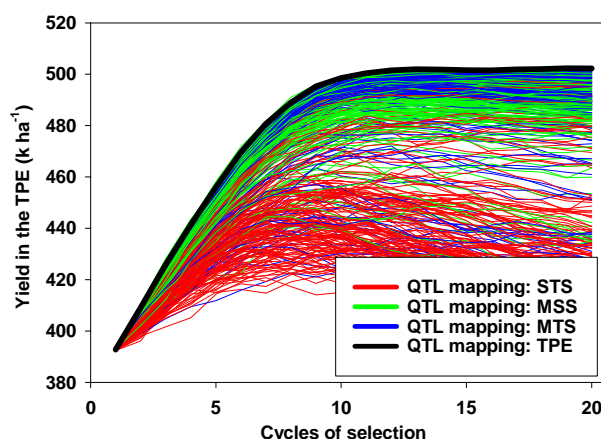


Figure 5. Average yield in the total population of environments (TPE) over 20 cycles of selection for a range of marker-assisted selection (MAS) scenarios. Each trajectory represents results using a QTL analysis based on a single environment. All environments in the TPE were used to derive the range of scenarios examined. Trajectories are coloured depending on the environment type of the environment used for the underpinning QTL analysis. The black line shows the outcome when all environments in the TPE are used to define QTLs.

Can trait physiology and modelling add value to plant breeding?

Although the underlying genetic controls assumed for the traits used in this analysis were very simple, the results nonetheless suggested that trait physiology and modelling could add value to plant breeding by unravelling environment and possibly gene context dependencies that cause inefficiencies in MAS. The success of molecular breeding relies on an effective prediction of phenotypic variation based on allelic variation. Current approaches to MAS for complex traits rely heavily on the use of statistical approaches that are based on linear models. Their predictive power is poor when interactions among genes and/or environments (i.e., context dependencies) are important. The added value from a trait physiology and modelling framework arises because consequences of these interactions on the resultant phenotype are an emergent property of the framework. Hence, predictive power and the effective implementation of MAS are enhanced.

To realise the added value of a trait physiology and modelling framework will require its effective integration into plant breeding programs. Better characterising production environments and the more effective use of molecular markers by defining intrinsically stable QTLs associated with complex traits offer realistic initial targets. Beyond this, there are opportunities for guiding gene discovery and for improved evaluation of potential of specific transgenics. Environment characterisation requires improved attention to soil and climate conditions encountered in breeding trials so that models can be used as virtual entries. This facilitates weighting of particular trials relative to their importance in unravelling genotype-environment interactions and their representation in the TPE (Chapman et al., 2000). However, to define stable QTLs requires far more attention to phenotyping than is normal practice in breeding programs. It is likely that specialist studies facilitating more in-depth physiological dissection among lines of interest will be required. This could be combined with broader screening in breeding populations based on selection indices that reflect the underpinning physiological basis for trait variation. It will be necessary to test the stability of QTLs identified in this way using validation studies based on their predictive capability. Reymond et al (2003) report an example of this approach. They used an ecophysiological model to identify QTLs for control of leaf growth in maize and then validated their stability by using the QTLs to predict responses of other genotypes. The challenge is now to determine whether this approach can be effective at whole plant/crop level for more complex traits.

To be useful, the physiological frameworks used for trait dissection and modelling at whole plant/crop level must realistically capture the functional basis of the genetic variation for complex traits of interest. Most existing crop models, which were constructed to deal mostly with agronomic issues, are not well structured in this regard, as found by Dingkuhn (1996) for carbon and nitrogen partitioning and by Jeuffroy et al (2002) for capture and use of nitrogen. The crop physiological modes of action of the complex trait must be understood and quantified and the crop model must be sufficiently detailed to simulate the consequences on growth and development generated by the interaction of those modes of action with the environment (Hammer et al., 1996). Appropriately specifying and quantifying the response and control equations are critical in effective crop modelling. The control equations most likely reflect the basis of metabolic signalling in plants and thus provide focal points for links to underlying genetic systems (Hammer et al 2002). Tardieu (2003) demonstrates this concept using an ecophysiological model of plant water use. He identified stable 'meta-mechanisms' at plant level that reflected the parameterisation of the response and control equations of the model. Hammer et al (2004) argue that these 'meta-mechanisms' provide the bridge across levels of biological organisation that will link molecular biology and crop improvement via 'in silico plant' technologies.

There remains some uncertainty as to the degree of residual aggregation in 'meta-mechanisms' in crop models that will be most effective for linking phenotype complexity to underlying genetic systems. Modelling concepts framed around source-sink balance among organs and regulation of the supply-demand dynamics for carbon, nitrogen and water provide a pathway forward (Dingkuhn, 1996) but their implementation may become highly parameterised (eg Drouet and Pages, 2003). We suggest that a key feature of the development of effective models in this domain will be the retention of simplicity, while simultaneously improving the rigour and generality of dealing with functional control. Our operating hypothesis is that physiologically sound but simple whole crop models will provide the balance between capturing process understanding and the predictive utility needed to effectively link phenotype to genotype. The 'meta-mechanisms' identified using such models will remain some distance away from

gene complexes on the scale of biological organisation, but by combining with statistical quantitative genetics approaches they should provide sufficient unravelling of environment and gene context dependencies to have significant impact on breeding strategies. We are progressing development of this approach via participative action research in the sorghum breeding program of the Queensland Department of Primary Industries and Fisheries in Australia.

Conclusion

Use of a crop growth and development modelling framework can link phenotype complexity to underlying genetic systems in a way that enhances the power of molecular breeding strategies. Such a framework facilitates meaningful characterisation of production environments and physiological dissection of complex traits. Both aspects aid in identifying intrinsically stable QTLs by reducing environment and gene context dependencies, which inhibit the utility of traditional statistical approaches used in molecular breeding. Implementing this gene-to-phenotype capability in crop improvement will require enhanced attention to phenotyping, developing the ecophysiological modelling framework required, and conducting the validation studies needed to test the stability of QTLs identified.

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