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Discovery and development of alleles contributing to sorghum drought tolerance

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***Objective 1. Fine-scale characterization of qualitative factors related to drought tolerance*.** Drought tolerance is a complex outcome of the actions of and interactions among a spectrum of genes with phenotypic effects that range from qualitative to minute, and which function in a wide range of biochemical pathways and biological processes. Diallel analysis of key combinations of *stay-green* QTLs from donor B35 is the next logical (and indeed, necessary) step toward their exploitation in applied sorghum improvement in the tropics. The relative efficacy of various QTL combinations under stress conditions in Ghana and India, will be essential in prioritizing *stay-green* QTL combinations for deployment. This information will also guide prioritization of *stay-green* QTLs for mitigation of linkage drag issues, by searching segregating populations to be generated in this study for new recombinants. *Anticipated outcomes:*

a. Provide empirical tests of post-flowering drought tolerance conferred by three pair-wise combinations of stay-green QTLs, revealing the relative efficacy of various combinations under stress conditions representative of those in India (3 locations), and West and Central Africa (Ghana).
b. Produce segregating populations for obtaining additional recombinants near QTLs that prove to carry undesirable traits by linkage drag, either individually or as a result of interaction with other stay-green QTL regions in the diallel. The sorghum sequence will provide us with the means to target comparative DNA markers to such specific regions
c. Improved germplasm is expected to result from both a and b (above).
d. Additional diagnostic DNA markers will also be a result of b (above).

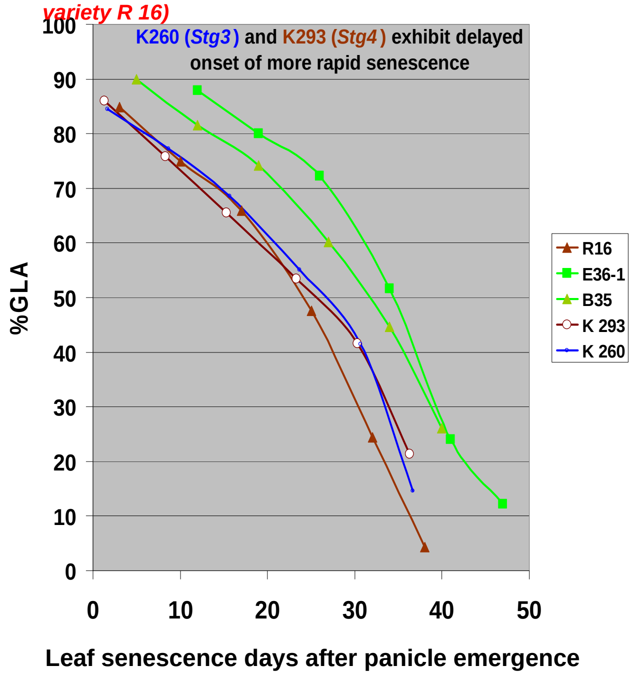
***Objective 2, Activity 1. Identification and analysis of functional SNPs useful for deterministic breeding in 'MAGIC' populations*.** Careful analysis of massively-parallel resequencing data, using methods that are routine in the lab of the PI for SNP calling and for classification of genes into biochemical pathways and biological processes, will provide for development of hypotheses that we will begin to test using genetic and phenotypic data for one RIL set already available from parents included in the MAGIC populations (the remainder to be a future focus when they are complete). We will initially focus on 8 genotypes that span a maximum of diversity, and which are being used as parents of the four sorghum MAGIC populations: B 35; E 36-1; IS 9303; IS 9830; IS 15401; M 35-1; N13; and S 35. *Anticipated outcomes are*:

a. Identification of a large SNP set suitable for developing high-throughput genotyping resources for the target populations (MAGIC, RIL, stay-green NIL) and others;

***Objective 2, Activity 2. Empirical testing of relationships between drought tolerance (and other phenotypes) and specific genes, gene families and functional SNPs*.** In addition to functional SNP discovery detailed above, comparison of the sorghum and rice sequences suggests several sorghum-specific gene family expansions that warrant further study for a relationship to drought adaptation. For example, the single rice miRNA169g, up-regulated during drought stress, has five sorghum homologs. The computationally predicted target of the sbi-miR169 subfamily comprises members of the plant nuclear factor Y (NF-Y) B transcription factor family, linked to improved performance under drought for both *Arabidopsis* and maize . Cytochrome P450 domain-containing genes, often involved in scavenging toxins such as those accumulated in response to stress, are unusually abundant in sorghum with 326 family members versus 228 in rice. Another gene family that could be linked to durability of sorghum is characterized by an expansin domain, which serves as chemical 'grease' in cell walls, with 82 copies in sorghum versus 58 in rice and 40 each in *Arabidopsis* and poplar.
An attractive first step to test hypotheses associating potentially-large numbers of SNP alleles, genes, and gene families with drought tolerance is genetic mapping, narrowing the field of candidates to a subset that are positionally associated with a relevant phenotype (in this case, drought). In the long term, we seek to apply this approach to the MAGIC populations (hence their selection for SNP discovery), taking advantage of the high resolution afforded by intermating. However, these populations will not be ready in time to use them here. Accordingly, we will begin to evaluate associations of drought-related phenotypes and the functional SNPs and candidate gene classes detailed above, in a single F8 RIL population based on the cross of stay-green drought tolerant parent E 36-1 with N13, which is a source of *Striga* resistance. *Anticipated outcomes are*:

a. Initial testing of hypotheses (above) about candidacy of specific alleles, genes, and gene families in direct contributions to sorghum drought tolerance;
b. Segregants (germplasm) that are fully genotyped and combine drought tolerance with other traits addressing production constraints in West and Central Africa, Eastern and Southern Africa, and South Asia, permitting us to single out particularly promising lines for further development either directly or as parents in mainstream cultivar breeding;
c. Any QTLs and diagnostic markers for charcoal rot resistance, *Striga* resistance, and/or *stay-green* that escaped detection in early-generation studies.

Fig 1. Difference in green leaf area maintenance after flowering in two stay-green QTL introgression lines K 260 and K293 (Stg3 and Stg4, respectively, from donor B35 in background of highly senescent but elite postrainy season variety R 16)



Introgression line trial at flowering (above)

and preharvest (below)