

Breeding Maize for Drought Tolerance in the US Corn-Belt

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Abstract

The objective of this review is to promote discussion on some of the key issues we consider to be involved in successful long-term genetic improvement of corn for drought tolerance. Many of the concepts have been discussed elsewhere for different crops, traits and different breeding targets. Here we focus on how to organize a comprehensive effort to enable breeding for drought tolerance in a heterogeneous target population of environments where drought is a component of the set of environmental challenges that the plant breeder has to deal with. Particular attention is given to mapping Quantitative Trait Loci (QTL) and the opportunities for their utilization in corn breeding.

Introduction

Drought influences the yield of hybrid corn in the US corn-belt. The incidence of drought changes with location and year in the corn-belt (Fig. 1) and is frequently severe enough to significantly reduce yield (Fig. 2). Thus, there has always been interest in breeding corn for improved levels of drought tolerance (Barker et al. 2004). Plant breeders and physiologists recognize drought tolerance as a complex trait. The difficulty of dealing with such complexity has motivated many studies to unravel the genetic and physiological basis of drought tolerance in field crops (Ludlow and Muchow 1990, Ribaut 2006). Given the ubiquitous nature of drought in many crop production systems throughout the world, drought tolerance is frequently identified as a target for research programs that seek new solutions to achieve higher levels of drought tolerance in subsistence and commercial crops. Those that have invested the majority of their careers in breeding for drought tolerance know that breeding for drought tolerance is easier to say than to do. For any research to have a chance of impacting the genetic improvement of drought tolerance in field crops the research effort must be established as a long-term commitment and have clear objectives. Plants can achieve drought tolerance through different approaches, involving combinations of escape, avoidance and tolerance of the effects of water deficit on the growth and development processes that impact yield. Thus, many different traits have been proposed as selection targets to improve levels of drought tolerance and yield under water deficit conditions. Many of these putative drought tolerance traits have been recommended for indirect selection and used as targets for genetic analysis (Ludlow and Muchow 1990; Edmeades et al. 2000). Genotype-by-environment interactions commonly confound interpretation of experimental results, realized genetic gain is often lower than predicted and most attempts to select for improved drought tolerance via indirect selection based on putative drought tolerance traits have not contributed to successful commercial improvements of drought tolerance in the hybrids grown by farmers. In general most improvements in crop drought tolerance have come from

incremental improvements achieved by long-term breeding efforts. The same is true for corn in the US corn-belt.

Pioneer Hi-Bred International has been breeding temperate corn hybrids with improved drought tolerance for more than 80 years. This long-term breeding effort has developed a sequence of successful commercial hybrids that have been grown widely throughout the US corn-belt (Fig. 3, Duvick et al. 2004). The modern hybrids of the current decade possess both improved yield potential and drought tolerance for the range of environments encountered in the US corn-belt. Further, the germplasm that contributed to the parents of the drought tolerant hybrids of previous decades and the elite germplasm that contributes to the modern drought tolerant hybrids grown today continue to contribute important sources of genetic diversity to the current breeding cycles of the Pioneer breeding programs whenever drought is an important component of their target environments. Thus, breeding for drought tolerance in elite commercial corn germplasm focuses on adding novel and improved levels of drought tolerance to hybrids that already demonstrate commercially viable levels of drought tolerance.

While we can measure genetic gain for drought tolerance in many ways (e.g., Fig. 3), success in breeding for drought tolerance in a commercial program is ultimately measured by hybrid yield in drought prone environments that is improved over that which can be achieved by the current set of grower preferred commercial hybrids. To achieve further improvements in drought tolerance, Pioneer applies molecular technologies in combination with novel pedigree breeding methods. This review paper has two main objectives: (1) Discuss some of the key considerations involved in efforts to breed corn for improved levels of drought tolerance; emphasis is given to mapping Quantitative Trait Loci (QTL) to enable molecular enhanced breeding, and (2) Review some novel methods, inspired by recent advances linking trait QTL to physiological processes captured within crop growth models, that can be used to provide testable predictions of how multiple traits interact to contribute to crop performance in drought prone environments.

Mapping QTL and Molecular Breeding for Drought Tolerance: Key Considerations

There is long-standing interest in mapping trait QTL. This interest has been strongly motivated by the expectation that the QTL provide useful entry points that will ultimately help us understand the genetic architecture of traits. Here we adopt a definition of genetic architecture comparable to that used by others (Mackay 2001, Holland 2007); involving the definition of the number of genes, their position in the genome, the effects of the genes and their alleles on the traits of interest. The plant breeder anticipates that knowledge of the genetic architecture traits, based on the identified QTL, can be applied to predict new genetic combinations that could be created by molecular enhanced breeding. For example, the breeder can use markers to select for specific alleles of individual QTL, specific combinations of alleles for multiple QTL, or for the combined effects of many QTL without specific attention to individual QTL.

Given the large number of publications that report the results of studies designed to map QTL for traits, it is reasonable to assume that it is widely accepted by many geneticists that a QTL mapping approach can be used to dissect traits into useful component pieces for further genetic analysis and marker-assisted selection. In some cases, for important QTL, such genetic analysis can lead to fine-mapping and map-based cloning of the sequence variation that underlies the QTL (Salvi and Tuberosa 2005). Whenever possible we would like to extend the genetic knowledge obtained from such trait dissection studies to include an understanding of how the QTL (or genes) and their alleles regulate the plant physiological processes that determine trait phenotypic variation and ultimately variation for grain yield. Thus, as well as being able to *dissect* traits into genetic components by mapping we would like to have the capacity to *integrate* the genetic components and predict whole plant performance. It has been argued that if we can determine how allelic variation for trait QTL impacts the physiological determination of yield within an environment there is an opportunity to use appropriately designed models of crop growth and development to predict the potential value of novel allele combinations for multiple QTL within the context of the reference population of genotypes and the target population of environments of a breeding program (Chapman et al. 2003, Tardieu 2003, Hammer et al. 2005, Messina et al. 2009). However, unlike trait dissection by QTL mapping the integration of QTL effects within crop growth and development models to predict the effects of their allele combinations on trait phenotypes for different genotypes in different environments is less familiar to plant breeders and thus less widely adopted.

The complementary methodology for mapping to *dissect* a complex trait, such as drought tolerance, into underlying genetic components on the one hand, and modeling to *integrate* the genetic components to predict whole plant performance on the other, have the potential to be used together to enable the definition of testable genetic hypotheses in the form of target genotypes for complex traits for creation by the breeder as an integral component of the breeding program. If a target genotype based on multiple QTL and gene allele combinations can be defined, then this can be created in a breeding program and evaluated for its performance in the target environments and compared to genotypes developed by other breeding strategies. If the combined dissection-integration QTL modeling strategy described above leads to the development of novel genotypes that have superior performance in the target environments, then the breeder has additional tools to enable molecular enhanced breeding for drought tolerance.

Some key considerations that have been found useful for efforts to map QTL for traits in general and with some specific considerations for their roles in breeding for drought tolerance are discussed below.

Breeding Objectives: For any long-term breeding effort to be successful it is critical to strike the appropriate balance between defining clear breeding objectives and ensuring flexibility within the breeding program to respond and accommodate new genetic, germplasm and environmental knowledge as this becomes available. Given the many proposed breeding strategies for improving drought tolerance that can be found in the literature, the breeder needs a clearly defined set of check hybrids as internal references to decide whether the chosen breeding strategy

is achieving adequate progress. Having access to a well characterized set of hybrids that represents the set of benchmarks to be improved upon (Fig. 3) is an advantage for two general types of breeding objectives: (1) Lesion Repair, where the objective is to transfer superior performance from germplasm that already has the desired performance, but may be unacceptable for other traits, into elite backgrounds. This approach is commonly associated with the use of backcross breeding methods to transfer disease resistance from donors into target elite germplasm. However, this approach can also be applied for more complex traits, such as drought tolerance. For this objective, molecular markers can be used to map the donor sources of drought tolerance and for selection to accelerate allele transfer across multiple backgrounds for evaluation of the impact of the introgressions. Such donors of genetic variation for drought tolerance may come from within the elite reference population of genotypes of the breeding program or exotic sources from outside of the breeding program. (2) Novel genotypes, where the objective is to create new allele combinations given the genetic architecture of the traits of interest. Here the objective is to create new hybrids that are beyond the levels of performance that are already available. For specific bi-parental crosses transgressive segregation, achieved by recombining the genetics contributed by the two parents, is a common example where the breeder creates novel progeny with superior performance to that of their parents. The concept is extended to multiple parents over multiple cycles of breeding within the context of a pedigree breeding program. To improve drought tolerance the breeder can either target specific combinations of putative drought tolerance traits or target yield evaluations of large populations of genotypes in appropriate environmental conditions. In the latter case there is scope to determine which trait combinations have contributed to improved performance and subsequently apply this knowledge to define breeding targets for testing (Podlich et al. 2004, Hammer et al. 2006). It is likely that a combination of both methods will be applied with different approaches attempted depending on the breeding populations and knowledge of traits contributing to improved drought tolerance in the target drought environments.

Germplasm: The reference population of elite genotypes available to the plant breeder represents integrated genetic combinations that can be used by the breeder to create new cycles of genetic variation for further breeding. Identifying parental genotypes that can be combined to create relevant genetic variation for traits contributing to drought tolerance is a key component for any breeding program and for any QTL mapping study conducted to enable molecular breeding. It is critical in commercial breeding systems to ensure that the base of germplasm used for studies of drought tolerance is a relevant pool of genetic variation in non-drought environments as well. That is, the study of the genetic and physiological basis of drought tolerance must be accomplished within the constraints of breeding programs developing new elite genetics for a target population of environments that comprises of a complex mixture of drought and non-drought conditions. While detection and characterization of QTL is important, it is ultimately the goal of the breeder to use this information for the development of new products; by ensuring that QTL mapping populations are meaningful to the existing germplasm base, the breeder is able to readily leverage promising QTL results in current elite populations by selecting on regions of interest or by designing new breeding populations.

Phenotyping: For all studies of the genetics of drought tolerance the ability to conduct uniform experiments and measure the correct traits at the correct time with adequate precision is critical to success. While this could be said for all traits it is particularly challenging for the complex traits associated with plant responses to drought. The same requirement of consistent and reliable phenotyping applies for drought tolerance breeding strategies. Thus, much time has been invested into high throughput methods for screening large numbers of genotypes for traits considered to either indicate or contribute to drought tolerance. These have been attempted in controlled environment facilities, glasshouse conditions and in managed stress field locations. Whatever approach is adopted, the expression of genetic variation for plant responses and the measurements taken must be predictive of the genetic variation for drought tolerance that contributes to improved yield under water deficit conditions in the target on-farm conditions. Quantitative genetic theory provides many useful statistical methods for determining the reliability of any phenotyping strategy for predicting genetic variation for drought tolerance in the target environments. Some examples of the application of this theoretical framework for drought tolerance breeding can be found in Cooper et al. 1995, 1997 and Cooper and Hammer 1996.

Environmental Characterization: Understanding the environmental conditions that contribute to drought and the expression of genetic variation for drought tolerance is critical to the success of any attempts to breed for drought tolerance. To assess the relevance of the genetic and phenotypic variation revealed in any experiment attention must be given to characterizing the pattern of development of water-deficit in the experiment and how this imposes drought stress on the different genotypes. For example, with access to specific environmental variables from different stages of crop growth and development, Boer et al. (2007) were able to relate QTL-by-environment interactions for grain yield to specific environmental co-variables and demonstrate differential expression of the QTL effects could be explained by differences in the environmental conditions sampled in experiments from across an East to West transect of the US corn-belt for two years. With some understanding of the environmental conditions in play within specific experiments it is possible to relate the specifics of individual experiments to the likelihood of occurrence of these conditions within the target population of environments for the US corn-belt. Löffler et al. (2005) demonstrated the use of the EnClass[®] system for characterizing the different environmental conditions of the US corn-belt. Messina et al. (2009) discussed applications of this methodology for the genetic improvement of drought tolerance. Cooper and Podlich (1997) applied simulation methodology to demonstrate how weighting results from individual experiments based on their likelihood of occurrence in the target population of environments could result in greater rates of genetic gain when genotype-by-environment interactions are important. In addition to helping the breeder to interpret the results of specific sets of experiments, this information can be used to design strategies for deploying experiments to locations to increase the chances of sampling relevant environmental conditions across multiple years. Equally, knowledge of the key environmental conditions in the target population of environments can be used to design appropriate managed stress conditions (Cooper et al. 1995, 1997, Barker et al. 2004).

Large Population Sizes: Empirical and theoretical QTL mapping studies conducted at Pioneer Hi-Bred have consistently demonstrated the importance of large mapping population sizes to avoid over estimation of QTL effects (Beavis 1998, Cooper et al. 2007). With the availability of a large number of molecular markers that can be cost efficiently measured on a large number of individuals, it is feasible and practical for commercial breeding programs to work with large mapping populations. The large populations can be developed from specific bi-parental crosses (Boer et al. 2007) or from multiple pedigree related populations (Graham and Podlich 2006, Blanc et al. 2006).

Mixed Model Analysis Methodology: Many methods have been proposed for applying genome scan approaches for QTL mapping applying linkage models and more recently for Association Mapping applying linkage disequilibrium methods. While different algorithms can be applied, the development of software for implementation of both common and more advanced mapping methods within a mixed model framework is the recommended methodology for dealing with the complex data sets generated across multiple environments for identifying QTL and their utilization within a breeding program.

Predicting trait and QTL contributions to improved Drought Tolerance: In addition to the identification of QTL for further study, plant breeders are particularly interested in methods for the integration of the QTL effects to predict the expected performance of genotypes. Applying mixed model methodology predictions can be constructed for the expected genotype performance for small numbers of genes and QTL (Eagles et al. 2002) to the whole genome prediction methods (Meuwissen et al. 2001). For the corn breeder these methods need to be applied within a hybrid breeding context. In addition to the statistical prediction methods that can be implemented within the mixed model framework, other approaches for prediction have been attempted and applied to corn. Messina et al. (2009) discussed the use of a crop growth and development framework for predicting the yield performance of genotypes based on QTL for component traits in a range of environments differing for degrees of water deficit. Hammer et al. (2009) used the same framework to evaluate the potential contributions of component traits to the long-term genetic gain of corn yields in the US corn-belt and emphasized the importance of root system architecture for improvements in water capture, tolerance of water deficits and increased yield under drought conditions. Figure 4 shows an example of performance landscapes, as determined by genetic variation for two traits and the equations included in the crop modeling framework, for two contrasting environment types, differing for degree of drought. The analyses of such multi-trait landscapes, describing how traits combine to contribute to drought tolerance, helps advance our understanding of genotype by environment interactions for yield under drought conditions, identify component traits that contribute to genetic variation for drought tolerance and yield, and can be used to define plausible selection trajectories leading to improved performance to be tested in the breeding program. While there is a heavy investment in methodology development required to implement either the statistical or crop modeling approaches to prediction, these approaches are feasible and practical and can be designed to provide predictions within the context of the cycle of a breeding program to assist selection decisions at many stages of the breeding program.

Testing Predictions: One final point that is emphasized here is that in addition to making predictions of how to improve drought tolerance of corn hybrids, it is important to design experiments or create genotypes within a breeding program to test any predictions. The literature on the study of genetic variation for drought tolerance is rich in predictions but poor on tests of the predictions. Two programs that have been reviewed previously for their long-term genetic gain results are the improvement of temperate corn hybrids in the US corn-belt by Pioneer Hi-Bred program and the CIMMYT program focused on tropical corn (Cooper et al. 2006).

Conclusions

The emphasis of this review is on specific components of a breeding program focused on improving drought tolerance of corn for the US corn-belt. While universal answers cannot be given for all cases, examples of successful results were provided to demonstrate the types of research that will have to be undertaken. While drought tolerance in corn for the US corn-belt is a complex trait, long-term genetic gain has been made and the experimental evidence and theoretical results indicate that further genetic improvements can be made. An important point to make is that the issues emphasized here focused on the study of the genetic architecture of drought tolerance by QTL mapping and utilization of genetic diversity associated with the QTL for molecular enhanced breeding. Similar arguments will apply to the utilization of transgenic sources of genetic diversity and combined uses of natural and transgenic sources of genetic diversity.

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Figure 1 Summary of the geographical distribution of different environmental conditions across North America for a sample of four years. The incidence of water deficits contributing to drought changed across the four years displayed.

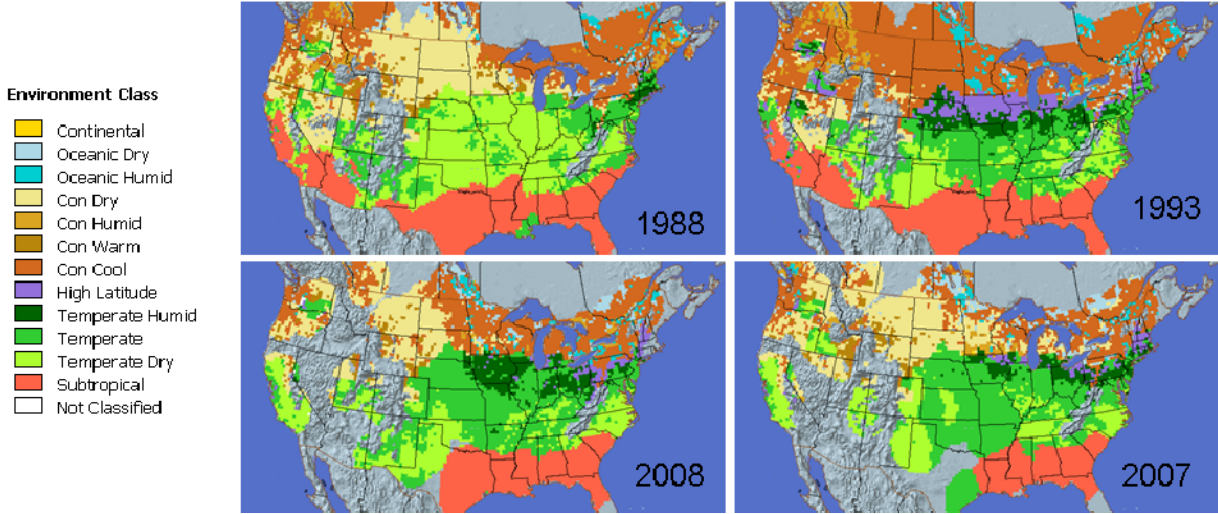


Figure 2: Grain yield impact of water deficit during different stages of corn development

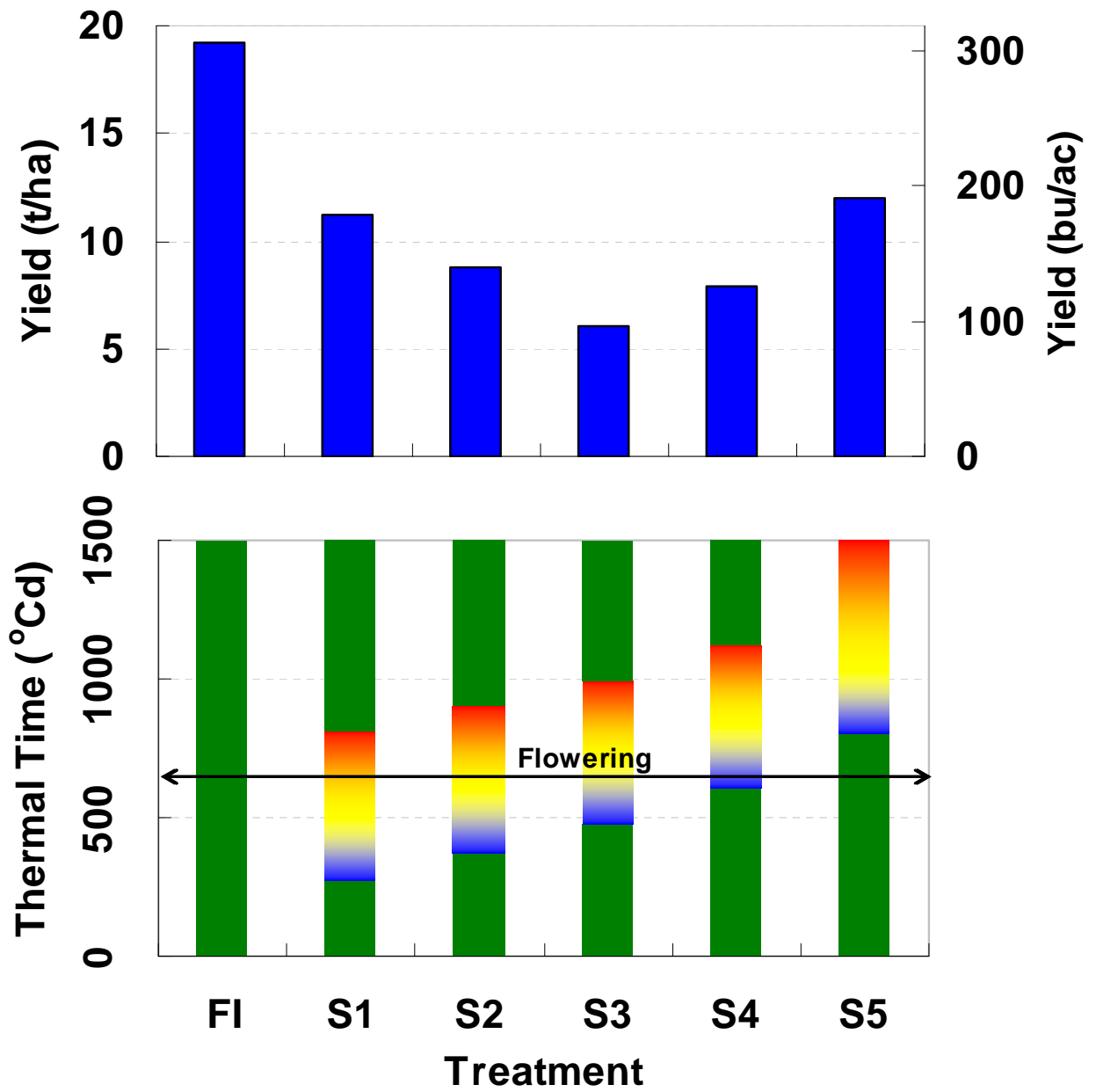


Figure 3. Grain yield for a sequence of hybrids released by Pioneer hybrid from the 1930s to the present. The environments are grouped into three categories: (1) High-Input irrigated environments to evaluate the yield potential of the hybrids, (2) Drought environments where irrigation was managed to impose water deficit on the hybrids during the flowering or grain filling stages (see Figure 2), (3) Target Population of Environments (TPE): Random environmental conditions sampled at 2 to 5 locations for each of the years from 1990 to 2007. For the TPE evaluation the hybrids were evaluated at three plant populations and the hybrid yield presented is for the plant population at which the hybrid achieved the highest grain yield.

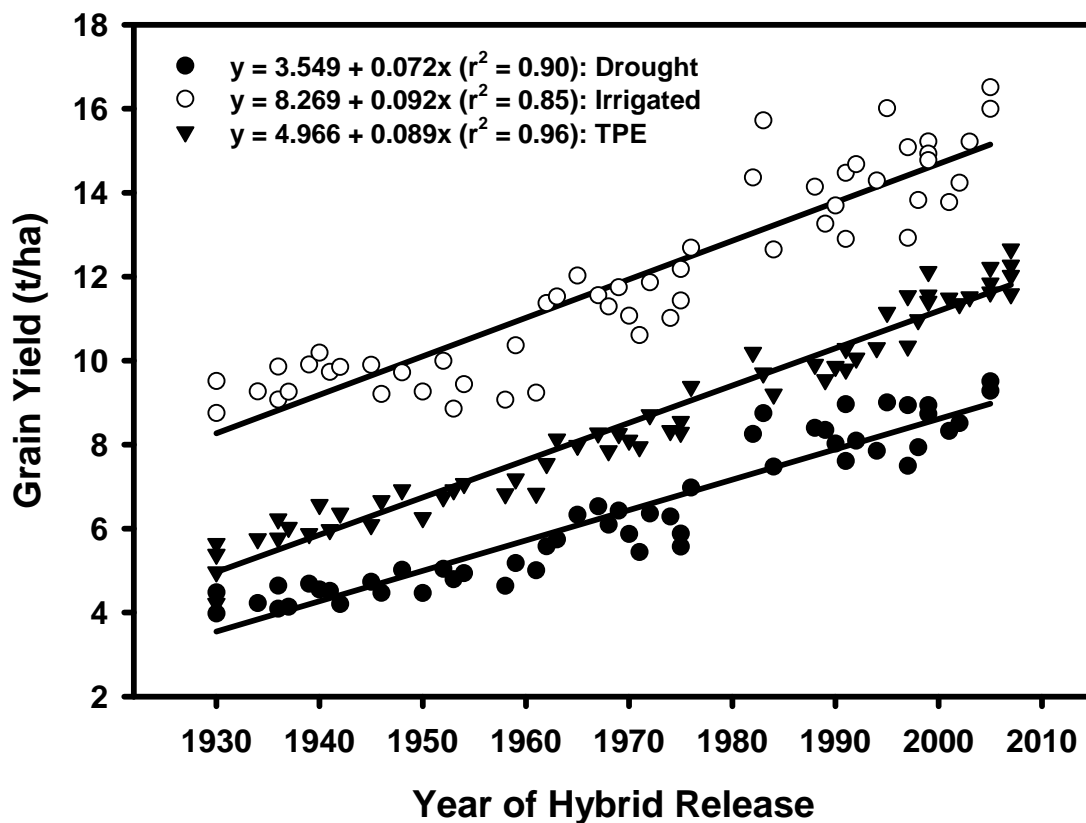


Figure 4. Simulated landscape for a breeding population varying for canopy size (x) and crop development (y); maturity increasing from 0 to 1. A sample of individual genotypes from the breeding population is plotted as spheres relative to the landscape response surface for an environment type characterized by moderate stress (red surface). A landscape response surface for an environment type characterized by severe drought stress is shown in blue. In this example the selected genotypes follow a ridge of superior grain yield given the environmental conditions experienced by the genotypes.

