## Discovering Stay-Green Drought Tolerance Genes in Sorghum: A Multidisciplinary Approach

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### Abstract

Drought is the major environmental factor constraining crop production globally. Identifying and understanding the function of genes and gene networks that contribute to improved plant drought resistance under water-limited conditions is a fundamental component of sorghum breeding programs in Australia and the United States. In particular, genes located in four genomic regions (Stg1, Stg2, Stg3 and Stg4) controlling the functional basis of the B35 source of 'stay-green' are being sought. Plants with the stay-green drought-resistance trait maintain green stems and upper leaves when water is limiting during grain filling. This paper discusses the multi-disciplinary approach to gene discovery implemented by Australian and U.S. scientists in pursuit of the key stay-green genes. In this project, map-based gene cloning is the primary approach to gene discovery. Multiple cycles of phenotyping and genotyping have enabled scientists to close-in on the genes of interest via fine-mapping, and will ultimately lead to the discovery of gene function. Candidate genes will be identified within each of the four genomic regions using an integrated genetic and physical map of sorghum, together with detailed physiological dissection of genotypes with different stay-green alleles. Proof of gene function will follow. Gene function in a range of environments will be assessed in silico using crop simulation modelling. Overall, this integrated approach to gene discovery will enable plant breeders to more efficiently custom-make sorghum varieties for specific water-limited environments.

### **Media summary**

The discovery of 'stay-green' genes in sorghum by Australian and U.S. scientists will lead to improved drought-resistance in sorghum, and potentially other major cereals.

# **Key Words**

Stay-green, gene discovery, drought-resistance, sorghum

### Introduction

In many areas of human endeavour, it is often the interface between fields of knowledge that proves to be the fertile ground in which significant ideas are sown, and subsequently reaped. So it is with 'gene discovery' in the world's most important cereal crops. The pursuit of drought-resistance genes in sorghum is a multi-disciplinary effort involving plant breeders, crop physiologists, molecular biologists, biometricians, functional genomicists and simulation modellers. It is at the interface of these disciplines that quantum leaps in knowledge are occurring. An international partnership involving scientists from Australia (DPIF Queensland) and three U.S. universities (Texas A&M, Texas Tech and Missouri) is leading the search for genes (*Stg1*, *Stg2*, *Stg3* and *Stg4*) associated with the 'stay-green' trait in grain sorghum. Keeping leaves alive for longer is a fundamental strategy for increasing crop production, particularly under water-limited conditions. During post-anthesis drought, genotypes possessing the stay-

green trait maintain more photosynthetically active leaves than genotypes not possessing the trait. The broad objective of this research is to identify and understand the function of genes and gene networks that contribute to improved plant drought resistance under water-limited conditions.

### Approaches to gene discovery

There are two general approaches to identify and isolate genes involved in drought resistance (Mullet et al., 2001). First, genes are targeted that show relatively rapid changes in expression at the RNA level in response to water limitation. Second, sorghum genes involved in drought resistance are identified and isolated using map-based gene discovery. This latter approach identifies genes based on allelic variation within sorghum germplasm that control sorghum responses to water limited environments. Since these genes may or may not respond to water limitation through changes in gene expression, they represent an important class of genes that can be missed by expression profiling technologies. The current stay-green project utilises map-based gene discovery undertaken by scientists at Texas A&M University, USA, although micro-array analysis is being used simultaneously to assist in gene discovery and to understand how sorghum responds to drought.

### Phenotyping, genotyping and physiological characterisation

### Phenotyping driving genotyping

Map-based cloning requires the accurate screening of phenotype and genotype of large segregating populations (Tanksley et al., 1995), highlighting the need for collaboration between plant breeders, crop physiologists and molecular biologists. Typically, plant breeders develop a range of populations for mapping (e.g., recombinant inbred lines), fine-mapping (e.g., segregating populations with breakpoints across the loci of interest), and physiological dissection (e.g., near-isogenic lines). Such populations are systematically phenotyped and genotyped by crop physiologists and molecular biologists, respectively, resulting in the identification of regions of genomes (trait loci) that modulate the expression of traits such as stay-green.

### Genotyping driving phenotyping

Following the mapping of drought resistance loci, efficient map-based cloning requires the availability of a high-resolution integrated genetic and physical map, large populations and careful phenotyping (Mullet et al., 2001). The construction of an integrated sorghum genome map is well underway. A genetic map with about 3,000 points has been constructed with AFLPs, SSRs, and RFLPs using a *Sorghum bicolor* recombinant inbred population (Menz et al., 2002). In addition, physical maps of the sorghum genome are being constructed using BAC libraries that provide about 20X coverage of the sorghum genome (Klein et al., 2000; Klein et al., 2003; Draye et al., 2001). Portions of the BAC libraries have been fingerprinted using various methods and the BAC contigs are being aligned to the sorghum genome sequence (i.e., Klein et al., 2003). As a consequence, an emerging high quality integrated genetic and physical sorghum genome map that is aligned with the rice genome sequence is available to assist map-based gene isolation (Klein et al., 2003).

### Physiological characterisation

The aim is to dissect complex traits such as stay-green into their functional components. The characteristics of such complex traits can be viewed as emergent consequences of the interactions between underlying determinants and the prevailing environmental conditions (Hammer, 1998). Integration of knowledge from gene to cropping system is also necessary. For example, stay-green can be viewed at a cell, leaf, whole plant, crop and system level. Understanding how gene networks respond to water deficits across these levels is critical to capturing traits like stay-green in plant breeding programs. At a cell level, the retention of chloroplast proteins such as LHCP2, OEC33 and Rubisco until late in senescence has been reported in sorghum containing the KS19 source of stay-green, indicating

that photosynthesis may be maintained for longer during senescence in these genotypes (de Villiers et al., 1993).

At a leaf level, maintenance of photosynthetic capability is closely related to nitrogen (N) status. A series of experiments have shown that specific leaf nitrogen (SLN) in stay-green hybrids remains above the 'threshold' senescence level for longer than in senescent hybrids for at least three reasons (Borrell and Hammer, 2000). First, the leaf N 'benchmark' at flowering is higher in stay-green than senescent hybrids; second, N uptake during grain filling is higher in stay-green than senescent hybrids; and third, the remobilisation of N from leaves of stay-green hybrids during grain filling is less compared with that of senescent hybrids.

Stay-green can be viewed as a consequence of the balance between N demand by the grain and N supply during grain filling at a whole plant level. Matching N supply from age-related senescence and N uptake during grain filling with grain N demand found that the shortfall in N supply for grain filling was greater in the senescent than stay-green hybrids, resulting in more accelerated leaf senescence in the former (Borrell et al., 2001). Preliminary simulation modelling to assess the value of stay-green in a range of environments found that improved N dynamics alone could not explain the observed yield increases under drought; enhanced transpiration efficiency (TE) was also required (Chapman et al., 2003). It is still not clear, however, whether stay-green genotypes use more water or use water more efficiently. While there is some evidence of enhanced TE (Borrell et al., 2000), this issue is the subject of on-going research.

### Identifying candidate genes

Initial mapping activities can generally map targeted loci to 1-5 cM regions of the sorghum genetic map (i.e., Klein et al., 2001). Analysis of large segregating populations (~1,000 plants) is usually required to provide sufficient genetic resolution for efficient map-based cloning. Fine-mapping can then reduce the target locus in euchromatic regions to less than 100 kbp, a size that can be readily sequenced using standard BAC-based shotgun sequencing approaches. Interestingly, ~100 kbp of sorghum DNA, on average, will encode ~10 sorghum genes. There are several ways to identify genes within the target genomic interval. First, if the targeted region is less than 500 kbp, shotgun sequencing of BAC DNAs spanning the region followed by BLASTX analysis can be used to identify sorghum genes that are related to other known protein coding genes. Second, the sorghum sequence can be compared to the sorghum EST database to identify the transcribed portions of the BAC sequence. Third, other genes encoded by the sorghum BAC sequence can be identified by aligning the sorghum sequence with orthologous rice or maize sequences. Finally, gene prediction programs such as FGENESH (http://www.softberry.com/) and riceGAAS (http://ricegaas.dna.affrc.go.jp/) can be used to identify regions of the sorghum sequence that may encode genes.

### Proof of gene function

Identification of a candidate gene(s) is not the endpoint. Direct validation that a candidate gene causes variation in the trait under investigation is still required. Currently, this step is difficult in sorghum because sorghum gene transformation technology is time consuming, throughput is low, and not all genotypes are easily transformed (Mullet et al., 2001). Other technologies such as RNAi are now under development in order to accelerate candidate gene validation (Holzberg et al., 2002).

### Assessing gene function across environments via simulation modelling

The phenotype exhibited by plants with the B35 source of stay-green is a result of the various stay-green genes (*Stg1*, *Stg2*, *Stg3* and *Stg4*) acting alone, or in combination, to produce specific physiological functions such as increased N uptake under drought and/or enhanced TE. The interaction of these genes with the environment (e.g., timing and severity of drought) adds another level of complexity. Determining the mix of genes necessary to optimise yield in a given environment requires simulation modelling.

Crop modelling contributes to the genetic regulation of plant performance and improvement in a number of ways (Hammer et al., 2002). For the purpose of this discussion, however, it is worth noting the role of simulation modelling as a means of both determining and assessing gene function *in silico*: the complexity arising from genes interacting with each other and their environments (both natural and managed) requires a mechanism to assimilate the many and varied combinations. Simulation modelling provides such a mechanism.

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