GENETIC ANALYSIS OF THE *SORGHUM BICOLOR* STAY-GREEN DROUGHT TOLERANCE TRAIT

A Dissertation

by

KAREN RUTH HARRIS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2007

Major Subject: Biochemistry

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Approved by:

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ABSTRACT

Genetic Analysis of the *Sorghum Bicolor* Stay-Green Drought

Tolerance Trait. (May 2007)

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Sorghum (*Sorghum bicolor* [L.] Moench) is the fifth most economically important cereal grown worldwide and is a source of food, feed, fiber and fuel. Sorghum, a C_4 grass and a close relative to sugarcane, is adapted to hot, dry adverse environments. Some genotypes of sorghum called stay-green have delayed leaf senescence during grain ripening under drought stress conditions which allows normal grain filling whereas most sorghum lines senesce early under post-anthesis drought. Eight sources of stay-green have been identified in the sorghum germplasm collection, most originating from Sudan and Ethiopia. The diversity of the eight sources of stay-green was analyzed using 55 simple sequence repeats (SSR) markers with genome coverage. This analysis showed that the sources of stay-green are quite diverse and can be divided into five groups based on race or working group. Three sources of stay-green have been used to identify 12 major quantitative trait loci (QTL) that modulate this trait. The origin of favorable alleles for stay-green was traced backward to ancestral lines and forward into breeding materials derived from stay-green germplasm. The analysis of the origin of favorable alleles for stay-green helped explain why subsets of stay-green QTL were identified in different studies and provided evidence that there may be more than

one favorable allele in the sorghum germplasm for several of the stay-green QTL. Analysis of stay-green breeding lines from three public sorghum-breeding programs revealed that one of the main QTL identified in mapping studies was not being used in the breeding programs (0/13), most likely due to its association with an allele for lemon yellow seeds. In addition, a subset of the regions containing favorable alleles for stay-green from the genotype BTx642 were over represented in stay-green breeding lines. Nearly isogenic lines containing favorable alleles from BTx642 for Stg1, Stg2, Stg3, and Stg4 in a RTx7000 (senescent) background were characterized and each NIL was shown to exhibit a stay-green phenotype. Based in part on this information, fine-mapping of Stg1 was undertaken by crossing the Stg1 NIL to RTx7000. Overall, these results revealed the origin of favorable alleles for stay-green and the current utilization of alleles for stay-green in public breeding programs. In addition, this study identified additional stay-green sources that could be used for further QTL analysis and highlighted the genetic complexity of the stay-green trait.

NOMENCLATURE

ABA	Abscisic acid
ABRE	Abscisic acid responsive *cis*-elements
AFLP	Amplified fragment length polymorphism
BAC	Bacterial artificial chromosome
BC	Backcross
BR	Brassinosteroid
CAM	Crassulacean acid metabolism
DAF	Days after flowering
DHZR	Dihydrozeatin riboside
DRE	Dehydration responsive element
EMS	Ethylmethane sulphonate
EST	Expressed sequence tag
FA	Favorable allele
FAM	Fluorescein
FISH	Florescence in-situ hybridization
GA	Gibberellin
HEX	Hexachlorofluorescein
ICRISAT	International crops research institute for the semi-arid tropics
INDEL	Insertion/deletion
iPA	Isopentenyl adenosine
IRD	Infrared dye

LG	Linkage group
LOD	Logarithm of the odds
MAS	Marker-assisted selection
NCBI	National Center for Biotechnology Information
NIL	Near isogenic line
NPGS	U.S. National Plant Germplasm System
PAC	p1-derived artificial chromosome
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RI	Recombinant inbred
RIL	Recombinant inbred line
RFLP	Restriction fragment length polymorphism
RO	Reverse osmosis
ROS	Reactive oxygen species
SBI	Sorghum bicolor chromosome
SD	Segregation distortion
SSR	Simple sequence repeat
Stg	Stay-green
TAES	Texas Agricultural Experiment Station
t-AR	*trans*-zeatin riboside
TET	Tetrachlorofluorescein
UPGMA	Unweighted pair group method with arithmetic average

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CHAPTER I

INTRODUCTION

Sorghum – origin, taxonomy, history, and diversity

Sorghum (*Sorghum bicolor* (L.) Moench), a C4 grass and a close relative of maize, is the fifth most economically important cereal crop grown worldwide (Figure 1.1) (Doggett 1988). This monocot is the second most important feed grain, with an annual value of $2 billion in the United States (Dahlberg *et al.* 1995), and is a staple food used in porridges and breads in parts of Africa and Asia (Mann *et al.* 1983). Sorghum includes three species; the rhizomatous taxa S. *halepense* and S. *propinquum* and all annual wild, weedy, and cultivated taxa belong to S. *bicolor*. Sorghum taxonomy based on Harlan and deWet, classifies *Sorghum bicolor* into five races based on spiklelet morphology. These races are Bicolor, Guinea, Caudatum, Kafir, and Durra (Figure 1.1) Because of the variability that is found in each race, and the existence of race intermediates, a classification scheme integrating Harlan and deWet's classification with working groups (sub-races) was established (Dahlberg *et al.* 2004). Seventy working groups based on "head opening" have been defined (Murty and Govil 1967).

This dissertation follows the style of Genetics.

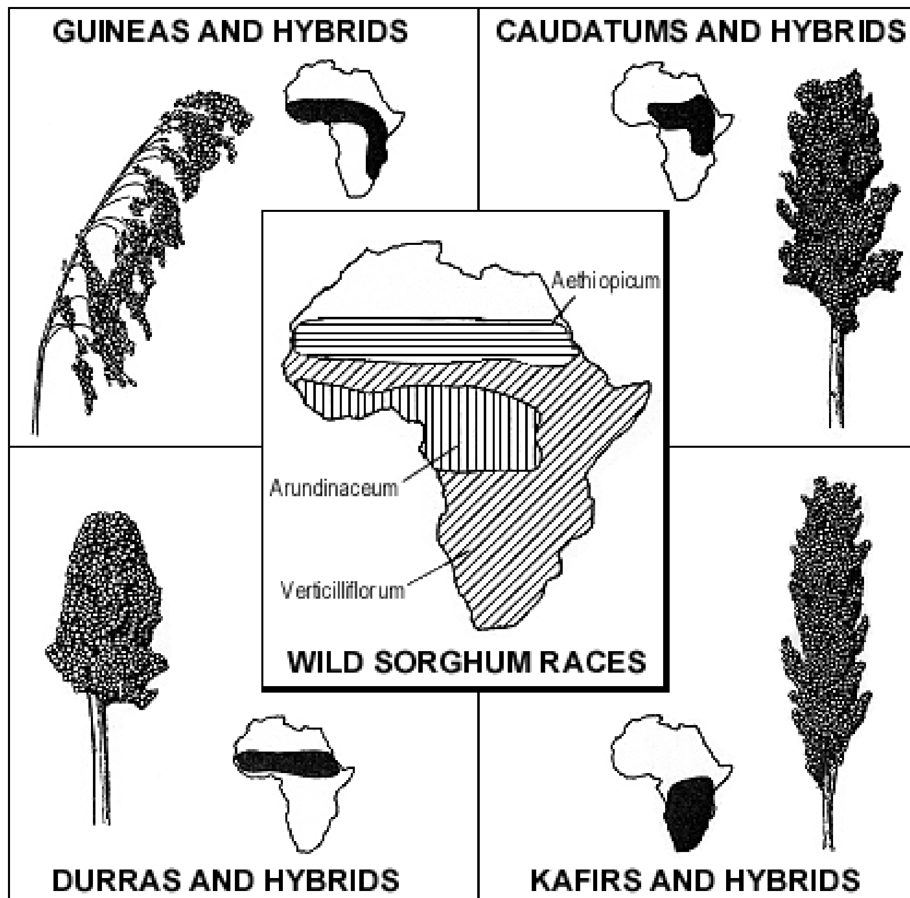

Figure 1.1 Races of sorghum based on spikelet morphology. Courtesy of J Hancock *et al.* (2004).

The races Caudatum, Kafir, Guinea, and Durra are thought to be derived from early Bicolors which introgressed with wild forms (Figure 1.1) (Dahlberg 1995). Early Bicolors are believed to have arisen from the subspecies *verticilliflorum* in central Africa (Dahlberg 1995). As the Bicolors moved west, they came into contact with wild *S. arundinaceum* from which the race Guinea evolved (Dahlberg 2000). The race Caudatum arose from the area of early Bicolor domestication and is thought to have been derived from an introgressed cross between an early Bicolor and a wild sorghum

(Dahlberg 2000). The race Kafir is thought to have been derived from crosses of the Bicolors of northern Africa with wild *verticilliflorum* and carried east and south by the Bantu speakers of Africa (Dahlberg 1995). The race Durra is thought to have originated in Ethiopia from early Bicolors which introgressed with wild *aethiopicum* adapted to drier conditions (Dahlberg 1995).

Sorghum is planted in the United States on 8-14 million acres per year with an annual crop value of $2 billion (Dahlberg *et al.* 2004). Studies of pedigree records and comparative molecular assays suggest the sorghum genotypes grown in the U.S. represent only a fraction of the full range of diversity that exists in the sorghum species (Ahnert *et al.* 1996). The increased vulnerability of low diversity crops to biological and abiotic hazards spurred the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the U.S. National Plant Germplasm System (NPGS) to augment their sorghum collections to approximately 35,000 and 40,000 accessions, respectively (Eberhart *et al.* 1997). To enable the use of these exotic lines in the U.S., the Texas Agricultural Experiment Station (TAES)-USDA Sorghum Conversion Project was created in 1960 to convert the exotic, tall, late, or non-flowering varieties into short, early maturing forms that can be grown in temperate zones (National Research Council 1996). Seven hundred and two sources of sorghum were converted using this approach by backcrossing the exotic lines to BTx406, an early maturing dwarf line adapted to the U.S. (Agriculture Research Service 2003). Some of the converted lines have desirable traits such as high yield potential, disease and insect resistance, drought resistance, and improved grain quality (Rosenow *et al.* 1997). Many of the improvements made in

sorghum hybrids over the past 30 years have thus been the result of the Sorghum Conversion Project (Rooney and Smith 2001).

Several diversity studies on sorghum have been done using Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPDs), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphisms (AFLPs), and sequencing (Menz *et al.* 2004; Ahnert *et al.* 1996; Casa *et al.* 2005; Cui *et al.* 1995; Deu *et al.* 1994, Smith *et al.* 2000; Dje *et al.* 2000; Hamblin *et al.* 2004) and general trends can be extracted. Sorghum has four-fold lower total sequence diversity compared to maize that may be due to sorghum being self pollinating and maize being an outcrosser (Hamblin *et al.* 2004). Cultivated lines can be classified by their race or working group (Menz *et al.* 2004; Cui *et al.* 1995; Deu *et al.* 1994; Ahnert *et al.* 1996; Casa *et al.* 2005) except for the race Bicolor (Deu *et al.* 1994; Dje *et al.* 2000). The race Bicolor is highly variable and includes many rare markers (Deu *et al.* 1994; Hamblin *et al.* 2004). Its accessions do not form a specific group (Deu *et al.* 1994), and accessions of the race Bicolor are distributed among the various clusters. This is consistent with the hypothesis that the race Bicolor is the progenitor *Sorghum bicolor* subsp. *bicolor* from which the other cultivated races evolved (Dje *et al*. 1994; Hamblin *et al.* 2004). The lowest genetic diversity was seen in the race Kafir (Casa *et al.* 2005; Dje *et al.* 2000; Menz *et al.* 2004; Hamblin *et al.* 2004), which may be due to Kafir being localized primarily in South Africa (Dje *et al.* 2000). Most of the elite sterility-maintaining (B) lines are derived from the Kafir germplasm (Menz *et al.* 2004). To increase the genetic

diversity in sorghum cultivated lines, new B-line germplasm should be developed by introgression with other diverse lines (Menz *et al.* 2004).

Principal Co-ordinate Analysis of 50 inbred lines using SSRs revealed that sorghum inbred lines fall into five broadly defined groups (Gabriel 2005; Menz *et al*. 2004). These groups have been designated as Kafir females, Zerazera derived females, Zerazera derived males, Milo (durra)-kafir derived males, and Feterita derived males (Gabriel 2005; Menz *et al*. 2004). A study examining if heterotic relationships exist between these proposed groups found that within-group crosses were inferior in specific combining ability effects and heterosis for grain yield compared to across-group crosses (Gabriel 2005). Furthermore, the genetic similarity estimates for parental line pairs were significantly correlated with specific combining ability and heterosis for yield for their respective hybrid pairs, thus linking the molecular data to the ability to predict heterosis (Gabriel 2005).

The sorghum genome and transcriptome

Sorghum belongs to the Poaceae family (tribe Andropogoneae), which includes rice, maize, barley, oat, rye, millet, and wheat. Despite the separation of sorghum from maize and rice approximately 15-20 million years ago and 50 million years ago respectively, significant conservation of gene order exists among the genomes of these plants, which facilitates comparative genome mapping approaches (Bennetzen 2000). Sorghum has a diverse germplasm, unusual tolerance to hot and dry environments, and a relatively small diploid genome of approximately 760-810Mbp (Arumuganathan and Earle 1991), well suited for genomic approaches. A high-density integrated genetic and

physical map of sorghum has been created using a combination of AFLP technology, six-dimensional bacterial artificial chromosome (BAC) pooling, and BAC DNA fingerprinting (Klein *et al.* 2000). A dense genetic map of sorghum was obtained by scoring 2454 AFLPs, 203 RFLPs, and 136 SSRs (Figure 1.2) in a recombinant inbred (RI) population, consisting of 137 lines derived from a cross of two *S. bicolor* genotypes BTx623 and IS3620C (Menz *et al.* 2002). The physical map was generated using three different BAC libraries with BAC contigs anchored every 1.5cM across all ten sorghum chromosomes. The integrated genetic and physical map permits map based cloning of important genes (Childs *et al.* 1997; Klein *et al.* 2005).

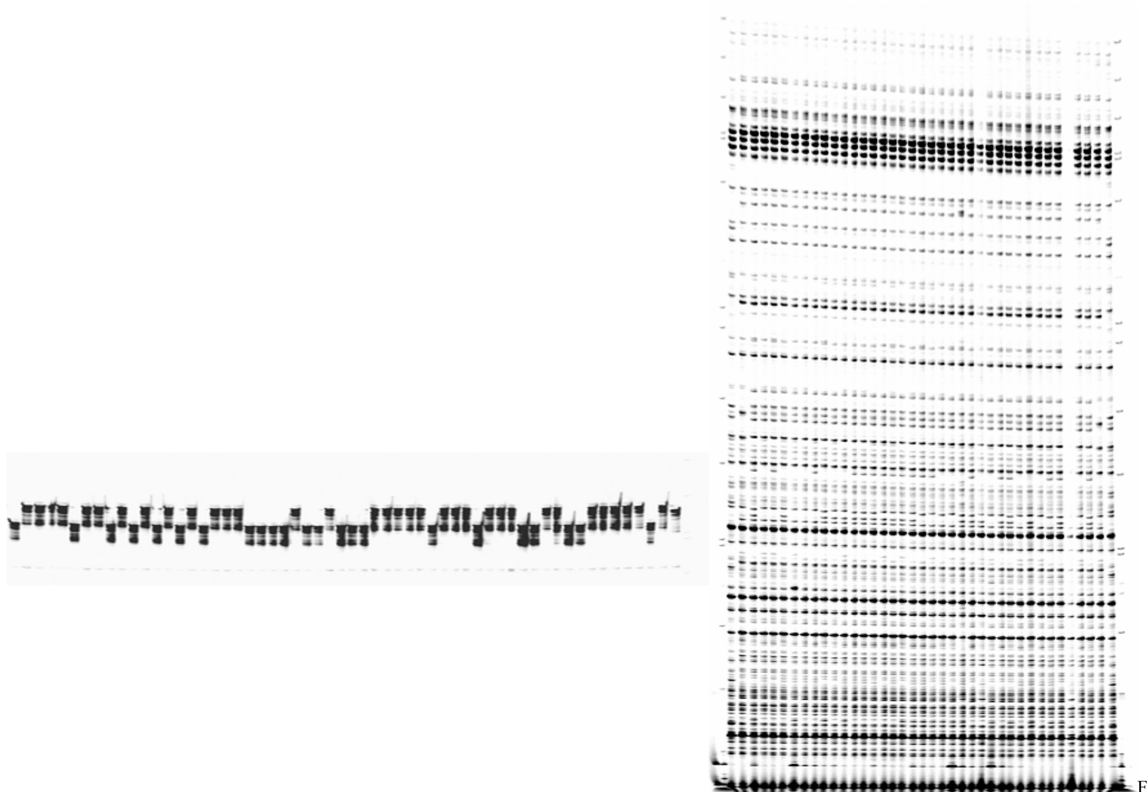

Figure 1.2 Example of an SSR (left) and an AFLP (right) gel image.

Genetic maps based on populations derived from a range of sorghum parental lines aided the creation of the two current high-density genetic recombination maps (Menz *et al.* 2002; Bowers *et al.* 2003). However, the lack of common linkage group nomenclature made it difficult to compare genetic maps between at least six groups (Kim *et al.* 2005a). Cytogenetic analysis of sorghum chromosomes allowed a chromosome size-based nomenclature to be developed (chromosomes SBI-01 to SBI-10 and linkage groups LG-01 to LG-10) (Kim *et al.* 2005a). Furthermore, the order of BAC-florescence in situ hybridization (FISH) loci along the chromosome was in agreement with the marker loci order along the linkage map of Menz *et al.* (2002) (Islam-Faridi *et al.* 2002; Kim *et al.* 2005b). An important finding of the BAC-FISH work is that very low recombination is characteristic of the pericentromeric regions of all sorghum chromosomes examined (Kim *et al.* 2005b; Islam-Faridi *et al.* 2002).

The sorghum genome of the genotype BTx623 is currently being sequenced by the Department of Energy Joint Genome Institute using a shotgun sequencing approach (Sorghum Genomics Workshop Planning Participants 2005). Sorghum will be sequenced using two *Sorghum bicolor* plasmid libraries to give 4X sequence coverage in paired-end sequencing and 15X coverage from one fosmid library (Paterson *et al.* 2006). The shotgun coverage with be combined with the paired-end sequencing from sorghum BACs as well as other publicly available sorghum sequences such as the 550,000 methyl filtered sequences (Bedell *et al.* 2005) to form pseudomolecules that cover most of the chromosomes (Paterson *et al.* 2006).

The sorghum transcriptome has been examined through an expressed sequence tag (EST) project at the University of Georgia (Pratt *et al.* 2005). From about 58,841 complementary DNA (cDNA) clones that were isolated from plants exposed to light, dark, abiotic and biotic stress, and floral libraries from three species of sorghum, 117,682 ESTs were obtained representing both 3’ and 5’ sequences. A total of 16,801 unique transcripts were identified and have been used for microarray studies (Buchanan *et al.* 2005; Salzman *et al.* 2005).

Drought resistance

Drought is the most significant cause of crop yield loss (Boyer *et al.* 2004). Water serves many vital roles in the plant, including acting as a solvent, a transport medium, and an evaporative coolant (Boyer 1982). Consequently, water limitation causes a decrease in whole plant growth and photosynthesis, wilting, stomatal closure, and is associated with changes in carbon and nitrogen metabolism (Sanchez *et al.* 2002). Physiologists have identified three general mechanisms of drought resistance involving avoidance, tolerance, and escape (Levitt 1980). Drought avoidance mechanisms allow plants to maintain cell turgor and cell water content under water-limiting conditions. This is accomplished by maintaining water uptake by the roots and/or reduction of water loss from transpiration and other non-stomatal pathways such as the cuticle. Most sorghum genotypes have a thick waxy cuticle that limits water loss during periods of water deficit. The possession of a deep, large root system, which has the ability to penetrate hard soil layers, is also often associated with plants that are able to maintain water supply during periods of low rainfall. C4 vs. C3 photosynthesis also improves

water use efficiency especially at high temperatures where the oxygenase activity of rubisco is favored over the carboxylation activity. C4 plants concentrate CO_2 in bundle sheath cells thus reducing photorespiration allowing these plants to decrease stomatal conductance and to conserve water without decreasing carbon fixation rates. Crassulacean acid metabolism (CAM) plants include plants such as cacti, which possess thick cuticles and fix CO_2 at night. CAM plants have stomata that remain open only at night allowing CO_2 entry with reduced water loss. Other types of avoidance mechanisms are based on leaf abscission, dormancy, and leaf angle/rolling that reduce water loss from transpiration. Reducing the evaporative surface area of the leaf is an effective means of decreasing transpiration.

Drought tolerance mechanisms allow plants to maintain metabolic activity during drought and under conditions of reduced plant water potential by osmotic adjustment and antioxidant capacity. Many plants can accumulate compatible solutes including sugars, organic acids, amino acids, sugar alcohols, or ions which accumulate in the cytosol, lowering the osmotic potential and maintaining turgor of both shoots and roots. Sorghum, for example, is known to accumulate glycine betaine and proline in response to water deficit (Buchanan *et al.* 2005). Antioxidant capacity is the ability of plants to detoxify reactive oxygen species (ROS) (Scandalios 2005). Reactive oxygen species can cause cell injury by lipid peroxidation or protein and nucleic acid modification. Plants have evolved mechanisms to prevent damage from free radicals by using antioxidant enzymes such as superoxide dismutase, catalases, and peroxidases and by using free radical scavengers such as carotenoids, ascorbate, proline, tocopherols, and glutathione

(Mundreee *et al.* 2002). The prevention of oxidative stress and reduction of the number of reactive oxygen species enhances the plant's tolerance to abiotic stresses such as drought.

Drought escape refers to early completion of the plant's life cycle, essentially flowering prior to the onset of drought. Early maturing varieties of sorghum avoid water deficit that in some regions often occurs later in the growing season.

Drought induced changes in gene expression

Genes induced by water-stress encode proteins involved in protection and signal transduction (Mundree *et al.* 2002). A hormone that acts as a major signal of water deficit is abscisic acid (ABA). Most drought-responsive genes are induced by exogenous ABA treatment, and are included in the ABA-dependent signal transduction. An additional gene set is induced by drought, but not by ABA, providing evidence for a second, ABA-independent signal transduction pathway (Mundree *et al.* 2002). Promoters of ABA inducible genes contain sequence-specific ABA-responsive *cis*-elements (ABRE's) with the sequence ACGTGGC (Mundree *et al.* 2002). These same *cis*-elements are found in sorghum genes that respond to ABA (Buchanan *et al.* 2005). Dehydrins, hydrophilic proteins thought to stabilize cell structures against dehydration, accumulate upon drought onset or ABA treatment and many studies have shown a positive correlation between the accumulation of dehydrins and drought tolerance (Cellier *et al.* 1998). Genes in the ABA-independent pathway contain a characteristic nine base pair sequence termed the dehydration responsive element (DRE). Proteins that bind the DRE include the ethylene-responsive element binding proteins, AP2 proteins,

and DRE binding factor 1 and 2. These factors bind and activate transcription of genes containing the DRE sequence (Mundree *et al.* 2002).

Leaf senescence

Leaf senescence, the terminal phase of leaf development leading to death, is the result of aging, or can be induced by water deficit, nutrient deficiency, shading, insects or disease, extreme temperatures, hormones, carbohydrate deficiency, or physical damage (Guo *et al.* 2004; Tollenaar and Daynard 1978). During leaf senescence, chlorophyll, proteins, lipids, and nucleic acids are degraded and nutrients are removed and recycled for growth elsewhere. The chloroplast is one of the first organs targeted for breakdown, while the nucleus and mitochondria are degraded last (Quirino *et al.* 2000). Besides the deterioration of existing proteins and the down regulation of gene expression, recent studies show the onset of leaf senescence involves *de novo* synthesis of proteins and the expression of a complex array of genes whose products are involved in senescence related changes (Gepstein 2004). The main functional categories of senescence-related ESTs are macromolecule degradation, nutrient recycling, defense and cell rescue, transcriptional regulation, and signal transduction (Guo *et al.* 2004).

Leaf senescence is regulated during development and is modulated by hormones, metabolism, and stress. Thus, there are many pathways involved in senescence (Figure 1.3). The hormonal control of senescence involves ethylene, jasmonic acid (JA), ABA, cytokinin, brassinosteriods, and gibberellins (GA). The *Arabidopsis* mutant *oresara 9* (*ore9*) links ethylene, JA, and ABA to a common senescence pathway (Woo *et al.* 2001). The *ore9* mutant does not show accelerated senescence when treated with ethylene, JA,

and ABA compared to wild type plants, but the mutant can perceive all known hormone signal transduction pathways. For example, *ore9* plants respond to ethylene with a triple response, and the addition of ABA suppresses germination (Woo *et al.* 2001). Furthermore, *ore9* is tolerant to oxidative stress. *ORE9* encodes an F-box protein, which is a component of the ubiquitin E3 ligase complex that acts as an E3 ligase in ubiquitin-dependent proteolysis (Woo *et al.* 2001). Thus it appears that *ORE9*, as part of the ubiquitin E3 ligase complex, degrades an unknown protein or proteins that functions to repress leaf senescence (Figure 1.3).

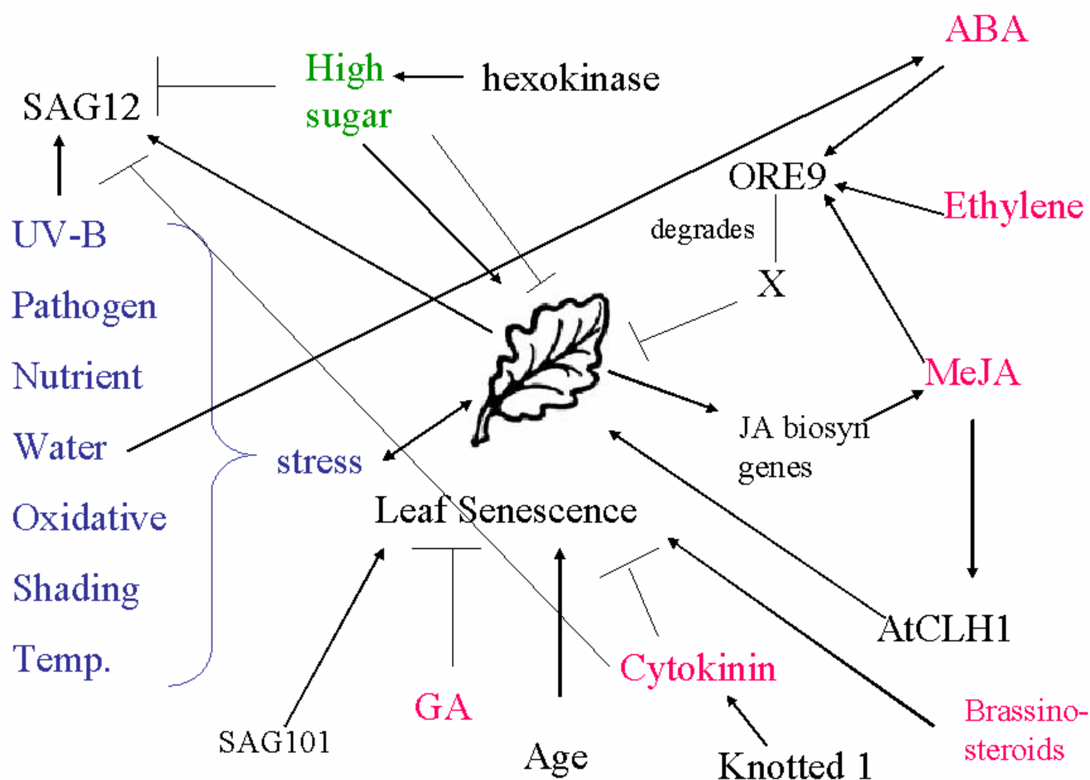

Figure 1.3 Pathways involved in leaf senescence. Plant hormones are shown in pink. X represents an unknown protein that likely is degraded by the E3 ligase complex and serves to repress leaf senescence. *AtCLH1* encodes an *Arabidopsis* chlorophyllase.

Cytokinin can delay leaf senescence, and a decrease in the level of cytokinin can lead to premature senescence (Masferrer *et al.* 2002). Transgenic tobacco plants that overexpress isopentenyl transferase (IPT), the enzyme for the first step of cytokinin biosynthesis, have increased chlorophyll and cytokinin levels and delayed leaf senescence (Ori *et al.* 1999). Similarly, plants expressing *Knotted 1 (KN1)*, a homeobox gene, under the control of the promoter of the senescence-associated gene *SAG12*, have increased levels of chlorophyll and cytokinin and delayed senescence (Buchanan-Wollaston *et al.* 2003). These results support the hypothesis that KN1 induces cytokinin production, thus delaying leaf senescence. Tissues with high levels of cytokinin are metabolic sinks suggesting a possible link between cytokinin and leaf metabolism. A leaf that has already undergone senescence can be induced to recover by treatment with cytokinin (Zavaleta-Mancera *et al.* 1999). Treatment of senescing leaves with cytokinin reactivates protein synthesis and increases chlorophyll and photosynthetic activity (Thomas and Howarth 2000).

The mechanisms of how cytokinin delays leaf senescence have been recently elucidated in tobacco. Cytokinin induces the expression of extracellular invertase that hydrolyzes sucrose in the apoplastic unloading pathway, and the newly synthesized hexose monomers are then taken up by sink cells (Lara *et al.* 2004). Extracellular invertase thus plays an important role in supplying carbohydrates to sink tissues (Lara *et al.* 2004). The induction of invertase production using a tetracycline-inducible promoter resulted in a delay of leaf senescence (Lara *et al.* 2004). When a tobacco apoplasmic invertase inhibitor was cloned behind a cytokinin-inducible promoter and cytokinin was

applied, no delay in senescence was observed (Lara *et al.* 2004). Extracellular invertase is therefore an essential component of cytokinin-mediated delay in leaf senescence (Lara *et al.* 2004).

Brassinosteroids (BRs) and gibberellins are hormones that affect leaf senescence. BRs, C_{27}, C_{28}, and C_{29} steroids, also affect stem elongation, leaf development, pollen tube growth, xylem differentiation, and photomorphogenesis (Yin *et al.* 2002). BRs enhance leaf senescence, as BR-deficient mutants such as *det2* have delayed leaf senescence (Yin *et al.* 2002). Gibberellins, a group of tetracyclic diterpenes, affect stem elongation, reproduction, and delay leaf and fruit senescence.

The effects of sugars on leaf senescence are controversial, and C:N ratios and ABA are more likely to affect senescence than absolute sugar levels (Gibson 2005). Addition of moderate concentrations of glucose (111mM) stimulates senescence in *Arabidopsis* but only if nitrogen levels are low (Gibson 2005). Also in nitrogen deficient *Arabidopsis* and tobacco plants, sugars accumulate and senescence is accelerated (Pourtau *et al.* 2004). ABA plays an important role in the various sugar responses, as the majority of sugar-insensitive mutants are either ABA-insensitive or ABA-deficient (Pourtau *et al.* 2004). *abi5-1,* an *Arabidopsis* line that has a mutation in a basic leucine-zipper transcription factor, is moderately glucose and mannose-insensitive during seedling development and has delayed leaf senescence (Pourtau *et al.* 2004).

During leaf senescence, lipids are degraded and are converted to phloem-mobile sucrose for transport out of the leaf. Antisense suppression of SAG101, a gene that encodes an acyl hydrolase that releases oleic acids from triolein, retards leaf senescence

and its overexpression promotes leaf senescence (Yoshida 2003). Similarly, phospholipase D alpha, a phospholipid-hydrolyzing enzyme, retards ABA- and ethylene-promoted senescence in *Arabidopsis* (Fan *et al.* 1997). Thus loss of enzymes involved in degrading lipids delays leaf senescence.

Stay-green

Stay-green is a term used to describe genotypes that have delayed leaf senescence as compared to a reference genotype (Thomas and Howarth 2000). The benefit of delayed senescence is that stay-green plants can assimilate more carbon and nitrogen than senescent plants because they retain more photosynthetically active leaves for a longer time. A stay-green phenotype can occur in three ways relevant to this study (Thomas and Smart 1993). Type A stay-green phenotypes have a delayed onset and a normal rate of senescence following its onset. Type B stay-green phenotypes initiate leaf senescence normally but the rate of senescence is comparatively slower. Type C stay-green phenotypes retain chlorophyll despite the normal onset and progression through senescence. Type C stay-green phenotypes have mutations in the chlorophyll degradation pathway and are thus considered cosmetic stay-greens.

Many crop plants express stay-green phenotypes. In cotton, the gene *GF14λ*, which encodes a 14-3-3 protein, was over expressed in cotton and transgenic lines showed a stay-green phenotype with improved drought tolerance and higher photosynthetic rates under drought conditions than wild type plants (Yan *et al.* 2004). 14-3-3 proteins are highly conserved regulatory proteins that are known to interact with over a hundred proteins and regulate primary metabolism, ion transport, cellular

trafficking, enzyme activities, and gene expression (Yan *et al.* 2004). In *Arabidopsis, G14λ* interacts with *ascorbate peroxidase 3* (*APX3*) and *ankyrin repeat-containing protein 2* (*AKR2*); these proteins have roles in protecting plants from water deficit and antioxidation metabolism (Zhang *et al.* 1997). How these 14-3-3 proteins function remains unknown, but similar effects have been demonstrated in potato. Overexpression of a 14-3-3 protein in potato resulted in delayed leaf senescence, whereas reduced expression of 14-3-3 proteins by antisense technology led to early leaf senescence (Yan *et al.* 2004)

In soybean, the homozygous combination of *d1d2* loci inhibits chloroplast, chlorophyll, chlorophyll-binding protein, and rubisco degradation, resulting in a Type C stay-green phenotype (Luquez and Guiamet 2002). The *d1d2* mutants do not have an increased life span of the leaves, respond normally to ABA, and are much more susceptible to drought stress than wild type genotypes (Luquez and Guiamet 2002). These results suggest that *D1* and *D2* are involved in a pathway controlling chloroplast disassembly and leaf water balance (Luquez and Guiamet 2002).

The stay-green tobacco line G28 loses chlorophyll, CO_2 exchange capacity, and rubisco content at a slower rate than the senescent KY14 line (Thomas and Smart 1993). These traits suggest G28 may be a Type B stay-green (Thomas and Smart 1993). Grafting a KY14 leaf onto a G28 stalk caused a delay in leaf senescence of the KY14 leaf while G28 leaves grafted to KY14 stalks caused an accelerated loss of chlorophyll in the G28 leaves (Thomas and Smart 1993). Thus, leaf senescence is influenced not by the individual leaf but by the rest of the plant.

A stay-green rice line Wuyujing 2 was crossed to a senescent line Zhenshan 97 to identify QTL associated with stay-green (Jiang *et al.* 2004). Most of the stay-green traits were negatively correlated with yield in this population, and no major stay-green QTL were identified (Jiang *et al.* 2004). A minor QTL identified in rice corresponds to a syntenic sorghum QTL, Stg3 on LG-03.

Stay-green pasta wheat lines were generated by ethylmethane sulphonate (EMS) as no natural stay-green wheat lines have been identified (Spano *et al.* 2003). The absence of natural stay-green lines is attributed to its being an ancient tetraploid. The EMS mutants are a combination of Type A and Type B stay-green phenotypes. In these mutants, chlorophyll loss is delayed by 10 days, and the stem becomes senescent before the flag leaf, which is an uncommon trait (Spano *et al.* 2003). Photosynthesis was maintained in the flag leaf at 40 days after flowering (DAF) whereas in wild type lines photosynthesis at 40 DAF was zero (Spano *et al.* 2003). Mutants had an increase in seed weight and grain yield as well as a decrease in embryo weight and grain nitrogen content as compared to the wild type lines.

Maize stay-green lines have delayed senescence, higher water and chlorophyll contents in the leaves at maturity, higher stalk sucrose content during grain fill, larger kernel weight, higher levels of sucrose, and protein in the husk and cobs and higher grain protein content than senescent lines (Gentinetta *et al.* 1986). Stay-green maize lines also show increased resistance to stalk-rotting pathogens (Ambler *et al.* 1987). A stay-green line in maize, P3845, when compared to a senescent line Hokkou 55, showed that the delay in leaf senescence in P3845 is correlated with increased levels of chlorophyll and

nitrogen (He *et al.* 2005). Of great interest, P3845 had more cytokinins (*trans*-zeatin riboside, t-ZR; dihydrozeatin riboside, DHZR; isopentenyl adenosine, iPA) and reduced levels of ABA in its leaves than Hokkou 55 (He *et al.* 2005). In roots, P3845 had increased levels of t-ZR, DHZR, and ABA but decreased concentrations of iPA. Thus the higher levels of cytokinins, a hormone known to delay leaf senescence, and the decrease in ABA, a hormone known to enhance leaf senescence, is most likely causing the delay in leaf senescence. In maize the inheritance of the stay-green phenotype was determined by crossing Lo876o2, an Italian line that is characterized by a delayed senescence of leaves even after physiological maturity, and B73, a 'Stiff Stalk' line from Iowa State University (Gentinetta *et al.* 1986). The inheritance study indicated that two alleles at a single locus are segregating, that the allele present in Lo876o2 delayed senescence and, that this allele is dominant over the allele present in B73 (Gentinetta *et al.* 1986). The same genetic factor responsible for delayed senescence was also shown to induce high sucrose levels.

Sorghum stay-green

In sorghum, stay-green is associated with increased tolerance to post-anthesis drought and sorghum stay-green lines are classified as either Type A or Type B. Water deficit during grain filling hastens leaf senescence in sorghum and kills the plant, (Rosenow and Clark 1981) however stay-green lines retain more green leaf area than senescent genotypes and continue to fill their grain under a post-anthesis drought (Rosenow *et al.* 1983). Stay-green lines in sorghum show increased resistance to disease/insects, reduced lodging and have greater biomass production (Rosenow 1983;

Rosenow and Clark 1981). Stay-green lines produce two to three more basal tillers per plant at black layer, have a greater stem diameter, have higher sugar concentrations at the base of the stem, maintain greater green leaf area longer, have a greater leaf area index than senescent lines, have a higher leaf relative water content, have higher specific leaf nitrogen, contain a higher level of cytokinins, and have enhanced transpiration efficiency (Dahlberg 1992; Ambler *et al.* 1987; Borrell *et al.* 2000; Borrell and Hammer 2000; Borrell *et al.* 1999; Duncan 1981; McBee 1984). Furthermore, stay-green genotypes do not show reduced yield under fully irrigated conditions, thus stay-green genotypes can be grown on both irrigated and non-irrigated land (Borrell *et al.* 2000).

Sorghum is an annual plant, but stay-green lines can survive for years through the generation of new tillers from the old plant base (Thomas and Smart 1993). In a study of annual and perennial sorghums, the annual sorghums had leaves that underwent senescence from the base of the plant upwards resulting in plant death. In stay-green lines, leaves senesce more slowly and the stem and plant base survive after grain set (Zartman and Woyedwodzic 1979). Interestingly, senescent lines have a greater root system than stay-green lines during the season, but 100 days after planting, root density declines in the senescent line while the stay-green line shows minimal loss (Zartman and Woyedwodzic 1979).

BTx642 (formerly called B35) is a stay-green genotype with a delay of the onset of senescence under water-limited conditions. BTx642 is a BC1 derived line resulting from a cross of BTx406 and IS12555 (Rosenow *et al.* 1983), a Durra sorghum from Ethiopia. BTx406 was used as a source of recessive *ma1* and *dw2*, therefore BTx642 is

early flowering and short in stature similar to most grain sorghums grown in Texas (Lin *et al.* 1995). BTx642 is used in many breeding programs in the United States and Australia. Four major stay-green quantitative trait loci (QTL) located on three linkage groups were identified using a recombinant inbred (RI) population developed from BTx642 (a stay-green line)/RTx7000 (a senescent line) (Xu *et al.* 2000). Two QTL, Stg1 and Stg2, located on LG-03 explain 20% and 30% of the phenotypic variability, respectively (Sanchez *et al.* 2002). Stay-green QTL Stg3 and Stg4 are, respectively, on LG-02 and LG-05 and account for ~16% and ~10% of the phenotypic variance (Sanchez et al. 2002). Thus, the ranking of stay-green QTL based on their relative contribution to the stay green phenotype expressed in this population is Stg2>Stg1>Stg3>Stg4 (Xu *et al.* 2000).

Seven populations have been utilized to map QTL for stay-green in sorghum. These mapping populations utilized BTx642, QL41, SC56, and E36-1 as a source of stay-green and RTx430, Tx7078, QL39, RTx7000, IS9830, and N13 as senescent parents (Table 1.1).

Table 1.1 Stay-green QTL mapping studies in sorghum.

Author	Parents	# of Sites	Contain Stg1-4?
Crasta *et al.* 1999	BTx642/RTx430	4 TX	Stg2, Stg4
Tuinistra *et al.*1997	BTx642/Tx7078	3 Mexico, AZ, IN	Stg1, Stg3
Tao *et al.* 2000	QL41/QL39	5 Australia	Stg3
Kebede *et al.* 2001	SC56/RTx7000	5 TX, KS	Stg2, Stg4
Subudhi *et al.* 2000	BTx642/RTx7000	2 TX	Stg1,Stg2, Stg3, Stg4
Xu *et al.* 2000	BTx642/RTx7000	5 TX	Stg1,Stg2, Stg3, Stg4
Haussmann *et al.* 2002	E36-1/IS9830	2 India	Stg1
Haussmann *et al.* 2002	E36-1/N13	2 India	Stg2, Stg3

Parents used in each QTL study, number of environments and locations where the phenotyping of the RI lines were done, and QTL that overlap with the Xu *et al.* (2000) Stg1-4 study are shown.

In the mapping population of BTx642/RTx430, where BTx642 is a Type A stay-green with a delay in the onset of senescence (Borrell *et al.* 2000), three major stay-green QTL (SGA, SGD, and SGG) contributed 42% of the phenotypic variability and four minor QTL (SGB, SGI.1, SGI.2, and SGJ) contributed an additional 25% of the phenotypic variability (Crasta *et al.* 1999). Only two QTL are shared between the BTx642/RTx7000 and BTx642/RTx430 QTL study. SGA and SGJ from the BTx642/RTx430 QTL analysis correspond to Stg2 and Stg4 from the BTx642/RTx7000 QTL analysis. In the BTx642/Tx7078 F5 population, six QTL for stay-green were identified that accounted for 53% of the variability in stay-green (Tuinistra *et al.* 1997). Two of these QTL on LgB and LgG of the BTx642/Tx7078 map, correspond to Stg1 and Stg3 of the BTx642/RTx7000 QTL analysis.

The RI lines from BTx642/RTx7000 were phenotyped for stay-green in an additional population beyond the Xu *et al.* (2000) study and Stg1, Stg2, Stg3, and Stg4 were identified again with the most important QTL being Stg2 (Subudhi *et al.* 2000).

These four QTL explained 53.5% of the phenotypic variation and QTL for chlorophyll content coincided with Stg1, Stg2, and Stg3 (Subudhi *et al.* 2000). Stg2 and Stg3 interacted together most favorably explaining 49.8% of the phenotypic variation, and although Stg1 alone accounts significantly for phenotypic variation, it does not act favorably in combination with other stay-green QTL (Subudhi *et al.* 2000). Subudhi *et al.* (2000) suggested that since RTx430 is less susceptible to post-flowering drought than RTx7000 (SG rating 3.4 vs SG rating 4.9, respectively) then BTx642 and RTx7000 are the most contrasting parents for stay-green mapping.

QTL mapping using RILs from the cross QL39/QL41, where QL41 is a stay-green line derived from the cross QL33/BTx642 and QL39 is senescent, identified three QTL associated with stay-green (Tao *et al.* 2000). Despite the small difference in the stay-green phenotype between QL39 and QL41, a stay-green locus was identified that corresponds to Stg3 identified in the Xu *et al.* (2000) mapping population (Tao *et al.* 2000).

QTL analysis of F7 RILs from SC56/RTx7000, where SC56 is a Caudatum-nigricans from Sudan with the stay-green phenotype and RTx7000 is a senescent parent, identified nine QTL for stay-green (Kebede *et al.* 2001). Two QTL from their mapping study, StgA and StgJ, correspond to Stg2 and Stg4 of the Xu *et al.* (2000) mapping study.

Two populations were made using E36-1, a Guinea-Caudatum hybrid race Type A stay-green line from Ethiopia, crossed with IS9830, a tall Sudanese Feterita, or N13, a Durra sorghum from India (Haussmann *et al.* 2002). Three QTL for stay-green on LgA,

E, and G were common to both E36-1/IS9830 and E36-1/N13 (Haussmann *et al.* 2002). The E36-1/IS9830 population have a QTL for stay-green that corresponds to Stg1 of the Xu *et al.* (2000) mapping population while the E36-1/N13 QTL analysis have QTL corresponding to Stg2 and Stg3 of the Xu *et al.* (2000) mapping population.

The dominance of the stay-green trait depends on the origin of stay green and the genotypes involved in crosses (Walulu *et al.* 1994). Stay-green in BTx642 shows partial dominance when crossed with RTx7000 (Walulu *et al.* 1994). When BTx642, RTx7000, and their resultant progeny (F1 or F2) were phenotyped using leaf and plant death scores at maturity, the progeny had better postflowering drought-resistance than the parent RTx7000 but less postflowering drought-resistance than the other parent BTx642. When scored 1 to 10, where a score of 1 is 0-10% leaf death and a score of 10 is 90-100% leaf death, BTx642 had a score of ~4.5 and RTx7000 had a score of 10 in a rainout shelter (Walulu *et al.* 1994). The F1 and selected F2 progeny had a score of 7 (Walulu *et al.* 1994). Similarly, when BTx642 was crossed with the senescent lines BTx623 and BTx378 partial dominance of stay-green was observed, and the stay-green phenotype was more evident in the F1 of BTx642/BTx623 and BTx642/BTx378 than BTx642/ RTx7000 (Figure 1.4)(Tenkouano *et al.* 1993). SC599-11E, a stay-green line derived from IS17459, a Nigricans-Feterita, was crossed with BTx623, and the F1 generation displayed reduced stay-green phenotype (Tenkouano *et al.* 1993). Conversely, when crossed with BTx378, the F1 generation had minimal stay-green phenotype (Figure 1.4). Interestingly, when BTx642 and SC599-11E, both of which possess stay-green, were crossed, stay-green from BTx642 was dominant to SC599-11E (Tenkouano *et al.* 1993).

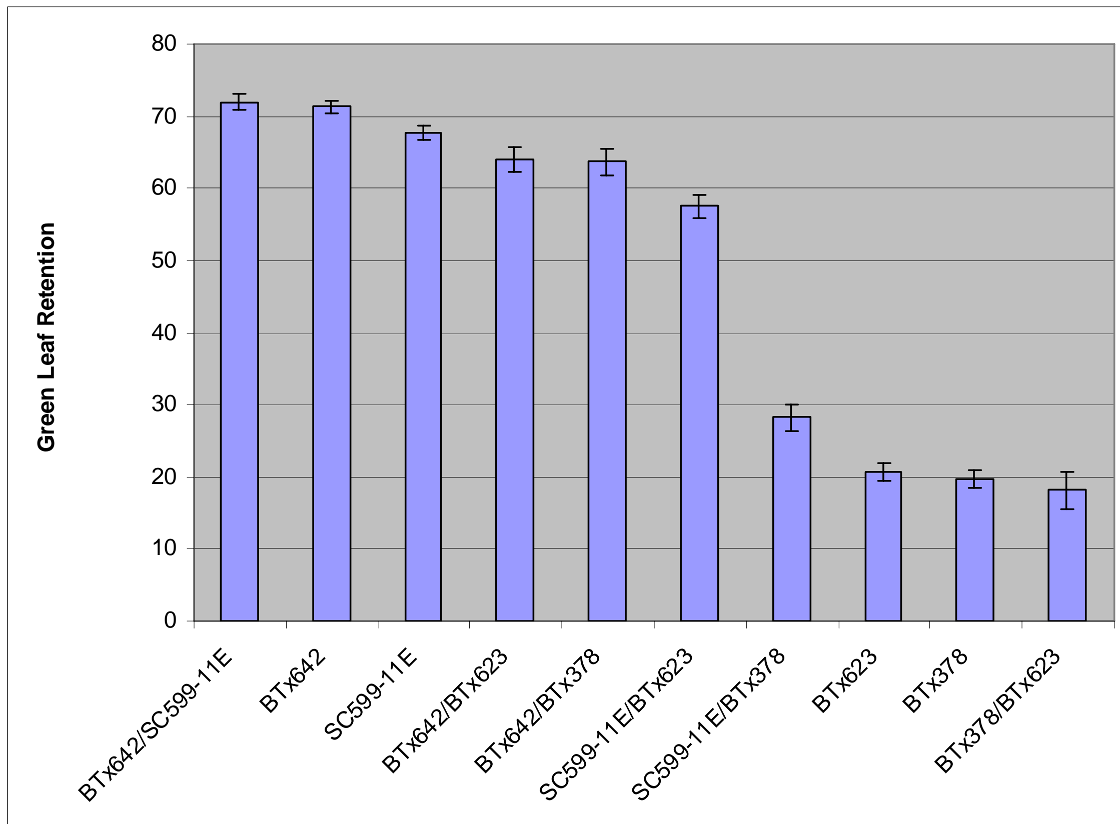

Figure 1.4 Green leaf retention of sorghum inbred cultivars BTx642, SC599-11E, BTx378, BTx623, and their F1 progeny (Tenkouano *et al.* 1993).

Sorghum is an important and diverse crop which has been the subject of many genome and transcriptome studies and its genome is soon to be sequenced. Sorghum has genotypes, called stay-green, that have delayed leaf senescence and increased drought tolerance. In this dissertation, the diversity and genetic mechanisms of the stay-green trait will be explored.

CHAPTER II

MATERIALS AND METHODS

Plant growth and DNA extraction

Sorghum seeds were treated with 30% bleach for 20 minutes and then treated with the fungicide Captan 400 (Gustafson LLC, Plano, TX, USA) at a 1:26 ratio of Captan to water and allowed to dry in a laminar flow hood. The treated seeds were then grown in a petri plate on Whatman chromatography paper (Whatman Inc, Florham Park, New Jersey, USA) for 8 days in the dark and were kept moist by the addition of 10mM $CaCl_3$ (Sigma-Aldrich Corp., St. Louis, MO, USA). Genomic DNA of etiolated samples was extracted using a FastDNA kit (Qbiogene, Irvine, CA, USA) with the FastPrep FP120 instrument to homogenize sorghum seedling leaf tissue according to the manufacturers protocol.

Generation of AFLP and SSR markers

AFLP template was prepared with *Eco*RI/*Mse*I or *Pst*I/*Mse*I-restricted genomic DNA. DNA template preparation and AFLP reactions for the *Eco*RI/*Mse*I as well as the *Pst*I/*Mse*I fragments were performed as described (Klein *et al.* 2000; Menz *et al.* 2002) except 100ng of genomic DNA was used. Visualization of AFLP amplification products, electrophoresis conditions, and data collection were as described (Klein *et al.* 2000).

Expressed sequence tags simple sequence repeats (EST-SSRs) markers within the stay-green loci were identified and each of these SSR markers was screened to identify polymorphisms that distinguish BTx642 from RTx7000. The alignment of rice

and sorghum chromosomes (Klein *et al.* 2003; Klein, unpublished) allowed the identification of rice p1-derived artificial chromosomes (PACs) that are collinear to the sorghum stay-green regions. Using the National Center for Biotechnology Information (NCBI) database search BLAST (NCBI: blast-http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide) genes encoded by rice PACs were used to identify sorghum ESTs that are likely to be collinear with the rice genes (Klein *et al.* 2003). Next, an SSR identifying program, SSRIT (Temnykh *et al.* 2001), was used to identify SSRs that are contained within ESTs that are predicted to map in the stay-green regions. Primers were designed to selected ESTs that contain an SSR repeat greater than four with a repeating unit less than a pentamer. These primers were used in PCR amplification reactions to identify polymorphisms between RTx7000 and BTx642. The EST-SSRs were also screened in IS3620C and BTx623, the mapping parents of a high-density genetic map (Menz *et al.* 2002). Any SSRs polymorphic in these lines was subsequently placed on the IS3620C/BTx623 genetic map by amplifying the SSR from each of 137 F6-8 RI lines.

SSRs were amplified and analyzed using fluorescent infrared dye (IRD)-labeled primers obtained from Li-COR (Lincoln, NE, USA) as described (Klein *et al.* 2000) or 5' Hexachlorofluorescein (HEX) or Tetrachlorofluorescein (TET) or Fluorescein (FAM) forward labeled primers (XXIDT, Coralville, IA, USA). For reactions containing HEX/TET/FAM labeled primers, the PCR conditions were identical to IRD labeled primers except the forward and reverse primer concentrations were 2.5 pmole/µl. Following amplification, the 10µl PCR products from the HEX/TET/FAM labeled

primers were diluted with 20µl of reverse osmosis (RO) water. Five µl of formamide:size standard mix at 1ml of HiDi formamide:25µl of 400 HD ROX (Applied Biosystems, Branchburg, NJ, USA) was added to a 96-well Perkin Elmer plate.and 1 µl of dilute PCR product was then added. Reactions were mixed, centrifuged briefly to remove bubbles and denatured at 95C for 5 minutes before loading the plate on an ABI 3700 Sequencer. Genotyping was performed on the sequencer by using the SUP2_POP5 module. Collected data was analyzed with Gene Scan version 3.7 Fragment Analysis Software (Applied Biosystems) and peaks were scored manually by using the Genotyper version 3.7 Fragment Analysis Software (Applied Biosystems).

SSR primer sequences as well as amplification product sizes are listed at http://sorgblast3.tamu.edu/ and an annealing temperature of 50C was used for all reactions.

Fine-mapping of Stg1

In order to fine-map the Stg1 locus, the Stg1 NIL 6078-1 was crossed to RTx7000 by Dr. David Jordan and 500 F2 plants were genotyped. From this population, plants containing recombinant chromosomes with breakpoints within the 36.2cM DNA segment spanning Stg1derived from BTx642 were identified. Two SSRs, txp38 and txp114, that flank the Stg1 locus, were used to genotype the F2 plants. Seventy-two plants were identified that contained a recombinant chromosome and a RTx7000 chromosome (henceforth called type 2 F2's). Initially, five F3 plants derived from each of the 47 F2 type 2 recombinants were genotyped using the flanking markers txp442 and Corn031 to identify plants homozygous for the recombinant chromosomes with

breakpoints in the Stg1 locus. The 34 plants that contained a homozygous crossover in the Stg1 locus were further genotyped with 14 additional AFLP and SSR markers to identify the location and size of the region from BTx642 present in RTx7000 NIL lines within the Stg1 area. Twelve plants with various recombination events within Stg1 were selfed by Dr. David Jordan and F4 plants from each line were phenotyped for the stay-green trait by Dr. Andrew Borrell in the summer of 2004 and 2005 in Australia.

Hydroponic growth conditions

Hydroponic growth conditions were as described (Buchanan *et al.* 2004). All plants were fertilized with 1x Hoaglands containing .005M $Ca(NO_3)_2$ x $4H_2O$, 5 mM KNO_3, 5 mM $MgSO_4$ x $7H_2O$, 5 mM $MgSO_4$, pH 6.5 with KOH, 1.4μM $ZnSO_4$ x $7H_2O$, 46 μM H_3BO_3, 14.1 μM $MnCl_2$ x $4H_2O$, .48μM $CuSO_4$ x $5H_2O$, 82.6 ηM $NaMoO_4$ x $2H_2O$, and .055g/L of Sprint 330 containing 10% Iron. Plants grown in soil were fertilized once a week.

Construction of the BTx642/ RTx7000 genetic map

An F7 population consisting of 97 individuals was created by a cross of the two inbred lines BTx642 and RTx7000 at Texas Tech University (Xu *et al.* 2000). BTx642 is a BC1 derivative of IS12555, a Durra sorghum from Ethiopia. RTx7000, variety Caprock, was derived from ‘Kafir’/ ‘Milo’ crosses. Parental lines and the 97 RILs were grown hydroponically in a growth chamber as described (Buchanan *et al.* 2004) and were phenotyped for seed color and mesocotyl length, which were used as morphological markers. DNA extraction, SSR, and AFLP generation were as described above.

The RILs were scored for the parental band of the female parent (BTx642, scored as B), male parent (RTx7000, scored as A), and heterozygous or missing values (scored as -), for each marker. A linkage map was created by using the computer program Mapmaker/EXP 3.0 (Lander *et al.* 1987) on a Sun ULTRA10 workstation (Sun Microsystems, Fremont, CA). The ten sorghum linkage groups were defined by using the 'make chromosome command' and 54 anchors were used consisting mainly of SSR markers previously mapped (Menz *et al.* 2002). A two point analysis was performed and markers were assigned to chromosomes with a minimum logarithm of the odds (LOD) score of 6 and a maximum recombination frequency of 0.39. The Kosambi function was used to convert recombination frequency into map distances in cM. Markers assigned to each chromosome were ordered using three point analysis with error detection on. The initial framework map for each linkage group was created using markers previously mapped (Menz *et al.* 2002) whose order was validated by the 'compare' command. For each marker addition to the framework, map order was statistically supported by the 'order', 'ripple', 'compare', and 'try' commands and each marker added did not increase the local linkage group length by more than 10%. Once the initial framework was set, all other markers assigned to each chromosome were placed between framework markers using the "place" command.

Genetic similarity analysis

For genetic similarity analysis using either AFLPs or SSRs, the presence of a band was represented as a "1" and the absence of a band was represented with a "0". Failed reactions were represented as a "9". The program NTSYS-pc (Rohlf 1994) was

used to generate a genetic similarity matrix among all pairs of lines by Dice coefficient of similarity (Nei and Li 1979). A dendrogram was created from the similarity matrix by the unweighted pair group method with arithmetic average (UPGMA). For bootstrapping, the program FreeTree (Hampl *et al.* 2001) was used and 500 bootstrap repetitions were used.

Screening RTx7000 NILs for the stay-green phenotype

Four of the RTx7000 NILs contained BTx642 DNA spanning all, or a portion of, only Stg1, Stg2, Stg3 or Stg4. These NILs were targeted for further physiological analysis to determine if the BTx642 DNA introgressed into each of these NILs contained genes that would contribute to the stay-green trait independent of the other stay-green loci. Dr. Andy Borrell conducted field experiments to physiologically characterize the NILs at the Hermitage Research Station (altitude 480 m, 28°10'S, 152°02'E) in Australia's north-eastern grain belt in two consecutive seasons: Experiment 1 (2004) and Experiment 2 (2005) as described in Harris *et al.* (2007).

CHAPTER III

SORGHUM DIVERSITY AND ORGINS

Introduction

To examine the diversity remaining after the Conversion Project, 50 diverse inbred sorghum lines including 13 elite temperate adapted inbreds and 18 exotic converted inbreds of U.S. agricultural importance were chosen for analysis (Menz *et al*. 2004). Lines were grouped based on genetic similarity by using 1814 AFLP and 100 SSR markers. Cluster analyses revealed that genetic similarity among these diverse lines were based on the sorghum working groups Zerazera, Kafir, Kafir-milo, Durra, and Feterita (Menz *et al.* 2004).

We aimed to add to the Menz *et al.* (2004) work to determine if the race Bicolor forms its own group as seen with the other races Durra, Kafir, and Caudatum (Zerazera is a working group of Caudatum). This study, to our knowledge, is the first analysis of genetic similarity of the race Bicolor using AFLP markers. The race Bicolor is thought to be the most primitive grain sorghum (Dahlberg 2000; Chapter 1). Bicolors consist of plants with open panicles and elongated grains and are low yielding and tend to have many tillers (Dahlberg 2000). Bicolor is distributed widely in Africa, Asia, and from India to Indonesia (Morden *et al.* 1989).

Results

Diversity

273 *Eco*RI-*Mse*I AFLP markers (Table 3.1) representing all ten linkage groups (Figure 3.1) were scored in sixty-one inbred lines (Table 3.2). From these data, genetic

similarity among genotypes was calculated and a dendrogram (Figure 3.2) was created by the unweighted pair group method with arithmetic average (UPGMA). The groups, based on a similarity score of .78, were the race/working groups Kafir (Cluster I), Kafir-Milo (Cluster II), Durra (Cluster III), Caudatum (Cluster IV), and Feterita (Cluster V) which agreed with the Menz *et al.* (2004) study and the genetic similarity within each group is .82, .84, .89, .77, and .81, respectively. With the exception of group Durra which includes only two individuals with one being the backcross of another, the Kafir groups made up of the Kafirs and Kafir-Milos, have the most similarity within their respective groups (.82 and .84) consisting of 11 and 5 inbred lines, respectively. The group with the most diversity is the Zerazera group with a genetic similarity score of .77. Zerazera is the largest group with 25 lines which represents the preference of breeders to use the race Caudatum as it provides high yield and excellent seed quality (Dahlberg 1995). The average genetic similarity among all sixty-one lines was .71.

Genetic similarly among genotypes ranged from 23% to 97%. Genotypes IS12666C and TAM2566 were the most similar (.94) whereas BTx406 and S. *propinquum* were the least similar (.23). Genotypes with the greatest similarity were those lines that were products of backcrosses with the corresponding genotype. Examples of this are TAM2566 and IS12666 (.97; BC_2), RTx218 and IS12661 (.90; BC_1), BTx642 and IS12555C (.88; BC_1), and TAM428 and IS12610 (.82; BC_2). The genetic similarity results were in general agreement with the available information regarding the pedigree of these lines.

Table 3.1 273 *Eco*RI/*Mse*I AFLP markers amplified on 61 inbred lines.

Marker	Linkage Group	cM
txa2704	LG-01	2.6
txa2723	LG-01	2.6-7.6
txa2959	LG-01	13.2-16.1
txa3154	LG-01	16.1-19.0
txa2773	LG-01	40.7-42.4
txa2765	LG-01	54.8-61.7
txa2953	LG-01	54.8-61.7
txa2716	LG-01	70.1-73.9
txa3538	LG-01	70.1-73.9
txa3782	LG-01	70.1-75.5
txa2770	LG-01	75.5-80.4
txa2112	LG-01	78.6-80.4
txa3161	LG-01	80.4-81.6
txa2760	LG-01	83.5-86.1
txa270	LG-01	89.4-93.7
txa8	LG-01	93.7-95.5
txa2583	LG-01	124.3-127.8
txa2588	LG-01	136.6-138.6
txa3555	LG-01	156.3-160.1
txa2129	LG-01	196.0-203.7
txa18	LG-01	205.5-209.8
txa2945	LG-01	218.4
txa2050	LG-02	7.9-11.1
txa2756	LG-02	16.4-20.4
txa3547	LG-02	34.8
txa2717	LG-02	42.6
txa2719	LG-02	52.6-56.9
txa3534	LG-02	58.0-60.3
txa3531	LG-02	63.6-64.8
txa2143	LG-02	64.8-68.3
txa3173	LG-02	64.8-68.3
txa3182	LG-02	73.3-75.7
txa3548	LG-02	73.3-75.7
txa2035	LG-02	75.7-75.7
txa2715	LG-02	75.7-77.3
txa274	LG-02	75.7-77.3
txa2939	LG-02	75.7-77.3
txa2724	LG-02	75.7

Table 3.1, continued

Marker	Linkage Group	cM
txa3153	LG-02	107.8
txa3542	LG-02	107.8-110.4
txa3779	LG-02	121.0-124.6
txa2576	LG-02	121.0-124.6
txa2938	LG-02	131.9-134.1
txa3541	LG-02	141.1-145.2
txa2965	LG-02	145.2-148.0
txa2575	LG-02	160.8-168.3
txa3557	LG-02	168.3-171.2
txa14	LG-02	196.9-201.5
txa2956	LG-02	off-end
txa2725	LG-03	off-end
txa2580	LG-03	1.9
txa2714	LG-03	41.4
txa3552	LG-03	49.4
txa267	LG-03	53.3
txa2967	LG-03	53.3-55.3
txa3781	LG-03	68.3-71.8
txa2030	LG-03	76.1-79.5
txa2586	LG-03	76.1-79.5
txa2759	LG-03	76.1-79.5
txa3780	LG-03	76.1-79.5
txa2768	LG-03	84.3-88.2
txa255	LG-03	125.2-127.5
txa3789	LG-03	149.1-150.3
txa2937	LG-03	151.9
txa2032	LG-03	169-174.5
txa2121	LG-04	9.7-12.5
txa3544	LG-04	9.7-12.5
txa6	LG-04	22.3
txa3554	LG-04	43.1-48.6
txa257	LG-04	57.7
txa2966	LG-04	67.3-72.5
txa3188	LG-04	67.3-72.5
txa2577	LG-04	83.3
txa2587	LG-04	85.9-88.2
txa2713	LG-04	85.9-88.2
txa2043	LG-04	85.9-91.6
txa2567	LG-04	89.7-91.6

Table 3.1, continued

Marker	Linkage Group	cM
txa2708	LG-07	50.9
txa16	LG-07	57.2
txa262	LG-07	66.4-71.8
txa17	LG-07	76.4-77.9
txa2046	LG-07	76.4-77.9
txa2047	LG-07	76.4-77.9
txa2700	LG-07	76.4-83.1
txa3	LG-07	76.4-83.1
txa3530	LG-07	76.4-83.1
txa3774	LG-07	76.4-83.2
txa3193	LG-07	77.9-78.1
txa2578	LG-07	77.9
txa3775	LG-07	79.4-83.1
txa20	LG-07	79.8-83.1
txa2033	LG-07	79.8-83.1
txa2702	LG-07	79.8-83.1
txa2712	LG-07	79.8-83.1
txa2947	LG-07	79.8-83.1
txa3549	LG-07	79.8-83.1
txa13	LG-07	83.1
txa11	LG-07	91.9
txa12	LG-07	91.9-96.9
txa2943	LG-07	128.8-134.4
txa2761	LG-07	143.7-146.1
txa3551	LG-08	0.0-6.1
txa2034	LG-08	26.3-30.1
txa2749	LG-08	72.7-76.4
txa2711	LG-08	72.7-80.2
txa2952	LG-08	72.7-80.2
txa2710	LG-08	77.5-80.2
txa2752	LG-08	77.5-80.2
txa2762	LG-08	77.5-80.2
txa3168	LG-08	77.5-80.2
txa3174	LG-08	77.5-80.2
txa3546	LG-08	77.5-80.2
txa3786	LG-08	77.5-80.2
txa9	LG-08	77.5-80.2
txa2703	LG-08	96.2-99.2
txa3771	LG-08	96.2

Table 3.1, continued

Marker	Linkage Group	cM
txa2582	LG-08	104.2-109.5
txa1	LG-08	122.7-126.1
txa2948	LG-08	152.3
txa2946	LG-09	43.6-45.5
txa3160	LG-09	62.8
txa254	LG-09	65.5-68.8
txa3788	LG-09	74.5-81.6
txa2585	LG-09	78.5-81.6
txa2589	LG-09	96.1-97.1
txa3772	LG-09	109.7
txa2764	LG-10	8.4-10.2
txa2968	LG-10	24.0
txa2720	LG-10	28.0
txa2750	LG-10	28.0-30.4
txa3185	LG-10	47.0-54.1
txa2584	LG-10	68.1-70.0
txa19	LG-10	70.0-72.0
txa2048	LG-10	70.0-72.0
txa3543	LG-10	70.0-72.0
txa3777	LG-10	70.0-72.0
txa3787	LG-10	70.0-72.0
txa3152	LG-10	72.0-75.1
txa3784	LG-10	85.3-89.7
txa2707	LG-10	97.8-99.8
txa259	LG-10	128.5
txa3175	LG-10	136.8-138.3
txa271	LG-10	off-end
txa10	-	-
txa15	-	-
txa2042	-	-
txa2114	-	-
txa2120	-	-
txa2122	-	-
txa2125	-	-
txa2126	-	-
txa2127	-	-
txa2128	-	-
txa2130	-	-
txa2131	-	-

Table 3.1, continued

Marker	Linkage Group	cM
txa2132	-	-
txa2136	-	-
txa2138	-	-
txa2561	-	-
txa2564	-	-
txa2590	-	-
txa260	-	-
txa2944	-	-
txa2949	-	-
txa2963	-	-
txa3156	-	-
txa3180	-	-
txa3183	-	-
txa3184	-	-
txa3192	-	-
txa3370	-	-
txa3553	-	-
txa3778	-	-
txa3791	-	-

cM values are based on the TAMU-ARS map and dashed represent markers that failed to map for the BTx623/IS3620C RI lines.

Table 3.2 Eleven lines added to the 50 diverse sorghum inbred lines study (Menz *et al*. 2004).

Line	Race	Working Group	Origin
SC1104	Bicolor	Bicolor-kafir	Uganda
SC991	Bicolor	Bicolor	Uganda
SC614	Bicolor	Bicolor	Tanzania
SC224	Bicolor	Dochna-leoti	Ethiopia
SC309	Bicolor	Dochna	Sudan
SC621	Bicolor	Dochna	
SC170-6	Caudatum	Zerazera	Ethiopia
Blackhull Kafir	Kafir		Africa
100M	Durra		USA
Australian Bicolor	Bicolor		
S. Propinquum	-	-	-

The Australian Bicolor used was an F2 from a cross of Australian Bicolor/BTx623.

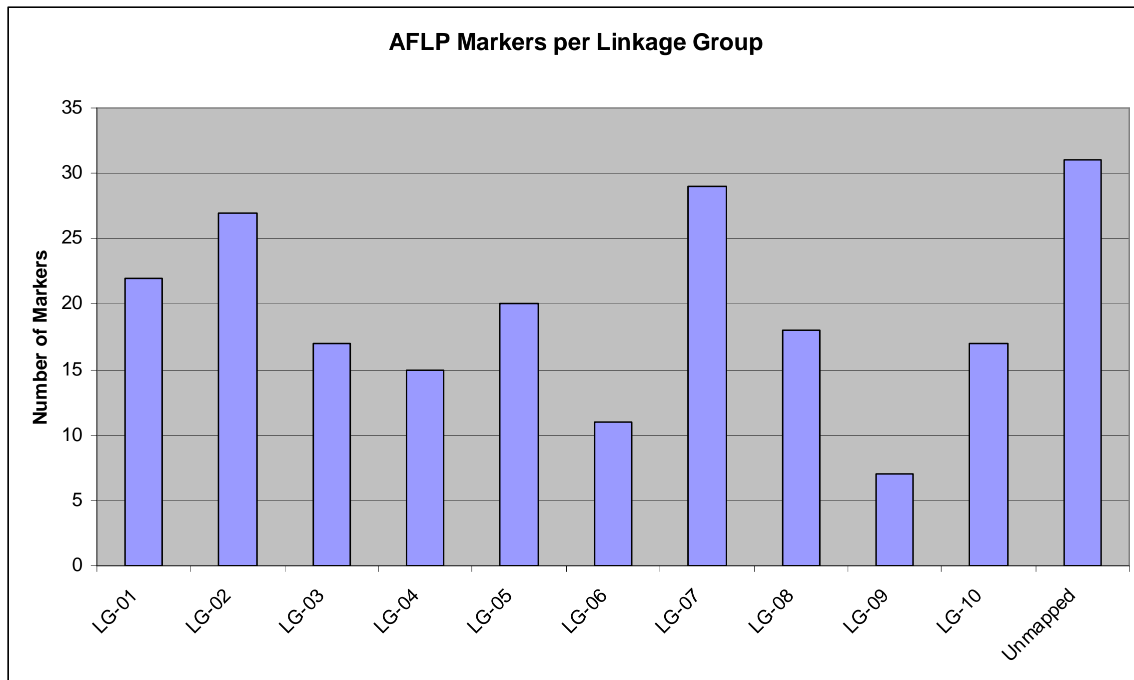

Figure 3.1 Number of markers per linkage group for the 273 *Eco*RI/*Mse*I AFLP markers run for the genetic similarity analysis.

Bicolor

None of the six genotypes of the race Bicolor fell within a group nor did they form their own group(s). The Bicolor lines SC991 and SC614 belong to WG10 Bicolor, SC1104 is classified as WG11-Bicolor/kafir, SC309 and SC621 belong to WG12-Dochna and SC224 is classified under WG13-Dochna/leoti (William Rooney, personal communication). If not considering the proposed lineage of the race Bicolor (Chapter 1), one might expect from the Menz *et al*. (2004) data, that one large group of the race Bicolor or two groups of the working groups Bicolor and Dochna may form from the six lines. Because these genotypes of the race Bicolor failed to group together, this gives support that the other races are derived from the race Bicolor. The Bicolors SC224, SC991, and SC621 are 58% to 63% genetically similar to one another and may be similar to material that did not form the other four races. Whereas, SC614 and SC309 may be similar to a progenitor of the bicolor in the Kafir lines, and SC1104 may be similar to a progenitor of the bicolor in the Caudatum lines. Of all the subspecies Bicolor, the race Bicolors are of the most dissimilar to one another with the exception of IS3620C (Figure 3.2), indicating that the race Bicolor is quite diverse. Furthermore, of the thirteen lines that failed to cluster in our study of the species Bicolor, eight of the lines are classified under the race Bicolor or have been bred with Bicolors.

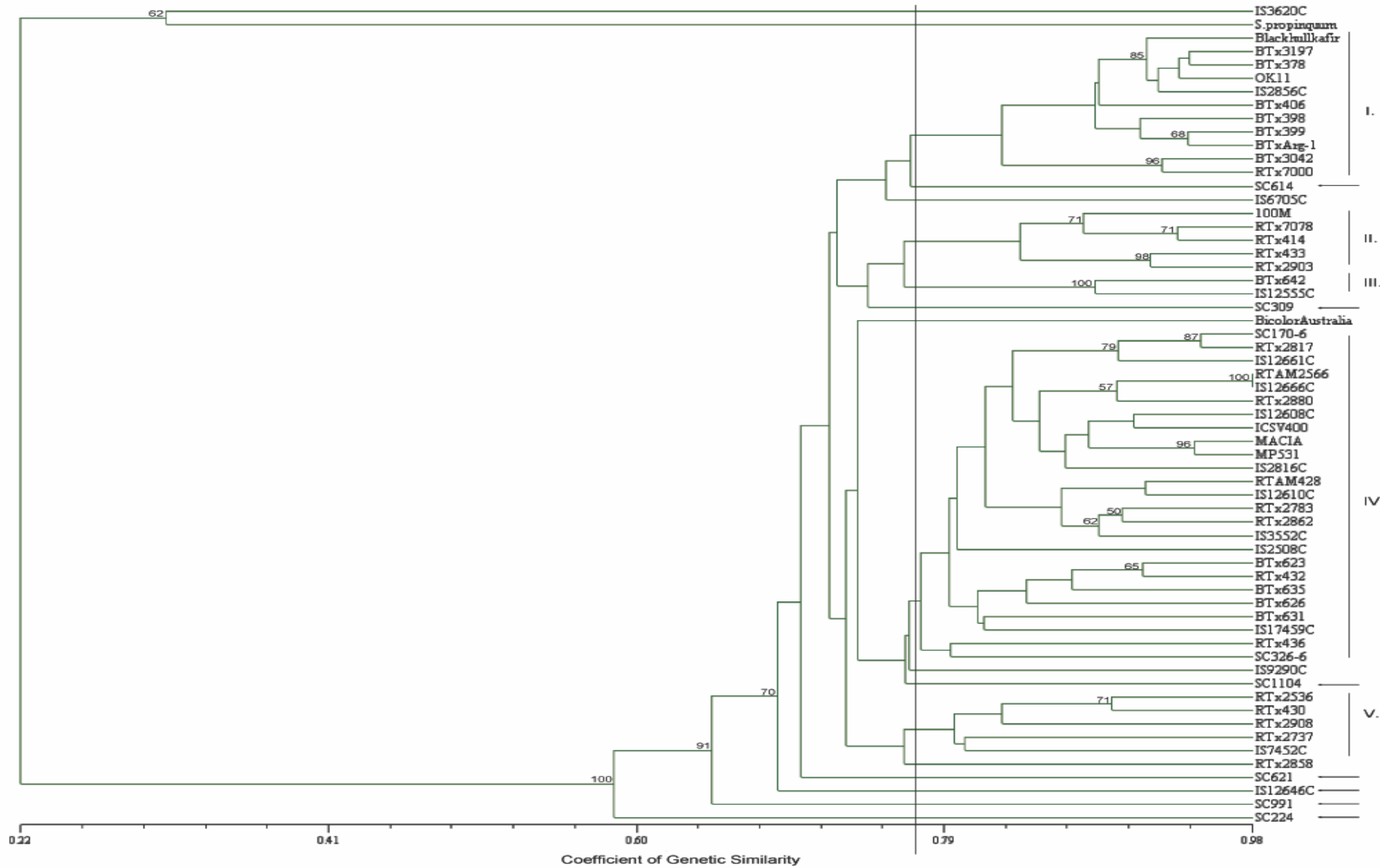

Figure 3.2 Dendrogram generated by UPGMA clustering analysis of 273 *Eco*RI/*Mse* I AFLP markers amplified from 61 sorghum inbred lines. Groups were formed using the genetic similarity of value .78 and bootstrapping was performed with 500 replicates. Bootstrap values greater than 50% were placed on the branches. The line bicolorAustralia refers to a F2 cross between Australian bicolor and BTx623. Cluster I includes the Kafirs, Cluster II includes the Kafir-Milos, Cluster III includes the Durras, Cluster IV includes the Caudatums, and Cluster V includes the Feteritas. Arrows indicate those accessions of the race Bicolor and IS12646C is of the hybrid race Durra-bicolor.

Propinquum

We also wanted to examine the genetic diversity between S. *bicolor* and its wild relative S. *propinquum* (Lin *et al.* 1999). Sorghum *propinquum* is a grain shattering perennial with stout rhizomes located in Sri Lanka, southern India, and Burma to the eastern islands of Southeastern Asia (Dahlberg 1995). S. *propinquum* and S. *bicolor* are fully fertile and belong genetically to one species but due to spatial isolation this perennial has been placed as a separate species (Lin *et al*. 1999). Despite the ability of these two species to form offspring, S. *propinquum* and S. *bicolor* share little genetic similarity, 25%-37% and S. *propinquum* does not cluster within a group (Figure 3.3). Thus the cultivated lines examined are highly diverged from S. *propinquum*.

Discussion

These results are in agreement with previous studies that show that the cultivated lines can be classified by their race or working group (Menz *et al.* 2004; Cui *et al.* 1995; Deu *et al.* 1994; Ahnert *et al*. 1996; Casa *et al.* 2005) with the exception of the race Bicolor (Deu *et al.* 1994; Dje *et al.* 2000). By comparison of the genetic similarity of each group, Zerazera was the largest and most diverse group which shows the importance of the race Caudatum in elite material (Menz *et al.* 2004).

The results of a genetic similarity analysis show that accessions of the race Bicolor contain alleles that are very dissimilar than most *Sorghum bicolors* and thus should be selected for conservation and potential introgression into cultivated lines, as these lines show the least amount of genetic redundancy. These data support previous work done on sorghum varieties, as it has been noted that with RFLP and microsatellites,

the Bicolor race was highly variable (Deu *et al*. 1994; Dje *et al*. 2000) and Bicolor accessions did not form a specific group, as they were distributed among the various clusters (Deu *et al*. 1994). These data support the hypothesis that the race Bicolor is a primitive sorghum from which the other races evolved (Dje *et al*. 1994; Hamblin *et al.* 2004).

The genetic distinctness of Margaritiferum, a working group of Guinea, sorghum from other Guinea sorghums has been well documented (Deu *et al.* 1994; Deu *et al*. 2006). All genotypes of *Sorghum bicolor* subspecies *bicolor* cluster together with the exception of IS3620C, a Margaritiferum. This suggests that IS3620C and perhaps the working group Margaritiferum should be classified outside the *sorghum bicolor* subspecies *bicolor* as IS3620C and *Sorghum propinquum*, a different species, are more similar to one another than any of the cultivated *Sorghum bicolor* subspecies *bicolor.*

These results are useful for crop improvement, as the use of diverse parents is expected to yield a higher frequency of heterotic hybrids. Also, these genetic similarity estimates can be used for development of segregating populations for mapping purposes.

CHAPTER IV

DIVERSITY OF SOURCES OF STAY-GREEN IN THE SORGHUM GERMPLASM COLLECTION

Introduction

Eight main sources of stay-green have been identified in the sorghum germplasm collection (Table 4.1). Two stay-green genotypes, BTx642 and KS19, have been used extensively in the United States and Australian sorghum breeding programs. BTx642 is a BC1 derived line from a cross of BTx406, a non stay-green Kafir-Milo cultivar and IS12555, a stay-green Ethiopian landrace (Rosenow *et al.* 1983). KS19 was selected from a cross of Short Kaura, an improved landrace from Nigeria, with the non stay-green genotype Combine Kafir 60 (Henzell *et al.* 1984). BTx642 and KS19 both delay the onset of senescence, but BTx642 derived lines have greater leaf area at flowering and a normal rate of leaf senescence once leaf senescence begins. KS19 derived lines have a smaller leaf area at flowering and a slower rate of leaf senescence (Mahalsksmi *et al.* 2002).

Table 4.1 Classification of eight sorghum stay-green sources used in this study.

Genotype	Race	Working Group	Origin
IS12555C	Durra	Durra	Ethiopia
SC33	Durra	Durra	Ethiopia
E36-1	Caudatum-guinea		Ethiopia
SC56	Caudatum	Caudatum-nigricans	Sudan
IS17459C (Rio)	Caudatum	Caudatum-nigricans	Sudan
SC170-6	Caudatum	Zerazera	Ethiopia
IS22380 (PI569826)	Caudatum	Caudatum-kafir	Sudan
Short Kaura (PI285193)	Caudatum	Caudatum-kaura	Nigeria

Other sources of stay-green include SC56, IS17459C (Rio), SC170-6, E36-1, IS22380, and SC33. Three Caudatum sources of stay-green SC56, SC599/Rio, and SC170-6 were compared to three non-stay green sorghum genotypes RTx2536, RTx7000, and RTx378 under irrigated conditions. The non senescent genotypes took two days longer to reach 50% anthesis, were three to four centimeters shorter, produced two to three more basal tillers per plant, had larger stem diameters, maintained higher basal stem sugar concentrations, and produced higher leaf blade chlorophyll contents than did the senescent genotypes (Duncan *et al.* 1981).

Mahalakshmi *et al.* (2002) sought to identify more sources of stay-green available at the International Crops Research Institute for SemiArid Tropics (ICRISAT). These authors analyzed 72 sorghum lines for green leaf area at 15, 30, and 45 days after flowering under terminal stress conditions and identified two groups of stay-green genotypes. The first group of genotypes (IS22380, KS19-derived, and BTx642) was non-senescent, had high green leaf area and flowered early to escape drought. The second group of genotypes (E36-1 derived and ICRISAT B/R line multifactor resistance population ICSR-2 B/R MFR) showed a stay-green phenotype but were late flowering (Mahalakshmi *et al.* 2002).

Of the eight sources of stay-green, three sources originated from Sudan, four from Ethiopia, and one from Nigeria. Three sources can be traced to the actual locations within these countries. SC599 was collected in southern Sudan, SC56 was collected in central Sudan, and SC170-6 was collected in northern Ethiopia. With the origins of the sources of stay-green genotypes in such close geographic proximity, the genetic

similarity of the sources of stay-green was investigated to understand their overall relationship and assess the similarity of DNA spanning regions containing favorable alleles for stay-green.

Results

Diversity of sorghum germplasm sources of stay-green

The genetic similarity among the eight sources of stay-green listed in Table 4.1 and 71 other inbred sorghum genotypes listed in Table 4.2 was assessed using 55 SSR markers selected with overall genome coverage but outside regions of the sorghum genome containing most of the favorable alleles for stay-green (Table 4.3). Some of the information on SSR allele diversity used in the analysis here was obtained from a previous study of 50 sorghum-breeding lines and their progenitors (Menz *et al.* 2004). The SSR markers identified 2 to more than 24 alleles within the collection of 79 sorghum genotypes analyzed. The information from SSR analysis was used to assess the genetic relatedness of the 79 genotypes analyzed in this study (Rohlf 1994; Nei and Li 1979). The results displayed in a dendrogram were created by first calculating the genetic similarity between all pairs of lines and then clustering these data (Figure 4.1).

Table 4.2 Genotype, pedigree, race, working group (WG), and country of origin for the 28 genotypes investigated in this study that were not included in the diversity analysis conducted by Menz *et al.* (2004).

Genotype	Pedigree	Race	WG	Origin
03BRON299	SC56-14E/86EON361			BL-USA
B.HF8	BTx643/BTx635			BL-USA
B01084	BTx623/(BTx642/B9501)			BL-USA
B03MN952	Tx399//Tx399/BTx642			BL-USA
B03MN954	Tx399//Tx399/BTx642			BL-USA
B03MN960	Tx3042//Tx3042/BTx642			BL-USA
B2-2	BTx625/BTx642			BL-USA
B402	BTx3042/(BTx625/BTx642)			BL-USA
B403	BTx3042/(BTx625/BTx642)			BL-USA
B923171	B872389-1-3/B886815-1-3			BL-AUS
B923296	B872389-1-3/B886815-1-3			BL-AUS
BTx625				BL-USA
E36-1		G-C		Ethiopia
IS12553C,SC33		D	Durra	Ethiopia
IS12568C,SC56		C	Caudatum-nigricans	Sudan
IS12661C,SC170-6		C	Zerazera	Ethiopia
IS22380,PI569826		C	Caudatum-kafir	Sudan
QL12	KS19*4/Kr13 x KS19			BL-AUS
QL33	QL23/B758864			BL-AUS
QL39				BL-AUS
QL41	BTx642/QL33			BL-AUS
R931945-2-2	R883373-3/(B872389-2-3/R890562-1-2)			BL-AUS
R974443-1-2	R940932/R931945-2-2			BL-AUS
RO4044	1790E/CE151			BL-USA
RO4047	1790E/CE151			BL-USA
RO4050	1790E/CE151			BL-USA
ROOMN7645	Tx430///(SC35//BTx642/80060			BL-USA
Short Kaura,PI285193		C	Caudatum-kaura	Nigeria

C, D, and G-C refer to the races Caudatum, Durra, and the hybrid race Guinea-caudatum, respectively. The abbreviation BL is used to denote that these genotypes are breeding lines created from US and Australian sorghum breeding programs.

Table 4.3 Markers used for the genome-wide diversity study.

Marker	Linkage Group	cM	Repeat	No. of alleles	Study
txp208	LG-01	23.4	(GGA)8	2	Genome
txp302	LG-01	31	(TGT)8	8	Genome
txp357	LG-01	70-73	(GT)10	5	Genome
txp149	LG-01	100-107	(CT)10	4	Genome
txp32	LG-01	114-117	(AG)16	13	Genome
Sb6_57	LG-01	156-160	(AG)18	8	Genome
Sb6_36	LG-01	182-186	(AG)19	12	Genome
txp319	LG-01	211	(TC)17	8	Genome
txp96	LG-02	5-7	(GA)24	9	Genome
txp297	LG-02	23-25	(AAG)24	24	Genome
txp84	LG-02	25-30	(AG)9	3	Genome
Cup74	LG-02	73-75	(TG)9	2	Genome
SbAGB03	LG-02	90-91		17	Genome
txp7	LG-02	157-160	(CT)14	8	Genome
txp228	LG-03	11-15	(TC)12	7	Genome
txp266	LG-03	11.9-15	(GT)8	2	Genome
txp489	LG-03	33-37		3	Genome
Corn023	LG-03	76-79	Pioneer	2	Genome
txi11	LG-03	169-174	indel	2	Genome
txi7	LG-03	174-177	indel	4	Genome
txi13	LG-03	174-177	indel	2	Genome
txi9	LG-03	174-177	indel	3	Genome
txi12	LG-03	177-179	indel	2	Genome
txp427	LG-03	184-190		8	Genome
txp425	LG-03	192-198		4	Genome
txp504	LG-04	leftmost		4	Genome
Sb4_121	LG-04	5-9	(AC)14	8	Genome
txp12	LG-04	94	(CT)22	9	Genome
txp60	LG-04	134-139	(GT)4GC(GT)5	3	Genome
Y12464	LG-05	0	(CT)4	4	Genome
txp65	LG-05	11.2	(ACC)4+(CCA)3CG(CT)8	6	Genome
txp23	LG-05	90	(CT)19	6	Genome
txp123	LG-05	115	(AT)9	6	Genome
txp434	LG-06	16-19		2	Genome
TS363	LG-06	26-29	Pioneer	4	Genome
CS030	LG-06	44-46	Pioneer	4	Genome
txp317	LG-06	56-59	(CCT)5(CAT)11	6	Genome
txp176	LG-06	80	(AG)4AAC(GA)4	7	Genome
txp40	LG-07	0	(GGA)7	4	Genome
txp312	LG-07	45.9	(CAA)26	14	Genome
txp92	LG-07	96	(GAA)5	2	Genome
Cup52	LG-07	147-149	(AATT)5	3	Genome
txp273	LG-08	0	(TTG)20	7	Genome

Table 4.3, continued

Marker	Linkage Group	cM	Repeat	No. of alleles	Study
txp47	LG-08	38	(GT)8(GC)5+(GT)6	4	Genome
txp294	LG-08	82.5	(TG)10(GT)4	5	Genome
txp321	LG-08	104-109	(GT)4+(AT)6+(CT)21	10	Genome
Sb6_34	LG-08	147-149	(AC/CG)15	7	Genome
txp289	LG-09	leftmost	(CTT)16+(AGG)6	10	Genome
txp410	LG-09	6-23		17	Genome
txp459	LG-09	6-23		7	Genome
Sb4_32	LG-09	118-129	(AG)15	11	Genome
txp20	LG-10	68	(AG)21	9	Genome
gap1	LG-10	99-101	(AG)16	10	Genome
Cup43	LG-10	136-138	(CTGCC)5	3	Genome
Cup7	LG-10	off end	(CAA)8	6	Genome
			Total	360	
			Mean	6.5	

Marker names, linkage group, map positions, repeat motif, number of alleles from 79 inbred lines, and average number of alleles per marker are listed. Some of these markers were previously published (Menz *et al.* 2002). Indel refers to those markers that contain an insertion or deletion. Pioneer refers to those markers that were created by Pioneer Hi-Bred International, Inc. Blank cells refer to those markers where the repeat motif could not be found.

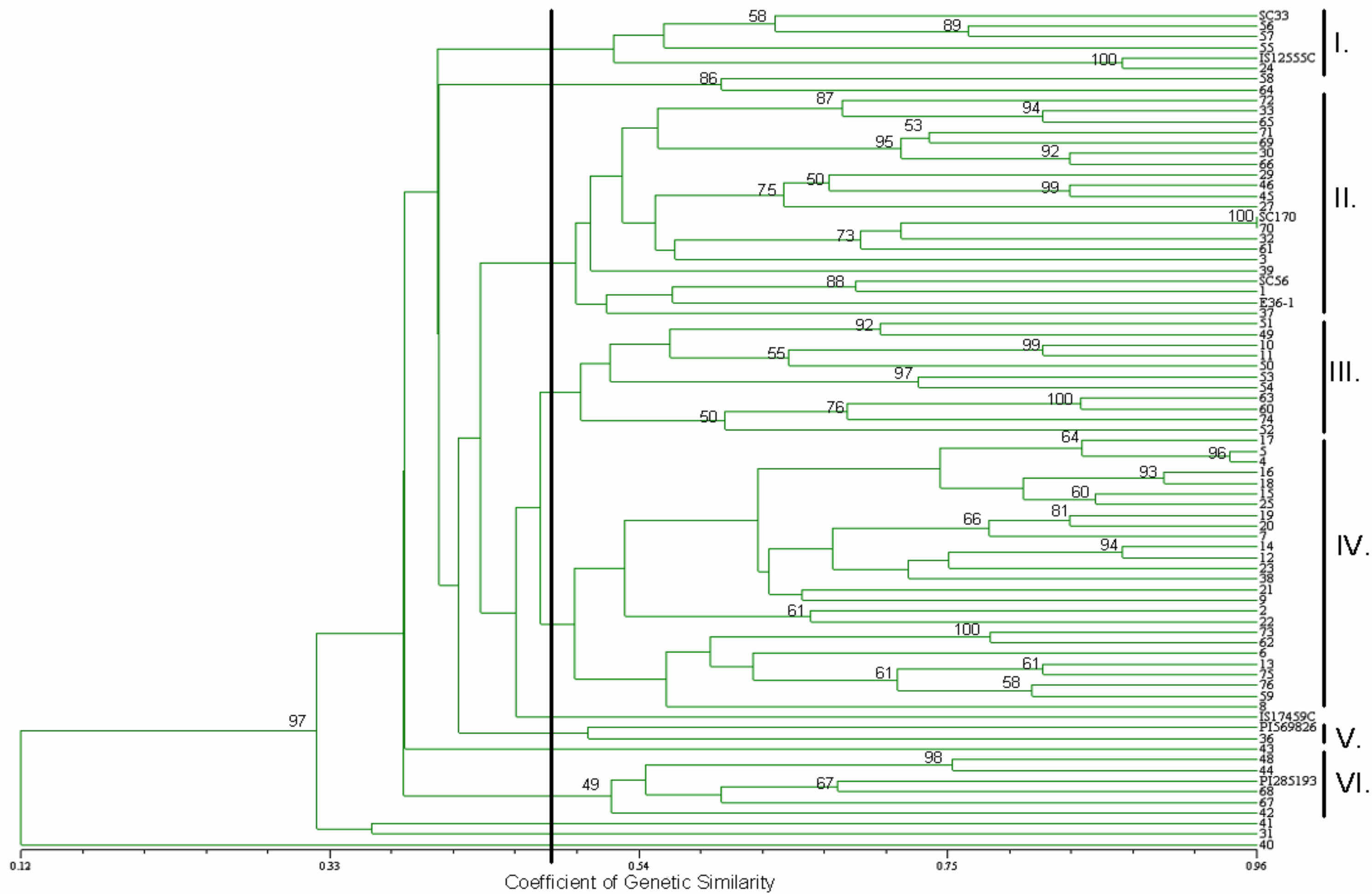

Figure 4.1 Dendrogram of 79 sorghum genotypes revealed by cluster analysis of genetic similarity using data collected from 55 genome-wide SSR markers. Numbers correspond to genotypes listed in Appendix A. Names of genotypes shown in the Figure correspond to the eight sources of stay-green. The thick black vertical line denotes the cutoff value for the clusters. Group I includes genotypes of the race Durra, Group II includes genotypes of the race Caudatum, Group III includes genotypes that are related to QL33/RTx430/RTx436, Group IV includes genotypes that belong to the race Kafir, Group V includes genotypes of the working group Caudatum-kafir of the race Caudatum, and Group VI includes genotypes from the working group Caudatum-kaura of the race Caudatum. 500 bootstrap repetitions were performed and values greater than 49 are shown.

Overall, the genotypes are distributed across the dendrogram based on race or working groups as previously described (Menz *et al.* 2004) (Figure 4.1, groups of genotypes marked by bars I-IV correspond to genotypes related to the sorghum races Durra (I), Caudatum (II), QL33/RTx430/RTx436 derived lines (III), Kafir (IV), working group Caudatum-kafir (V), Caudatum-kaura (VI). The eight stay-green germplasm sources did not cluster together but rather clustered by race or working group with the exception of IS17459C which failed to cluster with any group as noted previously (Menz *et al.* 2004). The stay-green germplasm sources ranged from ~27-56% genetic similarity where PI285193 and IS12555C were the least related and IS12555C and SC33 was the most similar (Table 4.4).

Table 4.4 Genetic similarity matrix, using DICE's coefficient of genetic similarity, for the eight sources of stay-green analyzed in this study.

	SC33	SC56	PI569826	SC170-6	PI285193	IS12555C	IS17459C	E36-1
SC33	100	37	37	39	35	56	35	44
SC56	37	100	53	55	36	40	45	55
PI569826	37	53	100	47	31	40	42	45
SC170-6	39	55	47	100	40	35	47	51
PI285193	35	36	31	40	100	27	31	41
IS12555C	56	40	40	35	27	100	36	36
IS17459C	35	45	42	47	31	36	100	36
E36-1	44	55	45	51	41	36	36	100

Analysis of genetic diversity within Stg1-Stg4, StgD, and StgG among eight sorghum stay-green germplasm sources

Although the eight sorghum genotypes that express stay-green have genomes that are not closely related overall, it is possible that DNA within regions of their genomes spanning the stay-green QTL is related. This possibility was investigated in the four regions of the sorghum genome corresponding to Stg1, Stg2, Stg3 and Stg4 identified by Xu *et al.* (2000) in a cross of BTx642/RTx7000 and two other regions corresponding to stay-green QTL identified in other studies. These two regions are the StgI2/StgG QTL region which has an overlapping QTL region (Crasta *et al*. 1999; Tao *et al*. 2000) and the StgD QTL region identified by Crasta *et al.* (1999). Hereafter the QTL will be denoted with the first letter of the author's last name followed by the name the author gave the QTL (ex. C-StgD for StgD identified by Crasta *et al.* (1999)). Six to thirteen SSR markers that mapped at intervals across each of the stay-green QTL were used to analyze the eight sources of stay-green and 71 other sorghum genotypes (Table 4.5, Appendix A).

Table 4.5 List of markers amplified in 79 inbred lines that map in the BTx642 QTL areas.

Marker	Linkage Group	cM	Repeat	No. of alleles	Study
txp464	LG-02	106	(GT)4+(GGC)7	5	X-Stg3
txp445	LG-02	110-113	(AT)4+(TA)5	5	X-Stg3
TS214	LG-02	110-113	(TC)14	7	X-Stg3
Cup29	LG-02	110-113		5	X-Stg3
txp430	LG-02	113-114	(GGC)7	5	X-Stg3
txp1	LG-02	114-118	(AG)34	10	X-Stg3
txp56	LG-02	118.5	(GA)39	10	X-Stg3
gap84	LG-02	118.5	(AG)14	11	X-Stg3
SDB049	LG-02	129-131	Pioneer	10	X-Stg3
txp179	LG-02	131-134	(GT)8AC(GT)5	3	X-Stg3
txp348	LG-02	137-145	(TAA)37	24	X-Stg3
Corn033	LG-02	189-193		3	C-StgD
CS033	LG-02	189-193		4	C-StgD
Cup40	LG-02	189-193		4	C-StgD
txp8	LG-02	193	(TG)31	24	C-StgD
TS451	LG-02	193-196	Pioneer	22	C-StgD
Cup69	LG-02	202-205	(ATGCG)4	3	C-StgD
CS056	LG-02	202-205		2	C-StgD
txp543	LG-03	98-102	(AT)10	3	X-Stg2
txp544	LG-03	102-104	(TC)11	7	X-Stg2
txp503	LG-03	105-108	(AT)21	2	X-Stg2
txp120	LG-03	110	(AT)18	8	X-Stg2
txp435	LG-03	110-111	(TA)5	3	X-Stg2
txp545	LG-03	111	(GT)10	7	X-Stg2
txp546	LG-03	111	(TC)10	4	X-Stg2
txp2	LG-03	111-118	(GT)10	6	X-Stg2
txp530	LG-03	111-118		7	X-Stg2
txp231	LG-03	118-125	(GGA)4+(GGA)5	3	X-Stg2
txp436	LG-03	118-125	(GCT)7	5	X-Stg2
txp59	LG-03	118-125	(GGA)5	3	X-Stg2
txp114	LG-03	134-135	(AGG)8	2	X-Stg1
Corn031	LG-03	140-142	Pioneer	5	X-Stg1
txp439	LG-03	145-147	(GA)16	8	X-Stg1
txp440	LG-03	145-147	(TTCT)16	10	X-Stg1
txp542	LG-03	147-149	(CGT)7	4	X-Stg1
txp441	LG-03	149-150	(TA)10	6	X-Stg1
SDB009	LG-03	150-151	Pioneer	2	X-Stg1
BE918289	LG-03	150-156	(CGA)8	2	X-Stg1

Table 4.5, continued

Marker	Linkage Group	cM	Repeat	No. of alleles	Study
txp446	LG-03	151-156	(ATA)25	18	X-Stg1
txp442	LG-03	151-156	(TAA)3+ (TA)16	9	X-Stg1
txp474	LG-03	156-161	(AG)19	16	X-Stg1
txp447	LG-03	156-158	(AG)14	8	X-Stg1
txp285	LG-03	158-161	(CTT)11CTC(CTT)16	13	X-Stg1
txp448	LG-03	158-161	(AG)15+(AG)5	6	X-Stg1
txp38	LG-03	158-161	(AG)17	8	X-Stg1
txp421	LG-03	158-161	(TC)14	5	X-Stg1
txp420	LG-03	161-165	(GA)22	7	X-Stg1
txp30	LG-05	34-35	(AAT)25	18	X-Stg4
txi18	LG-05	34-35	indel	2	X-Stg4
txp303	LG-05	35-41	(GT)13	5	X-Stg4
TS404N	LG-05	53-56	Pioneer	5	X-Stg4
txp225	LG-05	56.9	(CT)9(CA)8CCC(CA)6	5	X-Stg4
txp15	LG-05	58-61	(TC)16	7	X-Stg4
TS492	LG-05	58-61	Pioneer	10	X-Stg4
SDB064	LG-05	61-68	Pioneer	7	X-Stg4
txp258	LG-09	43-45	(AAC)19	6	T-StgG/C-StgI2
txp287	LG-09	43.6	(AAC)21	8	T-StgG/C-StgI2
SDB053	LG-09	43-45	(AG)41	20	T-StgG/C-StgI2
txp67	LG-09	52.5	(GA)28	15	T-StgG/C-StgI2
TS403	LG-09	62-65	Pioneer	2	T-StgG/C-StgI2
CS002	LG-09	65-68	Pioneer	7	T-StgG/C-StgI2
txp10	LG-09	81-85	(CT)14	7	T-StgG/C-StgI2
			Total	468	
			Mean	7.5	

Markers that lie within each stay-green QTL region are shown in the last column. Blanks refer to those markers whose repeat motif could not be found and Pioneer refers to those markers that were designed by Pioneer Hi-Bred International, Inc.

The data from this analysis were subsequently searched for groups of alleles of SSRs markers that map sequentially across the stay-green loci that are shared by more than one source of stay-green (haplotypes). The frequency of finding these haplotypes among the 79 genotypes was subsequently assessed to obtain further information about the distribution and frequency of the haplotypes within the larger set of genotypes. Haplotypes were required to span at least four SSR markers to increase the probability that the observed haplotypes were not just the result of chance. However, additional information, such as additional QTL studies, will be required to test the significance of haplotypes identified as described above.

The analysis of data from 13 SSR markers spanning the X-Stg1 QTL region revealed that SC170-6 and PI285193 (Short Kaura) share a four-allele haplotype that spans 5-10cM in this region (Table 4.6). In addition to SC170-6 and Short Kaura, this haplotype was also found in IS12661 (data not shown), a genotype related to SC170-6 by descent but in none of the other 76 genotypes analyzed.

Table 4.6 Genotypes of 13 SSR markers that map across the X-Stg1QTL region obtained from eight sources of stay-green and the conversion parent Tx406. Blue colored regions represent a common haplotype. Numbers designate allele types of a given SSR where numbers common between two genotypes indicates that the same allele was observed. XX represents that a PCR product could not be amplified from these lines. Blank cells represent a failed PCR reaction.

X-Stg1 region

Marker	LG-03, cM	TX406	SC33	IS12555C	IS17459C	SC56	SC170-6	PI569826	PI285193	E36-1
Corn031	140-142	3	3	3	1	1	1	1	1	1
txp439	145-147	4	2	2	2	2	4	8	5	7
txp440	145-147	4	4	1	2	6	4	6	4	
txp542	147-149	3	3	3	3	2	3	3	1,2	2
txp446	151-156	12	18	10	10	16	3,7	5	3	12
txp442	151-156	3	1	1	XX	1	1	1	1	4
txp447	156-158	4	1	1	4	3	4	4	4	4
txp448	158-161	2	5	3	1	4	4	4	4	4
txp285	158-161	3	7	5	7	4	4	7	6	7
txp38	158-161	1	1	7	2	3	2	5	3	7
txp474	158-161	3	1	7	12	7	13	7	5	16
txp421	158-161	4	1	1	6	1	1	1	1	1
txp420	161-165	2,5	5	5	1	5	2	1	4	2

Nine SSR markers were used to analyze the diversity of DNA spanning X-Stg2 among the eight sources of stay-green and the conversion parent Tx406 (Table 4.7). This analysis revealed a haplotype spanning the entire X-Stg2 region that was common to Tx406 and SC56. The lower portion of this same haplotype was also found in SC170-6. The nine-allele haplotype was found in six other genotypes, 03BRON299, QL12, BTx398, Tx7078, RTx414, RTx433 (data not shown).

Table 4.7 Genotypes of nine SSR markers spanning the X-Stg2 QTL region used to analyze the eight sources of stay-green and the conversion parent Tx406. Numbers designate alleles of a given SSR where numbers common between two genotypes indicates that the same allele was observed. Pink colored regions indicate the presence of a common haplotype.

X-Stg2 region

Marker	LG-03, cM	TX406	SC33	IS12555C	IS17459C	SC56	SC170-6	PI569826	PI285193	E36-1
txp543	98-102	1	2	2	2	1	1	1	1	1
txp544	102-104	2	4	5	3	2	4	6	5	4
txp435	110-111	2	2	2	3	2	3	2	1,2	3
txp120	111	4	4	4	5	4	3	3	7	8
txp545	111.6	2	5	1	2	2	2	1	2,6	7
txp546	112	2	2	2	3	2	2	2	2	2
txp2	111-118	1	5	2	1	1	1	2	1,6	1
txp530	111-118	2	2	3	2	2	2	1	4	4
txp436	118-125	1	5	1	1	1	1,2	4	2	4

The genetic diversity of DNA spanning the X-Stg3 QTL region was assessed in a similar manner using 11 SSR markers that map across this ~30cM QTL. The results in Table 4.8 show that PI569826 and E36-1 share a four-allele haplotype from 103-113cM on LG-02. Examination of 71 other genotypes showed that this haplotype is unique to these two stay-green lines.

Table 4.8 Genetic diversity of the germplasm sources of stay-green and the conversion parent Tx406 in X-Stg3 QTL region revealed by eleven SSR markers. Numbers designate different alleles of a given SSR where numbers common between two genotypes indicates the same allele was observed. Purple shading represents a shared haplotype between PI569826 and PI285193. A blank cell represents a failed PCR reaction.

X-Stg3 region

Marker	LG-02, cM	TX406	SC33	IS12555C	IS17459C	SC56	SC170-6	PI569826	PI285193	E36-1
txp464	103-108	3	2	2	2	4	2	2	4	2
txp445	110-113	1	4	4	1	3	2	2	2	2
TS214	110-113	1	5	1	2	6	4	7	4	7
Cup29	110-113	3	4	4	3	1	2	2	2	2
txp430	113-114	2	3	3	2	1	1	3	2	1
txp1	114-118	8	8	1	6	9	2	7	9	2
txp56	118.5	1	1	3	1	3	7	4	6	10
gap84	125-126	3	3	1	8	11	8	8	8	8
SDB049	129-131	4	3	4	2	3	2	2	9	2
txp179	131-134	1	1	1	1		1	1	1,2	1
txp348	137-145	22	1	11	12	15	1	23	4	1

The diversity of DNA spanning X-Stg4 was assessed using seven SSR markers that map across DNA spanning this QTL. This analysis showed that SC33 and IS12555C share a common four-allele haplotype spanning 34-56.9cM on LG-05 (Table 4.9). Five lines among the other genotypes examined also contain this haplotype, BTx642, B01084, RO4047, RO4050, Tx2737 (data not shown). BTx642 and B01084 were derived from IS12555 and RO4047 and RO4050 are derived from SC33.

Table 4.9 Genetic diversity of the sources of stay-green and the conversion parent Tx406 revealed by seven SSR markers spanning X-Stg4. Pink shading represents DNA derived from BTx406 while green shading highlights a shared haplotype between SC33 and IS12555C. Numbers designate allele types of a given SSR where numbers common between two genotypes indicates that the same allele was observed. XX represents that a PCR product could not be amplified from these lines.

X-Stg4 region

Marker	LG-05, cM	TX406	SC33	IS12555C	IS17459C	SC56	SC170-6	PI569826	PI285193	E36-1
txi18	34-35	1	1	1	1	1	1	1	1	2
txp303	35-41	3	1	1	3	2	3	2	4,5	1
TS404N	53-56	3	4	4	1	4	3	1	5	2
txp225	56.9	4	3	3	5	3	4	5	1	3
txp15	58.2-61	3	7	4	2	XX	2	XX	XX	6
TS492	58-61	6	6	9	2	4	6	8	5	3
SDB064	61-68	2	3	1	2	2	1	5	1	2

The region spanning T-StgG/C-StgI2 was analyzed using seven SSRs as described above. This analysis revealed no common haplotypes spanning four or more alleles among the sources of stay-green (Table 4.10).

Table 4.10 Haplotype of the sources of stay-green using six markers in the T-StgG/C-StgI2 QTL region. Numbers designate allele types of a given SSR where numbers common between two genotypes indicates that the same allele was observed.

T-StgG/C-StgI2region

Marker	LG-09, cM	TX406	SC33	IS12555C	IS17459C	SC56	SC170-6	PI569826	PI285193	E36-1
SDB053	43-45	7	3	15	14	11	1	4	9,10	19
txp258	43.4-45.5	6	4	6	4	6	2	4	3	3
txp287	43.6	2	4	2	4	2	5	4	8	7
txp67	52.5	7	6	7	9	13	5	9	4	15
CS002	65-68	4	2	1	2	4	1	5	6,7	1
txp10	81-85	6	1	3	4	6	3	3	6	7

The QTL region corresponding to C-StgD was analyzed for common haplotypes among the sources of stay-green using seven SSR markers (Table 4.11). This analysis showed that SC170-6 and PI569826 share a four-allele haplotype spanning ~189-196cM on LG-02 (Figure 4.11). This haplotype was present in ten other genotypes analyzed in this study, RTx430, BTx625, BTx623, Tx2880, BTx626, BTx635, SC1079, RTx432, Tx2817, and MACIA (data not shown). RTx430, BTx623, Tx2880, Tx2817 are all derived from SC170-6 and BTx626 and RTx432 share a common parent SC110-6.

Table 4.11 Haplotype of the stay-green sources using six markers in the C-StgD QTL region. Numbers designate allele types of a given SSR where numbers common between two genotypes indicated that the same allele was observed. The area highlighted in blue represents a haplotype shared by SC170-6 and PI569826.

C-StgD

Marker	LG-02, cM	TX406	SC33	IS12555C	IS17459C	SC56	SC170-6	PI569826	PI285193	E36-1
Cup40	189-193	2	1	1	1	2	1	1	2	1
Corn033	189-193	1	1	1	1	1	1	1	2	1
CS033	189-193	1	3	3	3	1	3	3	1	3
TS451	193-196	6	10	11	12	15	2	2	1	7
Cup69	202-205	1	1	2	1	1	2	1	2,3	1
CS056	202-205	4	4	2	4	4	2	4	4	4

Discussion

Data derived from fifty-five SSR markers that provide genome-wide coverage showed that the eight sorghum germplasm sources of stay-green analyzed in this study are not closely related to each other (Figure 4.1). Instead, the stay-green germplasm sources examined cluster with other genotypes of their race or working group. Cluster analysis based on genetic similarity showed that the stay-green lines SC33 and IS12555C are related to other genotypes of the race Durra. Similarly, SC170-6, SC56, and E36-1 belong to a group that includes genotypes of the race Caudatum whereas PI569826 (IS22380) is related to a group that includes genotypes of the working group Caudatum-kafir of the race Caudatum. PI285193 (Short Kaura) is related to a group of genotypes of the working group Caudatum-kaura of the race Caudatum, and IS17459C did not cluster within any group. While the sources of stay-green are quite diverse overall, none of these genotypes cluster with genotypes of the race Kafir. Genotypes of

this race are derived primarily from a broad region of southern Africa, a region quite distant from the region of Sudan and Ethiopia where most of the sources of stay-green germplasm were collected. This suggests that the stay-green response may not have been under strong selection in the environment present in southern Africa.

The eight sources of stay green were also analyzed for DNA diversity across genomic regions spanning six of the stay-green QTL that have been identified to date. This revealed several haplotypes within the stay-green loci common to two or more stay-green genotypes that spanned at least four DNA markers and ~5-10cM. For example, SC170-6 and PI285193 shared a common four-marker haplotype within X-Stg1. However neither of these genotypes has been involved in a stay-green QTL study so it is unclear if the common haplotype spans a favorable allele for stay-green. BTx406 and SC56 share a common haplotype spanning X-Stg2 and BTx406 and SC170-6 share the same haplotype from ~111cM to ~125cM. SC170-6 and SC56 are 'converted' lines that resulted from crosses of exotic germplasm to BTx406, the source of recessive genes conferring photoperiod insensitivity and short height. Therefore, it is likely that the haplotype shared by BTx406, SC170-6, and SC56 is the result of the conversion program. Of perhaps greater significance is the fact that a favorable allele for X-Stg2 is derived from SC56 (Crasta *et al.* 1999), and the data collected in this study indicates that this allele probably originates from or at least is in common with BTx406, a non-stay green genotype.

PI569826 and E36-1 were found to share a common four-allele haplotype within X-Stg3. A favorable allele for X-Stg3 was previously reported to be derived from E36-1

(Haussmann *et al.* 2002). Therefore PI569826 may also contain this favorable allele for stay-green (X-Stg3). The exact origin and pedigree of E36-1 is unknown, but E36-1 and PI569826 are both related to the Caudatum race and, although they are not closely related overall, the two genotypes originate from the same general region of Africa (Ethiopia/Sudan) so it is possible that the two genotypes share a common allele for X-Stg3.

SC33 and IS12555C shared a common four-allele haplotype in X-Stg4. A favorable allele for stay-green located in X-Stg4 was previously shown to originate from IS12555C (Xu *et al.* 2000). SC33 and IS12555C are both of the race Durra and originate from a similar region of Africa. Therefore, based on haplotype information and common origin, SC33 may also contain the favorable allele for X-Stg4 identified in IS12555C. Of course further stay-green mapping analysis, gene isolation and allele identification will be needed to test if the common haplotypes observed in this study are useful predictors of common alleles for stay-green. To date, the genetic basis of stay-green has been analyzed using four stay-green genotypes, BTx642, SC56, QL41 and E36-1 (Xu *et al.* 2000; Crasta *et al.* 1999; Tao *et al.* 2000; Kebede *et al.* 2001; Haussmann *et al.* 2002; Tuinstra *et al.* 1997). The results described here suggest that it would be useful to examine the genetic basis of stay-green genotypes in Short Kaura, PI569826 (IS22380), and IS17459C to capture the full range of genes and alleles involved in modulating the stay-green trait.

The overall analysis of genetic diversity among the eight sources of stay-green suggests that favorable alleles for stay-green may have been selected in a range of

diverse sources of sorghum germplasm. The fact that seven of the eight sources of stay-green originate from a common region of Africa that includes part of Ethiopia and Sudan could indicate that the stay-green trait provides a selective advantage for sorghum growing in this environment, regardless of race or working group. The mechanisms and genes that allow different sorghum genotypes to stay-green do not have to be shared by all of the genotypes and the different stay-green genotypes may utilize similar mechanisms but achieve their stay-green phenotype through the action of different genes or alleles. Because we do not know the exact pedigrees of the eight sources of stay-green, it is also possible that the same allele is present in the diverse genetic backgrounds of the sources of stay-green. Further information will be needed to test this possibility.

CHAPTER V

BTx642/RTx7000 GENETIC MAP

Introduction

Sorghum (*Sorghum bicolor* [L.] Moench), a C_4 grass and a close relative to sugarcane, is adapted to hot, dry adverse environments (Doggett 1988). It is the fifth most important cereal grown worldwide (Mullet *et al.* 2001) and is a source of food, feed, fiber and fuel (Sorghum Genomics Planning Workshop Participants 2005). With so many important qualities, numerous sorghum genetic linkage maps have been published in the last decade with eight created using stay-green lines (Table 5.1).

Genetic maps can be used for marker-assisted selection, map-based cloning, comparative genomics, diversity studies, targeted genome sequencing, and QTL studies (Mullet *et al.* 2001; Piquemal *et al.* 2005). Restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), and simple sequence repeats (SSRs) are useful DNA markers for map construction. RFLPs are locus specific and multi-allelic but processing RFLP markers is time consuming, expensive and requires a large amount of DNA (Song *et al*. 2005). AFLPs require no prior sequence knowledge and many alleles can be analyzed with each primer combination. AFLP analysis requires only small amounts of DNA but the polymorphisms usually result in the presence or absence of a DNA band and therefore these markers do not usually provide direct evidence for more than one allele (Vos *et al.* 1995). Thus AFLPs are more suitable for backcross or recombinant inbred (RI) lines (Song *et al.* 2005). SSRs are multi-allelic, easy to use, but lack the throughput of AFLPs (Kashi *et al.* 1997).

Table 5.1 List of published sorghum genetic linkage maps that utilized a stay-green genotype for recombinant inbred line (RIL) population development.

Author	Parents	# markers	# of RILs	#LGs
Subudhi *et al.* 2000	BTx642*/RTx7000	236	98	10
Xu *et al.* 2000	BTx642*/RTx7000	145	98	10
Crasta *et al.* 1999	BTx642*/RTx430	128	96	14
Tuinstra *et al.* 1997	BTx642*/Tx7078	170	98	17
Tao *et al.* 2000	QL41*/QL39	311	152	14
Kebede *et al.* 2001	SC56*/RTx7000	144	125	10
Haussmann *et al.* 2002b	E36-1*/IS9830	187	225	10
Haussmann *et al.* 2002b	E36-1*/N13	228	226	12

Stay-green parental lines are marked with a *. The number of DNA markers used in map construction, the number of RILs used, and the number of linkage groups (LG) identified in each study is listed.

BTx642 (formerly called B35) is a stay-green genotype with a delay in the onset of leaf senescence under water-limited conditions. BTx642 is a BC1 line derived from a cross of IS12555, a Durra sorghum from Ethiopia, and BTx406, a short early flowering non stay-green line used in the Sorghum Conversion Program (Rosenow *et al.* 1983). BTx642 has been used as a source of stay-green in many breeding programs in the United States and Australia and for understanding the genetic basis of stay-green. Four major quantitative trait loci (QTL) for stay-green were identified using a recombinant inbred (RI) population developed from BTx642 (a stay-green line)/RTx7000 (a senescent line) (Xu *et al.* 2000). Two QTL X-Stg1 and X-Stg2 located on LG-03 explained 20% and 30% of the phenotypic variability, respectively (Xu *et al.* 2000; Sanchez *et al.* 2002). X-Stg3 and X-Stg4 are located on LG-02 and LG-05, respectively and account for ~16% and ~10% of the phenotypic variance (Sanchez *et al.* 2002). Thus, the ranking of stay-green QTL based on their contribution to the stay-green phenotype is X-Stg2>X-Stg1>X-Stg3>X-Stg4 (Xu *et al.* 2000).

A previous genetic map of BTx642/RTx7000 was constructed using a limited number of RFLPs (Xu *et al.* 2000; Subudhi *et al*. 2000). In the present study a higher resolution genetic map based on the RIL population derived from BTx642 and RTx7000 was constructed using AFLP and SSR markers. This high resolution genetic map was constructed in order to help trace the origin of regions of the BTx642 genome that carry favorable alleles for stay-green and to facilitate map-based cloning of genes involved in stay-green by cross referencing the map to the integrated Texas A&M University and the

USDA Agricultural Research Service (TAMU-ARS) sorghum genome map (Menz *et al.* 2002; Klein *et al.* 2000).

Results

SSRs and AFLPs were amplified from a population of 97 F7 RILs derived from a cross of BTx642/RTx7000. These assays identified 525 polymorphic markers that could be placed on a 1255.2cM genetic map that contains ten linkage groups (Figure 5.1, Appendix B). Forty-five AFLP primer pairs, consisting of 32 *Eco*RI/*Mse*I primers and 13 *Pst*I/*Mse*I primers, generated 461 AFLP markers useful for genetic map construction. Each primer combination amplified 3-23 polymorphic DNA bands (Table 5.2). Sixty-three SSR markers were also used for genetic map construction. The number of markers mapped on each linkage group ranged from 18-92 with LG-09 containing the fewest mapped markers (Table 5.3). RTx7000 has seeds red in color while BTx642 has seeds that are lemon yellow in color. Therefore, red pericarp color was assayed and the locus controlling this trait was mapped to LG-03 as previously reported (Xu *et al.* 2000; Figure 5.1 designated 'seed color' on LG-03). The resulting map contains 223 markers in common with the high-density genetic map contructed from a cross between IS3620C and BTx623 which has over 3,000 markers linked to a physical map (Menz *et al.* 2002).

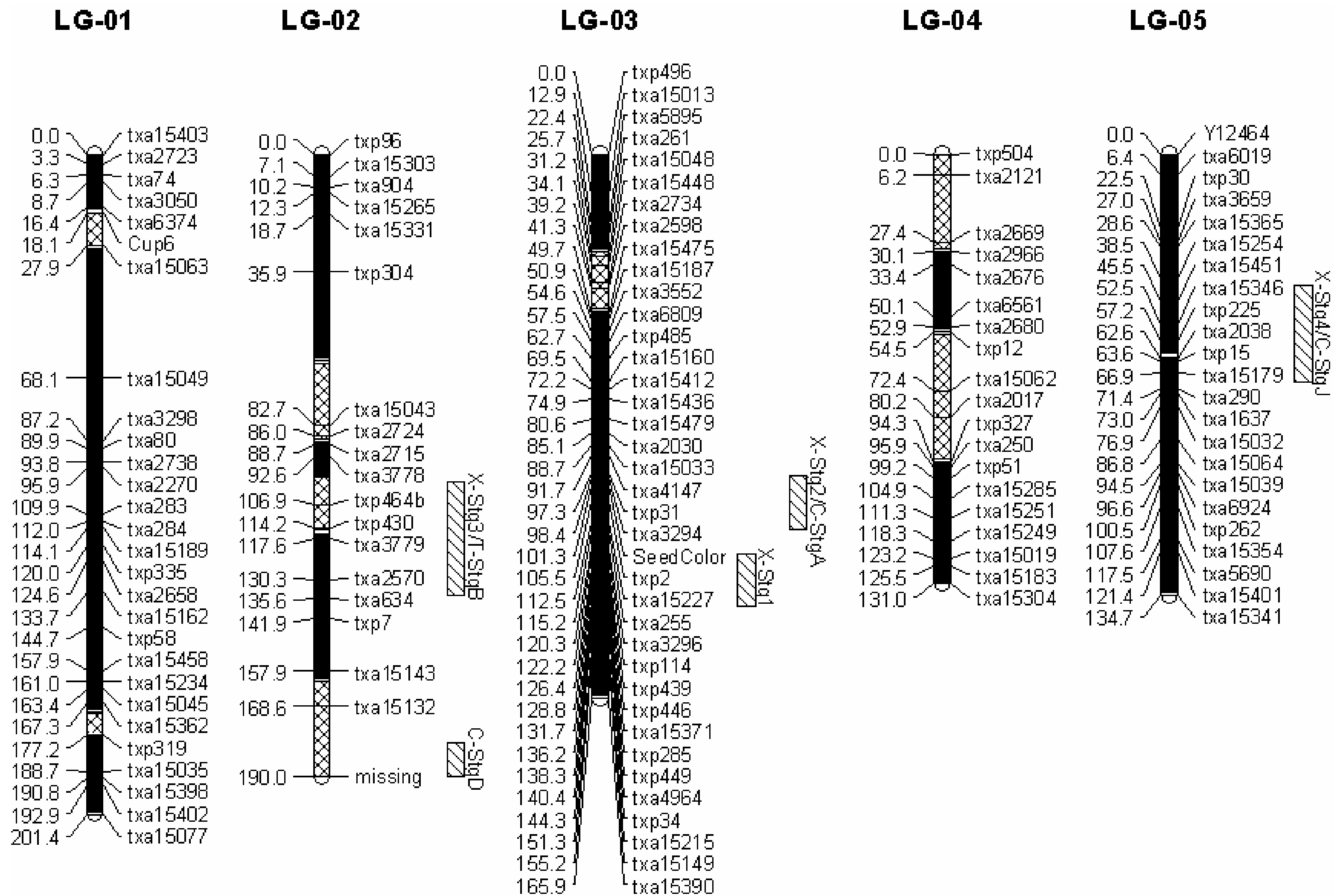

Figure 5.1 Genetic map based on a RIL population derived from BTx642/RTx7000.

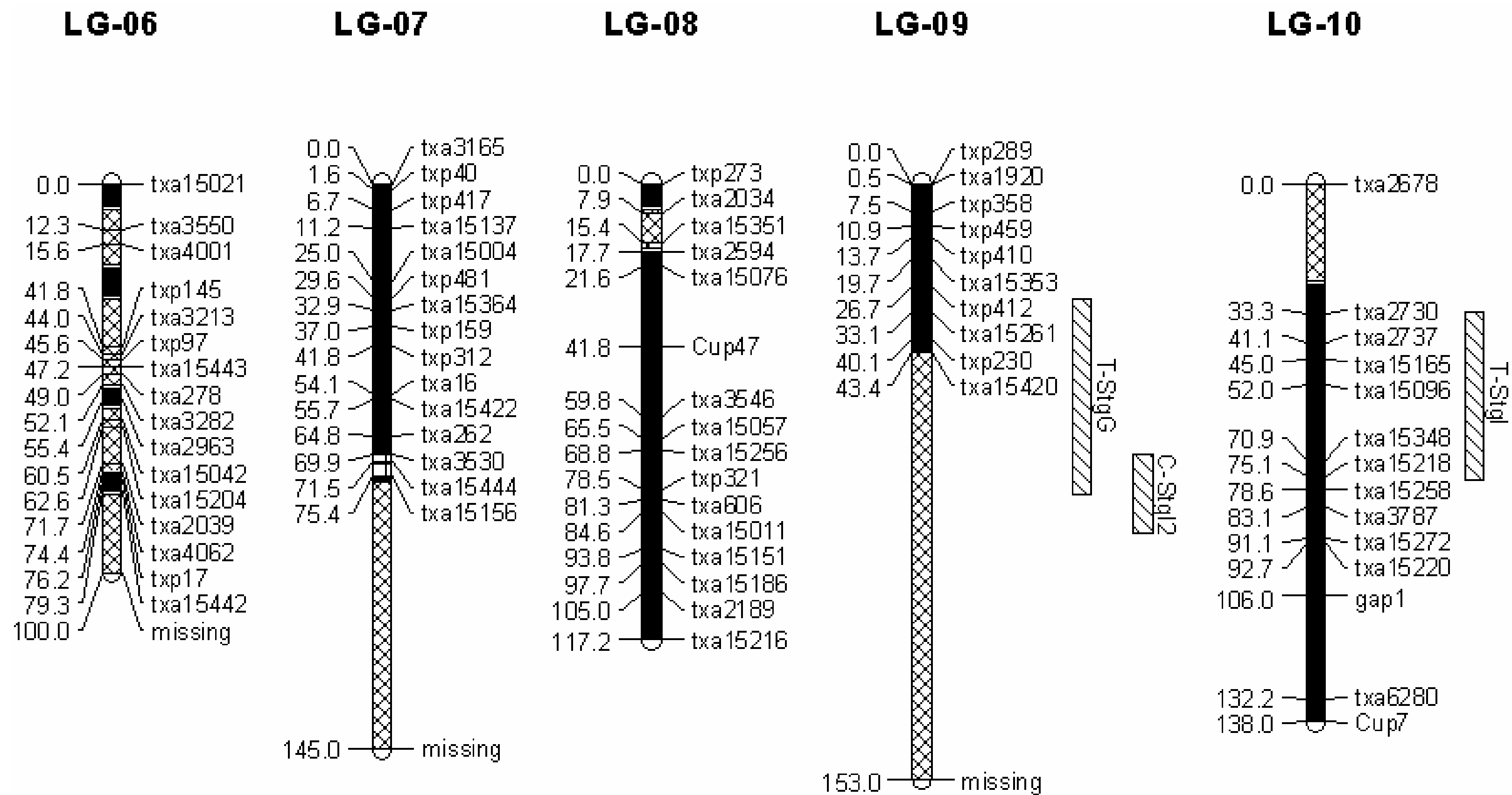

Figure 5.1, continued. Only the framework markers are shown in the figure. Black shading or hatch marks represents DNA derived from IS12555 or BTx406, respectively. Vertical and horizontal hatch marks on LG-07 represents DNA from some other source. Regions containing favorable alleles for stay-green (QTL) derived from BTx642 are shown to the right of each chromosome. Regions of the chromosome that are missing due to a lack of polymorphism between BTx642 and RTx7000 are designated “missing

Table 5.2. AFLP markers were amplified using 32 *Eco*RI/*Mse*I (E + three selective bases; M + three selective bases) and 13 *Pst*I/*Mse*I (P + three selective bases; M + three selective bases) selective-amplification primers revealing 461 AFLP markers useful for genetic map construction in a population derived from BTx642 and RTx7000.

Eco Selective	Mse Selective	# Markers
E-ACC	M-CAA	10
E-ACC	M-CAC	15
E-ACC	M-CAG	16
E-ACC	M-CAT	3
E-ACC	M-CGC	6
E-ACC	M-CTA	14
E-AGT	M-CAG	7
E-AGT	M-CAT	8
E-AGT	M-CCC	9
E-AGT	M-CCG	3
E-AGT	M-CGG	14
E-CAA	M-CAC	9
E-CAA	M-CAG	10
E-CAA	M-CAT	15
E-CAA	M-CCC	17
E-CAA	M-CTC	11
E-CTG	M-CAA	7
E-CTG	M-CTA	12
E-GAA	M-CAA	17
E-GAA	M-CCA	5
E-GAA	M-CGT	11
E-GGA	M-CAT	11
E-GGA	M-CCA	11
E-GGA	M-CGT	12
E-GGA	M-CTA	17
E-GGA	M-CTT	23
E-TAC	M-CAC	6
E-TAC	M-CAT	4
E-TAC	M-CTC	10
E-TGA	M-CAA	19
E-TGA	M-CAC	14
E-TGA	M-CTA	13
	Average	11.2

Pst Selective	Mse Selective	# Markers
P-AGA	M-CAC	3
P-AGA	M-CGG	7
P-CGT	M-CAA	8
P-CGT	M-CCG	6
P-CGT	M-CGT	12
P-CGT	M-CTA	7
P-CTC	M-CAA	9
P-CTC	M-CAC	5
P-CTC	M-CAT	7
P-CTC	M-CCG	4
P-CTC	M-CGG	9
P-CTC	M-CGT	11
P-CTC	M-CTA	14
	Average	7.8

Table 5.3 Information about the genetic map based on a RIL population derived from BTx642/RTx7000.

Linkage Group	# Total	SD	cM	IS3620C/BTx623 cM
LG-01	59	3	201.4	231.6
LG-02	74	5	168.6	205
LG-03	92	30	165.9	202.4
LG-04	49	2	131.3	174.4
LG-05	69	2	134.7	138.2
LG-06	40	2	79.3	115.6
LG-07	30	0	75.4	155.6
LG-08	45	5	117.2	152.3
LG-09	18	0	43.4	153
LG-10	49	41	138	148.4

The number of markers mapped to each linkage group (# Total), the number of markers showing segregation distortion (SD) on each linkage group, and the size of each linkage group is shown in cM. The column to the far right lists the length of each linkage group of the genetic map based on IS3620C/BTx623 (Menz *et al.* 2002).

The genetic map based on 97 RILs derived from BTx642 and RTx7000 spanned ~1255cM on 10 linkage groups (Figure 5.1,Table 5.3). Segregation distortion, the deviation ($P<0.05$) from the expected Mendelian 1:1 segregation ratio, was seen in marker allele ratios from all linkage groups except LG-07 and LG-09 (Table 5.3). In agreement with Xu *et al.* (2000), severe distortion occurred on LG-03 and LG-10 with 33% and 84% of markers, respectively, showing segregation distortion. LG-03 had a higher percentage of alleles from BTx642 in a 50cM region from txp31 to txa15113. A 59cM block was significantly skewed toward the male parent, RTx7000, on LG-10 between markers txa2730 and txa15220.

The 1255cM genetic map based on BTx642/RTx7000 constructed here is smaller than the 1713cM genetic map based on 137 RILs derived from BTx623/IS3620C (Menz *et al.* 2002). This difference could be due to the use of fewer RIL lines (97 vs. 137),

fewer DNA markers (525 vs. 2926) and/or regions not present in the BTx642/RTx7000 map due to regions of the parental lines that were genetically similar. LG-09 of the BTx642/RTx7000 map spans only ~54cM whereas LG-09 of the BTx623/IS3620C map spans ~153cM (Table 5.3). The basis of this difference was examined further using 120 DNA markers that mapped across LG-09 on the IS3620C/BTx623 genetic map (Menz *et al*. 2002). These DNA markers were used to search for potential polymorphisms in BTx642 and RTx7000. Nineteen of the markers mapping between 0-68cM on LG-09 were polymorphic between BTx642 and RTx7000. One additional polymorphic marker was found that mapped at 78cM but none of the markers mapping between 78cM and 153cM on the BTx623/IS3620C map were polymorphic in BTx642/RTx7000. This result suggested that BTx642 and RTx7000 contain related DNA in the region of their genome from ~68cM to 153cM of LG-09 (BTx623/IS3620C map coordinates), most likely due to a common parent in their pedigrees.

The origin of the region of LG-09 in BTx642 that was similar to RTx7000 was investigated further. The allelic state of 139 AFLPs on LG-09 was examined in the lines IS3620C, BTx623, BTx406, BTx642, RTx7000, and IS12555C (data derived in part from Menz *et al.* 2004). This analysis showed that BTx642 DNA on LG-09 from 0-45cM is nearly identical to IS12555 one of the parents of BTx642 (Table 5.4). In contrast, BTx642 DNA from ~54cM to the end of LG-09, is very similar to BTx406, the second parent used to create BTx642 (Table 5.4). BTx406 is a Kafir-Milo genotype similar to RTx7000, and both of these lines were developed in the U.S. This probably explains why there are few polymorphic differences between these genotypes in this

region of LG-09 and in several other regions of the BTx642/RTx7000 genetic map (regions marked 'missing' in Figure 5.1).

Table 5.4 Analysis of genetic similarity across LG-09 using AFLP and SSR markers among seven sorghum genotypes.

Marker	cM	IS2620C	BTx623	BTx406	BTx642	Tx7000	IS12555C	RTx430	Primer Combination
txa2315	6.6-23.5	1	0	0	0	0	0	0	E-GAA M-CTC
txa3463	6.6-23.5	0	1	0	0	1	0	1	E-CTG M-CCG
txa3464	6.6-23.5	1	0	0	0	0	0	0	E-CTG M-CCG
txa3616	6.6-23.5	1	0	0	0	0	0	0	E-TAC M-CAA
txa2757	24.1-30.2	1	0	0	1	0	1	0	E-ACC M-CAA
txa4125	24.1-30.2	ND							P-CGT M-CGC
txa3932	24.1-30.2	1	0	0	1	0	1	0	P-TAG M-CAG
txa3712	30.2-36.6	0	1	1	0	9	0	1	E-TAC M-CGA
txa6354	30.2-36.6	0	1	1	1	0	1	1	P-CTC M-CTT
txa2342	30.2-36.6	1	0	0	1	0	1	0	E-GAA M-CTT
txa4137	36.6	ND							P-CGT M-CTT
txa3845	39.4	0	1	1	0	1	0	1	E-CTG M-CGA
txa4213	39.4	0	1	0	0	0	0	0	P-TAG M-CTA
txa6214	41.7	0	1	0	0	0	0	0	P-CTC M-CGG
txa6132	41.7	1	0	1	0	1	0	1	P-CTC M-CCC
txa4026	43.6-45.5	1	0	1	1	1	1	1	P-TAG M-CCC
txa6344	43.6-45.5	1	0	1	0	0	0	0	P-CTC M-CTT
txa2946	43.6-45.5	1	0	0	0	0	0	1	E-ACC M-CTA
txa4100	43.6-45.5	1	0	0	0	0	0	0	P-TAG M-CTT
txa6342	43.6-45.5	1	0	0	0	0	0	0	P-CTC M-CTG
txa2326	45.5	0	1	1	1	1	1	1	E-GAA M-CTG
txa3526	47.8	0	1	0	0	0	0	0	E-CTG M-CGT
txa4184	50.1	0	1	1	1	0	1	1	P-TAG M-CGC
txa2816	50.1-52.5	0	1	1	1	1	1	1	E-ACC M-CCC
txa2542	54.4	0	1	0	0	1	1	1	E-GGA M-CGC
txa567	54.4	0	1	0	0	1	1	1	E-TGA M-CGC
txa2291	54.4-58.8	0	1	1	1	0	0	0	E-GAA M-CTA
txa6038	54.4-58.8	1	0	1	1	1	1	1	P-CTC M-CAA
txa2600		NS							E-GGA M-CTA
txa6038	54.4-58.8	1	0	1	1	1	1	1	P-CTC M-CAA
txa3160	62.8	1	0	0	0	1	0	0	E-AGT M-CAG
txa3160B	62.8	0	1	0	0	1	1	1	E-AGT M-CAG
txa2160	62.8-65.1	0	1	1	1	1	1	1	E-GAA M-CAG
txa2011	65.1	0	1	1	1	1	1	1	E-GGA M-CAG
txa3379	65.5	0	1	1	1	1	1	1	E-CTG M-CAA
txa3432	65.5-68.8	0	1	1	1	1	1	1	E-CTG M-CCA
txa2338	65.5-68.8	0	1	1	1	1	1	1	E-GAA M-CTG
txa2608	65.5-68.8	1	0	0	0	0	0	0	E-GGA M-CTA
txa2649	65.5-68.8	0	1	1	1	0	1	0	E-GGA M-CTG
txa3608	65.5-68.8	1	0	0	0	0	0	0	E-CTG M-CTT
txa4067	65.5-68.8	1	0	0	0	0	0	0	P-TAG M-CCT
txa6237	65.5-68.8	1	0	9	9	0	0	0	P-CTC M-CGT
txa254	65.5-68.8	1	0	0	0	0	0	0	E-TGA M-CAA
txa502	65.5-68.8	1	0	0	0	0	0	0	E-TGA M-CTT
txa3405	65.5-68.8	1	0	0	0	0	0	0	E-CTG M-CAG
txa4237	68.8-71.0	NS							P-TAG M-CGT

Table 5.4, continued

Marker	cM	IS2620C	BTx623	BTx406	BTx642	Tx7000	IS12555C	RTx430	Primer Combination
txa393	71	0	1	1	1	1	1	0	E-TGA M-CGA
txa2098	71.4	0	1	1	1	1	1	0	E-GGA M-CGA
txa2200	72.8-74.5	0	1	0	0	0	0	1	E-GAA M-CCA
txa2606	72.8-74.5	0	1	1	1	1	1	1	E-GGA M-CTA
txa3147	72.8-74.5	1	0	0	0	0	0	0	E-AGT M-CAC
txa3678	72.8-74.5	1	0	0	0	0	0	0	E-TAC M-CCC
txa3679	72.8-75.9	1	0	0	0	0	0	0	E-TAC M-CCC
txa2940	72.8-75.9	1	0	0	0	0	0	0	E-ACC M-CTA
txa3885	72.8-74.5	0	1	0	0	0	0	0	P-TAG M-CAC
txa3669	72.8-81.6	1	0	0	0	0	0	0	E-TAC M-CCC
txa3765	72.8-81.6	0	1	1	1	1	1	1	E-TAC M-CTA
txa2517	74.5	1	0	1	1	1	1	1	E-GGA M-CAC
txa2517B	74.5	0	1	0	0	0	0	0	E-GGA M-CAC
txa21	74.5	0	1	0	0	0	0	0	E-CAA M-CAC
txa3716	74.5	1	0	0	0	0	0	1	E-TAC M-CGC
txa3210	74.5-75.9	0	1	1	1	1	1	1	E-AGT M-CCC
txa3458	74.5-75.9	0	1	1	1	1	1	1	E-CTG M-CCG
txa3512	74.5-75.9	0	1	1	1	1	1	1	E-CTG M-CGT
txa6048	74.5-75.9	0	1	1	1	1	1	1	P-CTC M-CAC
txa2755	74.5-75.9	0	1	1	1	1	0	1	E-ACC M-CAA
txa2014	74.5-75.9	0	1	1	1	1	0	1	E-GGA M-CAG
txa3759	74.5-75.9	0	1	1	1	1	0	1	E-TAC M-CTA
txa3868	74.5-75.9	0	1	1	1	1	0	1	P-TAG M-CAA
txa3788	74.5-81.6	0	1	0	0	0	0	0	E-TAC M-CTC
txa184	74.5-75.9	0	1	1	1	1	1	1	E-CAA M-CTA
txa2063	75.9	0	1	0	0	0	0	0	E-GGA M-CCG
txa2316	75.9-76.3	0	1	1	1	1	0	1	E-GAA M-CTC
txa3380	76.3	0	1	0	0	0	1	1	E-CTG M-CAA
txa3315	76.3	0	1	0	0	0	0	0	E-AGT M-CGT
txa3733	76.7	0	1	1	1	1	1	1	E-TAC M-CGG
txa282	76.7-77.5	0	1	0	0	9	1	0	E-TGA M-CAC
txa2528	76.7-78.5	0	1	0	0	0	1	0	E-GGA M-CAC
txa447	76.7-81.6	0	1	0	0	0	0	0	E-CAA M-CGG
txa2731	76.7-78.5	0	1	1	1	0	0	1	E-ACC M-CAG
txa2933	77.5	0	1	0	0	0	0	1	E-ACC M-CGT
txa3338	77.5-78.5	0	1	1	1	1	1	1	E-AGT M-CTC
txa2535	77.5-81.6	1	0	0	0	0	0	0	E-GGA M-CGC
txa2881	77.5-78.5	0	1	0	0	0	0	1	E-ACC M-CGA
txa3807	77.5-78.5	0	1	0	0	0	0	9	E-TAC M-CTG
txa3983	77.5-78.5	1	0	0	0	0	0	0	P-TAG M-CCA
txa4206	77.5-78.5	1	0	1	1	1	0	1	P-TAG M-CTA
txa363	77.5-78.5	0	1	1	1	1	1	1	E-TGA M-CCC
txa3312	77.5-78.5	1	0	0	0	0	0	0	E-AGT M-CGT
txa3465	77.5-78.5	1	0	0	0	0	0	0	E-CTG M-CCG
txa2541	77.5-78.5	1	0	0	0	0	0	0	E-GGA M-CGC
txa2585	78.5-81.6	1	0	0	0	0	0	1	E-GGA M-CGT
txa3247	78.5-81.6	1	0	0	0	0	0	0	E-AGT M-CCT
txa6357	78.5-81.6	1	0	0	0	0	0	0	P-AGA M-CGG
txa603	78.5-81.6	1	0	0	0	0	0	0	E-TGA M-CTA
txa6275	81.6-85.0	1	0	0	0	0	0	0	P-CTC M-CTA
txa3869	81.6-85.0	0	1	1	1	1	1	1	P-TAG M-CAA

Table 5.4, continued

Marker	cM	IS2620C	BTx623	BTx406	BTx642	Tx7000	IS12555C	RTx430	Primer Combination
txa4113	85	0	1	0	0	0	0	1	P-TAG M-CTT
txa2932	85.0-87.7	0	1	1	1	1	1	1	E-ACC M-CGT
txa2553	87.7-99.4	1	0	0	0	0	0	0	E-GGA M-CGG
txa236	87.7-91.7	1	0	0	0	0	0	0	E-CAA M-CTT
txa6308	91.7	0	1	1	1	1	1	0	P-AGA M-CTG
txa3354	91.7-94.0	0	1	1	1	1	1	0	E-AGT M-CTT
txa2599	94	0	1	1	1	1	1	0	E-GGA M-CTA
txa3996	94.0-95.5	1	0	0	0	0	0	0	P-TAG M-CCA
txa2295	94.0-95.5	1	0	0	0	0	9	1	E-GAA M-CTC
txa509	95.5	0	1	1	1	1	0	0	E-TGA M-CCT
txa6329	95.5-96.1	0	1	1	1	1	1	0	P-CTC M-CTG
txa66	96.1	0	1	1	1	1	1	1	E-CAA M-CCA
txa2589	96.1-97.1	0	1	1	1	1	1	1	E-GGA M-CGT
txa590	96.7	0	1	1	1	1	1	1	E-TGA M-CGT
txa2135	97.1	0	1	0	0	0	0	1	E-GAA M-CAA
txa415	99.4	0	1	0	0	0	0	1	E-CAA M-CCT
txa2351	101.7	1	0	0	0	0	0	0	E-GAA M-CTT
txa2782	101.7-105.	1	0	0	0	0	0	0	E-ACC M-CAT
txa2289	101.7-109.	1	0	0	0	0	0	0	E-GAA M-CTA
txa547	105.3	1	0	0	0	0	0	0	E-TGA M-CTC
txa411	106.1	1	0	0	0	0	0	0	E-CAA M-CCT
txa3445	106.1-109.	1	0	0	0	0	0	1	E-CTG M-CCC
txa3693	106.1-109.	0	1	1	1	1	1	0	E-TAC M-CCG
txa542	106.1-109.	1	0	0	0	0	0	0	E-TGA M-CGG
txa553	106.1-109.	1	0	0	0	0	0	0	E-TGA M-CTC
txa3772	109.7	1	0	0	0	0	0	0	E-TAC M-CTC
txa3772B	109.7	0	1	1	1	1	0	1	E-TAC M-CTC
txa3014	109.7	1	0	0	0	0	0	0	E-ACC M-CTG
txa3012	109.7-109.	0	1	1	1	1	1	1	E-ACC M-CTG
txa6224	109.7-114.	0	1	1	1	1	0	0	P-AGA N-CGC
txa6343	109.7-114.	0	1	1	1	1	1	1	P-CTC M-CTT
txa6028	118.4-129.	0	1	1	1	1	1	1	P-CTC M-CAA
txa6058	118.4-129.	0	1	1	1	1	1	1	P-AGA M-CAG
txa3121	129.2-143.	NS							E-AGT M-CAA
txa562	129.2-143.	0	1	1	1	1	1	1	E-TGA M-CGC
txa2673	143.1-153.	1	0	0	0	0	0	0	E-GGA M-CTT
txa2807	143.1-153.	1	0	0	0	0	0	1	E-ACC M-CCA
txa3233	143.1-153.	1	0	9	0	0	0	9	E-AGT M-CCT
txa3593	143.1-153.	0	1	1	1	1	1	1	E-CTG M-CTG
txa6075	143.1-153.	1	0	0	0	0	0	0	P-CTC M-CAG
txa2228	off-end	0	1	1	1	1	1	1	E-GAA M-CCT

The analysis shows that BTx642 is similar to IS12555 (light blue shading) for the first ~45cM on LG-09, whereas from ~54.4cM to the end of LG-09, BTx642 is similar to BTx406 (pink shading). For AFLP markers (denoted with a "txa" prefix), 1's and 0's represent the presence or absence of a band, respectively, and 9's represent missing data. For SSR markers, numbers common between genotypes indicate that the same allele was observed. NS are markers that could not be scored and ND represents those markers not amplified in the diversity study of Menz *et al.* (2004).

The genetic relationship between regions of BTx642, BTx406, and RTx7000 were further analyzed in the following way. Twenty-two AFLP markers that mapped from 0-52cM on LG-09 were scored across the lines IS3620C, BTx623, BTx642, RTx7000, and IS12555C. A similarity matrix was created using DICE's coefficient of genetic similarity and a dendrogram was created with the unweighted pair group method with arithmetic average (UPGMA). Branches were supported by 300 bootstrap replications. As displayed in Figure 5.2, RTx7000 and BTx406 show 66.8% similarity from 0-52cM on LG-09. To examine the rest of the chromosome of LG-09, 110 *Eco*RI/*Mse*I and *Pst*I/*Mse*I markers were examined that map to LG-09 from 54cM to 153cM (the arm of LG-09 missing on the BTx642/RTx7000 map) relative to the IS3620C/BTx623 map. Figure 5.3 shows that RTx7000 and BTx406 are 94% similar to one another on LG-09 from 54cM to the end of the chromosome.

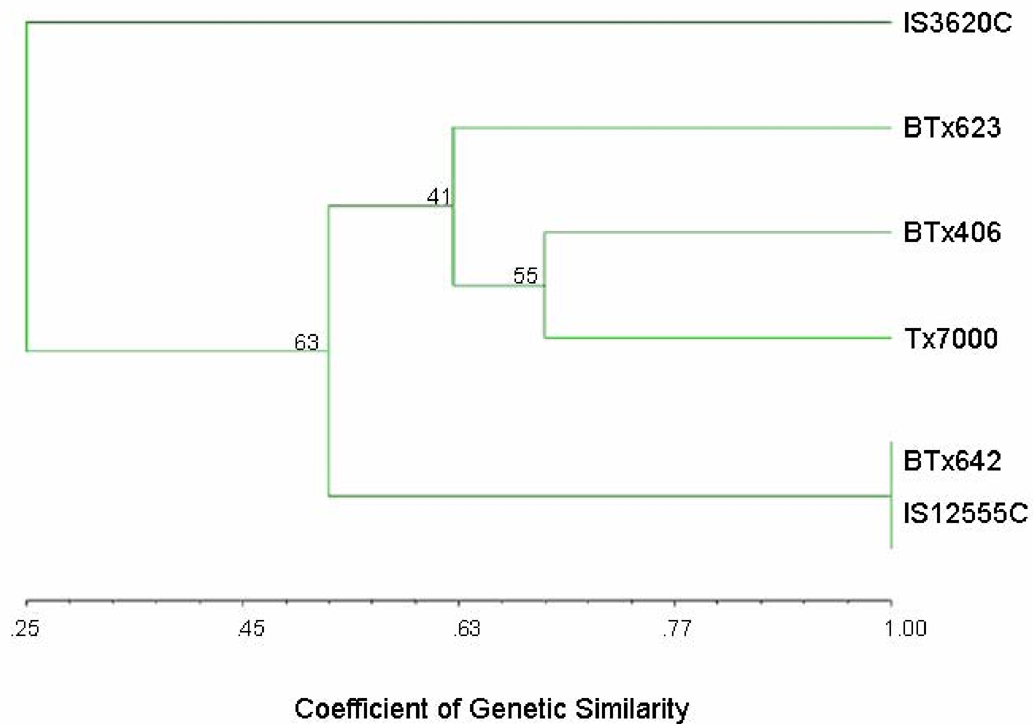

Figure 5.2 Genetic similarities of IS3620C, BTx623, BTx406, BTx642, RTx7000, and IS12555C from 0cM to 52cM on LG-09. DICE's coefficient of genetic similarity was used to create the similarity matrix and UPGMA clustering was used to build the dendrogram. 300 bootstrap repetitions were done to show the support of each branch. Twenty-two *Eco*RI/*Mse*I and *Pst*I/*Mse*I markers were used for this analysis.

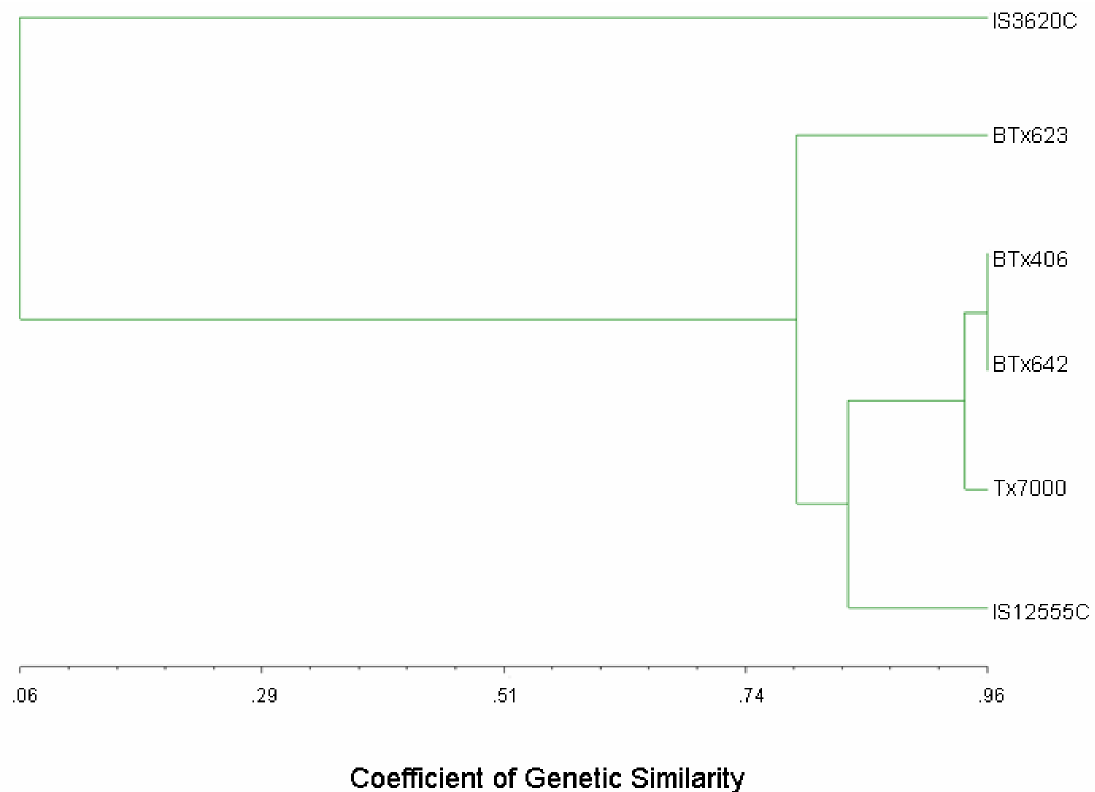

Figure 5.3 Genetic similarity analyses of IS3620C, BTx623, BTx406, BTx642, RTx7000, and IS12555C from 54cM to the end of LG-09 using DICE's coefficient of genetic similarity to create a matrix and UPGMA clustering to build the dendrogram. 300 bootstrap repetitions were done to show the support of each branch. 110 *Eco*RI/*Mse*I and *Pst*1/*Mse*I markers were used for this analysis.

Comparison of the size (cM) of linkage groups in the BTx642/RTx7000 map and the BTx623/IS3620C map showed that most of the linkage groups in the BTx642 map were smaller by 5% to 20% compared to linkage groups of the IS3620C/BTx623 map (Table 5.3). The exceptions to this general trend included LG-09 as discussed above and LG-07 that was 50% smaller than its counterpart in the BTx623/IS3620C genetic map. Analysis of DNA markers shared by the two linkage maps from LG-07 showed that the BTx642/RTx7000 based map could be aligned to the BTx623/IS3620C map from 0-

75cM but the remaining portion of this linkage group (75-159cM) was missing from the BTx642/RTx7000 map. Examination of alleles generated by DNA markers across this interval showed that BTx642 and RTx7000 had the same alleles suggesting that this region was derived from a similar source of DNA.

The extended regions of low genetic diversity in the BTx642/RTx7000 map were most likely due to the fact that BTx642 is a BC1 derived line from a cross of IS12555 and BTx406 and the fact that BTx406 is derived from the same group of Kafir-Milo lines as RTx7000. This finding indicates that favorable alleles for stay-green mapped in this population could be derived from IS12555 or from BTx406. Therefore, the origins of all regions of the BTx642/RTx7000 map were traced back to either BTx406 or IS12555C by determining if alleles of DNA markers spaced across the map were more similar to BTx406 or IS12555C (Menz *et al.* 2004). As expected for a BC1 derived line, large regions of BTx642 were derived from each parent (Figure 5.1, regions derived from IS12555C are shown in solid black, regions from BTx406 are hatched, missing regions of the map derived from BTx406 are designated 'missing'). The location of stay-green QTL mapped using BTx642 are also shown in this figure to the right of each linkage group. The data in Figure 5.1 indicate that X-Stg1, X-Stg2, X-Stg4, and T-StgI are derived from IS12555 whereas C-StgD and C-StgI2 are derived from BTx406. X-Stg3/T-StgB and T-StgG QTL regions encompass DNA from both IS12555 and BTx406. This topic will be investigated and discussed further in the next chapter.

Discussion

The genetic map based on BTx642/RTx7000 constructed in this study spanned 1255.2 cM, which is about 1.5 times larger than the previous map constructed using the same population (Xu *et al*. 2000). The increase in map length is likely due in part to the use of about four times more DNA markers in this study compared to the previous study (Xu *et al.* 2000). Significant segregation distortion was observed in the BTx642/RTx7000 genetic map. Segregation distortion has been commonly seen in mapping population of crops, forest trees, and fruit trees (Zhang *et al*. 2004). Although segregation distortion can be explained by sampling errors or an excess of heterozygotes, the distortion on LG-03 and LG-10 of the BTx642/RTx7000 map is so severe and extensive that genetic factors are likely involved. The preference of one parental allele can be due to compatibility, gamete or zygote viability, or general fitness such as resistance to disease (Menz *et al*. 2002). For example, in poplar, a recessive lethal allele affecting embryonic development was tightly linked to markers showing segregation distortion (Zhang *et al.* 2004). Darrell Rosenow, the creator of the BTx642/RTx7000 population, noted that some lines of this population flowered anonymously and those lines were discarded (D. Rosenow, personal communication). It is possible that the genes for this flowering phenotype may be located in the areas of segregation distortion.

Most of the linkage groups in the BTx642/RTx7000 map were smaller by 5% to 20% compared to linkage groups of a genetic map based on BTx623/IS3620C. The exceptions to this general trend included LG-09 and LG-07 that were much smaller than their counterparts in the BTx623 map. Analysis of DNA markers shared by the two

linkage maps of LG-07 showed that the BTx642/RTx7000 based map could be aligned to the BTx623/IS3620C map from 0-75cM but the remaining portion of this linkage group (75-159cM) was missing from the BTx642/RTx7000 map. Examination of alleles generated by DNA markers across this interval showed that BTx642 and RTx7000 had the same alleles suggesting that this region was derived from a similar source of DNA. Analysis of LG-09 revealed a similar situation where lack of allelic variation between the parental genomes across the lower portion of this chromosome resulted in the truncation of LG-09 in BTx642/RTx7000 relative to the map based on BTx623/IS3620C. The lack of allelic variation was attributed to the inheritance of these regions of BTx642 from BTx406, one of the parents of BTx642. BTx406 is derived from Kafir-Milo genotypes similar to those used to create RTx7000. Therefore, regions of BTx642 derived from BTx406 are expected to have little or no genetic variation relative to RTx7000 and this can account for the ‘missing’ regions of the genetic map based on this cross.

CHAPTER VI

COMPLEXITY, ORIGIN AND UTILIZATION OF FAVORABLE ALLELES FOR STAY-GREEN IN SORGHUM

Introduction

Eight main sources of stay-green have been identified in the sorghum germplasm collection. Two sources, BTx642 and KS19, have been used extensively in the United States and Australian sorghum breeding programs. BTx642 was selected from a converted version of IS12555, an Ethiopian landrace (Rosenow *et al.* 1983) and KS19 was selected from a cross of Short Kaura, an improved landrace from Nigeria, with Combine Kafir 60 (Henzell *et al.* 1984). The genetic basis of the stay-green trait has been investigated in six studies using seven populations and four genotypes that confer stay-green, BTx642, QL41, SC56, and E36-1 (Xu *et al.* 2000; Crasta *et al.* 1999; Tao *et al.* 2000; Kebede *et al.* 2001; Haussmann *et al.* 2002; Tuinistra *et al.* 1997). Four major stay-green QTL, X-Stg1-4, were identified through analysis of a RIL population derived from BTx642 (stay-green) and RTx7000 (senescent) (Xu *et al.* 2000). X-Stg1 and X-Stg2 located on LG-03 explained 20% and 30% of phenotypic variability, respectively and X-Stg3 and X-Stg4 accounted for ~16% and ~10% of phenotypic variance (Sanchez *et al.* 2002). A second stay-green QTL mapping study utilized a population derived from BTx642 and RTx430 to map seven QTL for stay-green (Crasta *et al.* 1999). However, only two of these QTL corresponded to QTL mapped by Xu *et al.* (2000). Tao *et al*. (2000) mapped five stay-green QTL in a cross of QL41, a line derived from BTx642 and QL39 (non stay-green). Only one of the five stay-green QTL identified

corresponded to X-Stg1-4 mapped by Xu *et al.* (2000). The lack of correspondence among stay-green QTL mapping studies has not been fully explained and is the subject of the studies described in this chapter. The resolution of this question is relevant to the utilization of favorable alleles for stay-green in sorghum breeding programs, therefore this topic will also be addressed here.

Results

Analysis of stay-green QTL identified in prior studies

Stay-green QTL have been identified in seven populations using four different genotypes that express the stay-green trait, BTx642, QL41, SC56, and E36-1 (Xu *et al.* 2000; Crasta *et al.* 1999; Tao *et al.* 2000; Kebede *et al.* 2001; Haussmann *et al.* 2002; Tuinistra *et al.* 1997). In these studies, a total of 39 QTL were identified that modulate the expression of stay-green under water limiting conditions (LOD scores>2) (Table 6.1). Five of the stay-green loci identified in these studies overlap with QTL that modified flowering time in the same population (C-StgG, H2-Stg3, K-StgG, K-StgF, H1-Stg4). These loci were eliminated from further consideration because of the confounding impact of flowering time on the expression of the stay-green trait (Rosenow and Clark 1995).

Table 6.1 List of thirty-nine QTL (LOD >2) identified in five studies (Xu *et al.* 2000; Crasta *et al.* 1999; Tao *et al.* 2000; Kebede *et al*. 2001; Haussmann *et al*. 2002) that modulate expression of stay-green under water limiting conditions.

#	Study-QTL	parental genotypes	Linkage group (cM)	FA origin	Evidence	LOD, % variance
	Xu, 2000					
1	X-Stg3	BTx642/Tx7000	LG-02:97-125	BTx642	3/5E	LOD=4.5, ~19%
2	X-Stg2	BTx642/Tx7000	LG-03:104-125	BTx642	5/5E	LOD=6.3, ~30%
3	X-Stg1	BTx642/Tx7000	LG-03:138-165	BTx642	5/5E	LOD=3.3, ~16%
4	X-Stg4	BTx642/Tx7000	LG-05:45-60	BTx642	2/5E	LOD=2.2, ~11%
	Crasta, 1999					
5	C-StgG	BTx642/RTx430	LG-01:30-60	BTx642	3/4E	LOD=5.8, ~25%
6	C-StgD	BTx642/RTx430	LG-02:190-200	BTx642	3/4E	LOD=5.0, ~22%
7	C-StgA	BTx642/RTx430	LG-03:104-130	BTx642	4/4E	LOD=6.6, ~28%
8	C-StgJ	BTx642/RTx430	LG-05:50-70	BTx642	1/4E	LOD=2.3, ~12%
9	C-StgI1	BTx642/RTx430	LG-09:0-20	RTx430	2/4E	LOD=3.8, ~17%
10	C-StgI2	BTx642/RTx430	LG-09:70-90	BTx642	3/4E	LOD=2.9, ~14%
11	C-StgB	BTx642/RTx430	LG-10:125-end	RTx430	2/4E	LOD=3.0, ~14%
	Tao, 2000					
12	T-StgC	QL41/QL39	LG-01:100-135	QL39	1/5E	LOD=3.1, ~13%
13	T-StgB	QL41/QL39	LG-02:114-140	QL41	3/5E	LOD=3.5, ~14%
14	T-StgA	QL41/QL39	LG-03:30-60	QL39	2/5E	LOD=3.5, ~15%
15	T-StgG	QL41/QL39	LG-09:30-80	QL41	1/5E	LOD=2.5, ~11%
16	T-StgI	QL41/QL39	LG-10:30-65	QL41	3/5E	LOD=2.6, ~11%
	Kebede, 2001					
17	K-StgG	SC56/Tx7000	LG-01:70-80	SC56	4/5E	LOD=2.9, ~11%
18	K-StgA	SC56/Tx7000	LG-03:95-120	SC56	1/4E	LOD=2.6, ~10%
19	K-StgJ	SC56/Tx7000	LG-05:53-70	SC56	2/4E	LOD=4.0, ~15%
20	K-StgF	SC56/Tx7000	LG-06:25-40	Tx7000	1/4E	LOD=2.8, ~11%
21	K-StgE	SC56/Tx7000	LG-07:60-80	SC56	1/4E	LOD=3.5, ~13%
	Haussmann, 2002					
22	H1-Stg6	E36-1/IS9830	LG-01:70-80	E36-1	1/1E	GL15(<2), GL30(2.6), GL45(2.9)
23	H1-Stg7	E36-1/IS9830	LG-01:140-150	E36-1	1/1E	GL15(<2), GL30<2), GL45(4.5)
24	H1-Stg1	E36-1/IS9830	LG-03:134-149	IS9830	1/1E	GL15 (14.9), GL30(6.4), GL45 (<2)
25	H1-Stg2	E36-1/IS9830	LG-04:104-114	IS9830	1/1E	GL15 (3.1), GL30(2.8), GL45(2.6)
26	H1-Stg8	E36-1/IS9830	LG-05:65-85	IS9830	1/1E	GL15(<2), GL30(2.6), GL45(2.6)
27	H1-Stg9	E36-1/IS9830	LG-05:126-136	IS9830	1/1E	GL15(<2), GL30(2.6), GL45(3.3)
28	H1-Stg3	E36-1/IS9830	LG-07:65-75	E36-1	1/1E	GL15 (2.6), GL30(<2), GL45(<2)
29	H1-Stg4	E36-1/IS9830	LG-10:68-78	E36-1	1/1E	GL15(3.5). GL30(2.9, GL45(<2)
30	H1-Stg5	E36-1/IS9830	LG-10:120-133	E36-1	1/1E	GL15(3.3), GL30(2.9), GL45(3.0)
	Haussmann, 2002					
31	H2-Stg2	E36-1/N13	LG-01:23-35	E36-1	1/1E	GL15(12), GL30(6.2), GL45(4)
32	H2-Stg3	E36-1/N13	LG-01:35-65	E36-1	1/1E	GL15(<2), GL30(2.9), GL45(4.1)
33	H2-Stg4	E36-1/N13	LG-01:93-108	E36-1	1/1E	GL15(<2), GL30(4.8), GL45(<2)
34	H2-Stg1	E36-1/N13	LG-01:195-219	E36-1	1/1E	GL15(3.1), GL30(3.0), GL45(3.0)
35	H2-Stg5	E36-1/N13	LG-02:114-148	E36-1	1/1E	GL15(2.5), GL30(3), GL45(4.9)
36	H2-Stg6	E36-1/N13	LG-03:108-133	E36-1	1/1E	GL15(<2), GL30(<2), GL45(2.8)
37	H2-Stg7	E36-1/N13	LG-07:37-87	E36-1	1/1E	GL15(6.8), GL30(7.5), GL45(7.6)
38	H2-Stg8	E36-1/N13	LG-07:139-156(end)	N13	1/1E	GL15(2.8), GL30(3.4), GL45(2.9)
39	H2-Stg9	E36-1/N13	LG-10:130-145(end)	E36-1	1/1E	GL15(<2), GL30(2.7), GL45(2.6)

Each stay-green QTL is designated by the first letter of the first author's last name followed by the QTL name given in that study. The location of each QTL listed is based on map coordinates from the IS3620C/BTx623 genetic map (Menz *et al.* 2002). The parental genotype contributing favorable alleles (FA) for stay-green is listed, as well as the number of environments where the stay-green trait was observed and the number of environments tested (column marked 'evidence'; # of environments where stay-green was expressed/# environments studied). The LOD score and percent variance explained by each QTL is also listed as well as significant differences in green leaf area (GL) at 15, 30, and 45 days post-anthesis reported by Haussmann *et al.* (2002).

To identify QTL that are common among the seven populations and studies, all of the previously mapped QTL were located on the TAMU-ARS genome map using DNA markers common to the genetic maps (Table 6.1). Twelve regions of the genome containing stay-green QTL were identified in more than one trial and further analysis was focused on this subset of the stay-green QTL (Table 6.2). Favorable alleles for stay-green from more than one genotype were mapped in the same region of the sorghum genome in several cases (Table 6.2 regions 1, 2, 5, 7, 8, 12). This could be due to DNA of common descent present in different genotypes, different alleles of the same gene for stay-green, or different genes that modulate stay-green within the same locus.

Origin of favorable alleles for stay-green derived from BTx642

BTx642 was the origin of favorable alleles for stay-green in six of the stay-green QTL listed in Table 6.2. BTx642 is a BC1 derived line from a cross of BTx406, a non stay-green genotype and IS12555, a stay-green landrace. Therefore, as described in Chapter V, different portions of the BTx642 genome originate from either BTx406 or IS12555. The origin of DNA conferring favorable stay-green alleles for the stay-green QTL derived from BTx642 was analyzed using six to thirteen SSRs that map across each of these QTL (Table 6.3). This analysis showed that BTx642 DNA spanning X-Stg1, X-Stg2, and X-Stg4 is derived from IS12555 whereas X-Stg3 contains DNA from both parents. A similar analysis of other stay-green QTL showed that the favorable allele(s) for stay-green corresponding to C-StgD and C-StgI2 are derived from BTx406 and that DNA spanning T-StgG contains DNA from both BTx406 and IS12555 DNA.

Table 6.2 Twelve regions of the sorghum genome that contain stay-green QTL identified in studies spanning more than one year, location, or population.

No.	Stg QTL	Parental Genotypes	Coordinates (cM)	FA origin
1	T-StgC	QL41/QL39	LG-01:100-135	QL39
1	H2-Stg4	E36-1/N13	LG-01:93-108	E36-1
2	X-Stg3	BTx642/RTx7000	LG-02:97-125	BTx642 (both)
2	T-StgB	QL41/QL39	LG-02:114-140	QL41 (IS12555)
2	H2- Stg5	E36-1/N13	LG-02:114-148	E36-1
3	C-StgD	BTx642/RTx430	LG-02:190-200	BTx642 (Tx406)
4	T-StgA	QL41/QL39	LG-03:30-60	QL39
5	X-Stg2	BTx642/RTx7000	LG-03:104-125	BTx642 (IS12555)
5	C-StgA	BTx642/RTx430	LG-03:104-130	BTx642 (IS12555)
5	K-StgA	SC56/RTx7000	LG-03:95-120	SC56
5	H2-Stg6	E36-1/N13	LG-03:108-133	E36-1
6	X-Stg1	BTx642/RTx7000	LG-03:138-165	BTx642 (IS12555)
7	X-Stg4	BTx642/RTx7000	LG-05:45-60	BTx642 (IS12555)
7	C-StgJ	BTx642/RTx430	LG-05:50-70	BTx642 (IS12555)
7	K-StgJ	SC56/RTx7000	LG-05:53-70	SC56
7	H1-Stg8	E36-1/IS9830	LG-05:65-85	IS9830
8	K-StgE	SC56/RTx7000	LG-07:60-80	SC56
8	H2-Stg7	E36-1/N13	LG-07:37-87	E36-1
8	H1-Stg3	E36-1/IS9830	LG-07:65-75	E36-1
9	C-StgI1	BTx642/RTx430	LG-09:0-20	RTx430
10	C-StgI2	BTx642/RTx430	LG-09:70-90	BTx642 (BTx406)
10	T-StgG	QL41/QL39	LG-09:40-80	QL41 (BTx406)
11	T-StgI	QL41/QL39	LG-10:30-65	QL41 (IS12555)
12	C-StgB	BTx642/RTx430	LG-10:125-end	RTx430
12	H1-Stg5	E36-1/IS9830	LG-10:120-133	E36-1
12	H2-Stg9	E36-1/N13	LG-10:130-end	E36-1

Linkage group coordinates are based on the IS3620C/BTx623 genetic map (Menz *et al.* 2002).The genotypic origin of favorable alleles (FA) for stay-green is listed. Genotypes listed in parenthesis are the parents in which the favorable allele was inherited. ‘Both’ refers that this QTL contains DNA from both IS12555 and Tx406.

Table 6.3 Genotypes of BTx642 and its parents IS12555 and BTx406 in the DNA regions spanning X-Stg1, X-Stg2, X-Stg3, T-StgG/C-StgI2, and C-StgD. Numbers designate allele types of a given SSR where numbers common between two genotypes indicates that the same allele was observed. Green shading represents DNA from IS12555 and pink shading represents DNA from BTx406.

X-Stg1 region

Marker	LG-03, cM	IS12555C	BTx406	BTx642
Corn031	140-142	3	3	3
txp439	145-147	2	4	2
txp440	145-147	1	4	1
txp542	147-149	3	3	3
txp446	151-156	10	12	10
txp442	151-156	1	3	1
txp447	156-158	1	4	1
txp448	158-161	3	2	3
txp285	158-161	5	3	5,9
txp38	158-161	5	1	5
txp474	158-161	7	3	7
txp421	158-161	1	4	1
txp420	161-165	5	2,5	5

X-Stg4 region

Marker	LG-05, cM	IS12555C	BTx406	BTx642
txi18	34-35	1	1	1
txp303	35-41	1	3	1
TS404N	53-56	4	3	4
txp225	56.9	3	4	3
txp15	58.2-61	4	3	4
TS492	58-61	9	6	9
SDB064	61-68	1	2	1

X-Stg2 region

Marker	LG-03, cM	IS12555C	BTx406	BTx642
txp543	98-102	2	1	2
txp544	102-104	5	2	5
txp435	110-111	2	2	2
txp120	111	4	4	4
txp545	111.6	1	2	1
txp546	112	2	2	2
txp2	111-118	2	1	2
txp530	111-118	3	2	3
txp436	118-125	1	1	1

T-StgG/C-StgI2 region

Marker	LG-09, cM	IS12555C	BTx406	BTx642
SDB053	43-45	15	7	15
txp258	43.4-45.5	6	6	6
txp287	43.6	2	2	2
txp67	52.5	7	7	7
CS002	65-68	1	4	4
txp10	81-85	3	6	6

X-Stg3 region

Marker	LG-02, cM	IS12555C	BTx406	BTx642
txp464	103-108	2	3	3
txp445	110-113	4	1	1
TS214	110-113	1	1	1
Cup29	110-113	4	3	3
txp430	113-114	3	2	2
txp1	114-118	1	8	8
txp56	118.5	3	1	3
gap84	125-126	1	3	1
SDB049	129-131	4	4	4
txp179	131-134	1	1	1
txp348	137-145	11	22	11

C-StgD region

Marker	LG-02, cM	IS12555C	BTx406	BTx642
Cup40	189-193	1	2	2
Corn033	189-193	1	1	1
CS033	189-193	3	1	1
TS451	193-196	11	6	4
Cup69	202-205	2	1	1
CS056	202-205	2	4	4

Comparison of stay-green QTL mapped in BTx642/RTx7000 and BTx642/RTx430

Four QTL for stay-green were identified using a population derived from BTx642/RTx7000 (Xu *et al*. 2000) and six QTL for stay-green were identified in a population derived from BTx642/RTx430 (Crasta *et al.* 1999). However, only two of the stay-green QTL identified in these studies mapped to the same interval of the sorghum genetic map (Table 6.4, X-Stg2 and C-StgA, X-Stg4 and C-StgJ).

Table 6.4 Comparison of stay-green QTL mapped in BTx642/RTx7000 and BTx642/RTx430.

BTx642/RTx7000		BTx642/RTx430	
Stg QTL	Origin of FA	Stg QTL	Origin of FA
X-Stg1	IS12555	not mapped	
X-Stg2	IS12555	C-StgA	IS12555
X-Stg3	IS12555/BTx406	not mapped	
X-Stg4	IS12555	C-StgJ	IS12555
not mapped	(BTx406)	C-StgD	BTx406
not mapped	(IS12555)	C-StgI1	RTx430
not mapped	(BTx406)	C-StgI2	BTx406
not mapped	(IS12555)	C-StgB	RTx430

The failure to map a QTL in one of the two populations listed in Table 6.4 might be due to lack of allelic variation in genes that modulate stay-green in one of the two crosses, small population sizes, or differences in the environments used for screening among other possibilities. Potential lack of allelic variation in one of the two crosses was analyzed by examining allele variation in DNA markers that span each stay-green QTL. This analysis showed that two regions not mapped in BTx642/RTx430 corresponding to X-Stg1 and X-Stg3 showed significant allelic variation among markers mapping to these regions (data not shown). This indicates that the two regions were

genetically different but that there was no significant functional variation of alleles for stay-green in this interval in this population.

Four QTL for stay-green were mapped in BTx642/RTx430 but not in BTx642/RTx7000 (Table 6.4, C-StgD, C-StgI1, C-StgI2, C-StgB). Analysis of allelic variation in DNA from BTx642 and RTx7000 across these regions revealed two different results. In the genomic regions corresponding to C-StgD and C-StgI2, there was little or very limited allelic variation among DNA markers from these regions in BTx642 and RTx7000. For example, examination of C-StgI2 on LG-09 from 70-90cM using 55 AFLP markers revealed only one marker that was polymorphic between BTx642 and RTx7000. Similarly the C-StgD region from 190-200cM on LG-02 was examined with 12 AFLP and SSR markers and all marker alleles were identical between BTx642 and RTx7000. The analysis in Chapter V showed that these two regions of BTx642 were derived from BTx406. The lack of allelic variation in these regions is consistent with failure to map QTL corresponding to C-StgI2 and C-StgD in BTx642/RTx7000. In contrast, there was significant allelic variation in DNA markers mapping in BTx642/RTx7000 in the regions corresponding to C-StgI1 and C-StgB and both these regions in BTx642 originate from IS12555. However, the favorable alleles for stay-green mapped in BTx642/RTx430 originate from RTx430 and this is probably why these QTL were only mapped in this population.

Stay-green QTL mapped in QL41/QL39 compared to BTx642/RTx7000

QL41 is a stay-green line commonly used in the Australian sorghum breeding program that was derived from a cross between BTx642 and QL33 (Tao *et al*. 2000).

Five QTL were identified for stay-green in a population derived from QL41/QL39 and favorable alleles for three of these QTL, T-StgB, T-StgG, and T-StgI originate from QL41 (Table 6.5). T-StgB corresponds to X-Stg3, T-StgG overlaps with C-StgI2, whereas T-StgI does not correspond to any other stay-green QTL mapped to date (Table 6.5). Surprisingly, no stay-green QTL corresponding to X-Stg1, X-Stg2, or X-Stg4 were identified in the QL41/QL39 population. QL41 was derived from a cross of BTx642 and QL33 (senescent), therefore it was possible that QL41 did not inherit favorable alleles for X-Stg1, X-Stg2, or X-Stg4 from BTx642. This question was investigated by analyzing the origin of these regions in QL41 using DNA markers. The data in Table 6.5 shows that QL41 inherited QL33 DNA in the regions spanning X-Stg2 and X-Stg4 QTL and since QL33 is a senescent line, this probably explains why no stay-green QTL were mapped in these regions in the cross of QL41 and QL39. The situation for X-Stg1 was less clear. QL41 inherited a region on LG-03 from 140-151cM from QL33 and DNA from BTx642 extending from 151-161cM.

Table 6.5 Haplotypes of IS12555C, Tx406, BTx642, QL41, QL33, and QL39 in the genomic regions spanning X-Stg1, X-Stg2, X-Stg3, and X-Stg4. DNA derived from IS12555 is shaded light green, DNA derived from BTx406 is shaded pink, and DNA derived from QL33 is shaded orange.

X-Stg1 region

Marker	LG-03, cM	IS12555C	TX406	BTx642	QL41	QL33	QL39
Corn031	140-142	3	3	3	1	1	1
txp439	145-147	2	4	2	6	6	6
txp440	145-147	1	4	1	4		5
txp542	147-149	3	3	3	2	2	2
txp446	151-156	10	12	10	10	6	14
txp442	151-156	1	3	1	1	3	1
txp447	156-158	1	4	1	1	4	7
txp448	158-161	3	2	3	3	2	1
txp285	158-161	5	3	5,9	9	1	9
txp38	158-161	5	1	5	5	1	4
txp474	158-161	7	3	7	7	3	7
txp421	158-161	1	4	1	1	4	1
txp420	161-165	5	2,5	5	7	5	5

X-Stg4 region

Marker	LG-05, cM	IS12555C	TX406	BTx642	QL41	QL33	QL39
txi18	34-35	1	1	1	1	1	1
txp303	35-41	1	3	1	2	2	2
TS404N	53-56	4	3	4	2	2	3
txp225	56.9	3	4	3	2	3	4
txp15	58.2-61	4	3	4	2	2	2
TS492	58-61	9	6	9	2	2	6
SDB064	61-68	1	2	1	1	1	1

X-Stg2 region

Marker	LG-03, cM	IS12555C	TX406	BTx642	QL41	QL33	QL39
txp543	98-102	2	1	2	3	3	1
txp544	102-104	5	2	5	3	3	4
txp435	110-111	2	2	2	2	2	1
txp120	111	4	4	4	4	4	4
txp545	111.6	1	2	1	2	2	2
txp546	112	2	2	2	2	2	2
txp2	111-118	2	1	2	1	1	1
txp530	111-118	3	2	3	2	2	2
txp436	118-125	1	1	1	1	1	1

T-StgG/C-Stgl2 region

Marker	LG-09, cM	IS12555C	TX406	BTx642	QL41	QL33	QL39
SDB053	43-45	15	7	15	15	1	20
txp258	43.4-45.5	6	6	6	6	2	5
txp287	43.6	2	2	2	2	4	3
TXP67	52.5	7	7	7	7	7	1
CS002	65-68	1	4	4	4	4	1
txp10	81-85	3	6	6	6	2	4

X-Stg3 region

Marker	LG-02, cM	IS12555C	TX406	BTx642	QL41	QL33	QL39
txp464	103-108	2	3	3	3	3	2
txp445	110-113	4	1	1	1	1	2
TS214	110-113	1	1	1	1	1	4
Cup29	110-113	4	3	3	3	3	2
txp430	113-114	3	2	2	2	2	1
txp1	114-118	1	8	8	8	8	3
txp56	118.5	3	1	3	3	1	3
gap84	125-126	1	3	1	1	3	4
SDB049	129-131	4	4	4	4	4	4
txp179	131-134	1	1	1	1	1	1
txp348	137-145	11	22	11	11	XX	13

C-StgD region

Marker	LG-02, cM	IS12555C	TX406	BTx642	QL41	QL33	QL39
Cup40	189-193	1	2	2	2	2	1
Corn033	189-193	1	1	1	1	1	1
CS033	189-193	3	1	1	1	1	3
TS451	193-196	11	6	4	6	6	17
Cup69	202-205	2	1	1	1	1	2
CS056	202-205	2	4	4	4	4	2

Utilization of favorable alleles for stay-green from BTx642 in public breeding programs

BTx642 has been used as a source of stay-green in public sorghum breeding programs in Texas, Kansas, and Australia. These programs crossed BTx642 with various elite lines and selected for progeny that expressed the stay-green phenotype in environments subject to water deficit. Breeding lines derived from BTx642 that express stay-green were provided for analysis from three sorghum-breeding programs (Texas A&M University Sorghum Breeding Program, Kansas State University Sorghum Breeding Program, Australian Sorghum Breeding Program). DNA markers that map across X-Stg1-4 were analyzed to determine if segments of the BTx642 genome containing favorable alleles for stay-green were present in the 13 breeding lines submitted for analysis. In most cases, DNA from all parental lines in the pedigrees was available for analysis allowing identity by descent to be established. The data summarized in Table 6.6 show that ten out of thirteen BTx642 derived breeding lines contained BTx642 DNA spanning a portion of the genomic region corresponding to X-Stg3, six had BTx642 DNA spanning a portion of X-Stg1, and one had BTx642 DNA spanning X-Stg4.

Table 6.6 Analysis of stay-green breeding lines for DNA derived from BTx642 within genomic regions corresponding to X-Stg1-4.

Line	X-Stg1	X-Stg2	X-Stg3	X-Stg4	Program
QL41	-/+	-/-	?/+	-/-	AUS
*B923296	-/-	-/-	-/+	-/-	AUS
*R931945-2-2	-/+	-/-	+/+	-/-	AUS
*R974443-1-2	-/+	-/-	+/+	-/-	AUS
*B923171	-/-	-/-	-/+	-/-	AUS
ROOMN7645	-/-	-/-	-/-	-/-	KSU
B03MN960	-/+	-/-	?/?	-/-	KSU
B03MN954	-/-	-/-	?/+	-/-	KSU
B03MN952	-/-	-/-	?/+	-/-	KSU
B402	-/+	-/-	+/+	?/-	TAMU
B403	+/+	-/-	-/+	-/-	TAMU
*B01084	-/-	-/-	-/?	+/+	TAMU
B2-2	-/-	-/-	+/+	-/-	TAMU
TOTAL:	6	0	10	1	

In most cases, results are based on identify by descent but in some cases (*) haplotype origin was inferred. The designation -/+ indicates presence of BTx642 DNA in the upper part of the region spanning the Stay-green QTL and +/- indicates presence of BTx642 DNA spanning the lower part of the QTL. +/+ indicates genotype has DNA from BTx642 spanning the Stay-green QTL and -/- indicates that no DNA from BTx642 was present. The three sorghum breeding programs providing lines are the Australian Sorghum Breeding Program (AUS), the Kansas State Breeding Program (KSU), and Texas A&M University Sorghum Breeding Program (TAMU). The '?' represent regions where the parental lines have the same haplotype.

Discussion

The genetic basis of stay-green has been analyzed using six different populations and three sources of stay-green (Xu *et al.* 2000; Crasta *et al.* 1999; Tao *et al.* 2000; Kebede *et al.* 2001; Haussmann *et al.* 2002; Tuinstra *et al.* 1997). These studies identified 39 QTL for stay-green, however, 16 of the QTL were observed in only one environment or one year suggesting that these QTL are highly dependent on a specific environment (Xu *et al.* 2000; Crasta *et al.* 1999; Tao *et al.* 2000; Kebede *et al.* 2001; Haussmann *et al.* 2002; Tuinstra *et al.* 1997). The remaining 23 QTL that modulated expression of stay-green were located in 12 regions of the sorghum genome. The co-localization of stay-green QTL from different studies to the same genomic region could indicate that allelic variation of the same gene (or genes) was mapped in different studies. This would not be surprising because three of the QTL studies on stay-green utilized BTx642 or QL41, a genotype derived from QL41, as a source of favorable alleles for stay-green (Xu *et al.* 2000; Crasta *et al.* 1999; Tao *et al.* 2000). In addition, favorable alleles for stay-green for a region conferring stay-green were derived from more than one genotype. This suggests that sorghum germplasm is likely to have more than one favorable allele for many of the stay-green QTL.

Six of the 12 regions spanning major stay-green QTL had favorable alleles for this trait derived from BTx642 or QL41 a line derived from the stay-green genotype BTx642. Haplotype analysis of parental DNA of BTx642 showed that the favorable alleles for stay-green in X-Stg1, X-Stg2, X-Stg4, and T-StgI were derived from IS12555, in X-Stg3 favorable alleles could have come from either IS12555 or BTx406, and in C-

StgD and C-StgI2, favorable alleles for stay-green came from BTx406. The identification of favorable alleles for stay-green in BTx406, a non stay-green line, was not unexpected because alleles contributing to a specific trait are often identified in populations even though the parental lines do not exhibit a strong phenotype for the trait (Young *et al.* 1996). The two specific QTL of this type, C-StgD and C-StgI2 were expressed in 3 out of 4 environments and mapped with relatively high LOD scores (5.0, 2.9) (Crasta *et al.* 1999). These QTL were not mapped in the BTx642/RTx7000 (Xu *et al.* 2000) population but this can be explained by a lack of allelic diversity in the regions spanning C-StgD and C-StgI2 in this cross.

Stay-green QTL studies based on BTx642/RTx7000 and BTx642/RTx430 identified two of the same stay-green QTL and six QTL that were specific to one of the two populations (Xu *et al.* 2000; Crasta *et al.* 1999). The explanation for this observation was investigated by analyzing the origin and diversity of DNA in the two parents of each population. This analysis showed that that C-StgD and C-StgI2 were probably not mapped in BTx642/RTx7000 due to low allelic diversity among the parental lines in these regions of the genome. Second, haplotype analysis showed that favorable alleles for C-StgI1 and C-StgB were derived from RTx430, explaining why these loci were not mapped in BTx642/RTx7000. On the other hand, X-Stg1 and X-Stg3 were not mapped in BTx642/RTx430, even though BTx642 was the source of the favorable alleles mapped in these loci. X-Stg1 is from 140 to 165cM on LG-03 and the BTx642/RTx430 map has a 35cM gap just after 147cM on LG-03 where cM values are relative to the TAMU-ARS map (Crasta *et al.* 1999). Thus the lack of markers likely

prevents X-Stg1 derived from BTx642 from being identified in the BTx642/RTx430 population. In the X-Stg3 area from 103 to 145cM on LG-02, three markers exist in the BTx642/RTx430 map that is in the X-Stg3 region (csu6.3, umc5, txs2042). It is possible that RTx430 and BTx642 contain a similar allele for stay-green in both of these loci or that epistatic interactions in the BTx642/RTx430 population prevent expression of phenotypes modulated by these alleles.

Five QTL for stay-green were mapped in a population derived from QL41/QL39 (Tao *et al.* 2000). In this case, QL41 is a stay-green parent derived from BTx642. While C-StgB corresponds to X-Stg3 and T-StgG overlaps with C-StgI2, no stay-green QTL corresponding to X-Stg1, X-Stg2 or X-Stg4 were mapped in this population. Haplotype analysis of the QL41 pedigree (BTx642/QL33) showed that QL41 obtained DNA from QL33, a non stay-green line, in the regions corresponding to X-Stg2 and X-Stg4. This explains why these QTL were not mapped in QL41/QL39. The situation for X-Stg1 is less clear. A portion of the DNA in QL41 spanning this locus was derived from BTx642. However, this region of the QL41/QL39 genetic map published by Tao *et al.* (2000) was not well represented, possibly explaining why this locus was not mapped in their study.

The stay-green trait contributes significantly to the yield of sorghum hybrids in environments subject to terminal drought (Borrell *et al.* 2000). Therefore, sorghum breeding programs have used BTx642 as a source of stay-green in Texas, Kansas, and Australia. Until recently selection of the stay-green trait in breeding materials was done by screening progeny for the trait as well as other agronomic indicators. This type of

selection is quite different from a QTL mapping study where all lines are analyzed for expression of the trait regardless of yield or other characteristics of progeny. Therefore, it was of great interest to analyze stay-green sorghum breeding material derived from BTx642 to see which if any regions containing favorable alleles for stay-green were present in the breeding lines. The analysis of 13 breeding lines from Texas, Kansas and Australia showed that 10 of the 13 lines analyzed contained DNA spanning all or a portion of X-Stg3. In contrast, none of the breeding lines contained DNA from BTx642 containing favorable alleles for X-Stg2. This result was very surprising because among the four stay-green QTL mapped in BTx642/RTx7000, X-Stg2 explained the largest proportion of the stay-green phenotype. A possible explanation for lack of the X-Stg2 region from BTx642 in the breeding lines was the presence of an allele for lemon yellow seeds in the same region of the genome. Discussion with the sorghum breeders involved in this study revealed that they selected for red seeds not realizing that this selected against the favorable allele for X-Stg2. It was also apparent that favorable alleles for stay-green in X-Stg4 were not highly represented in the breeding lines and in no case were more than two of the four favorable alleles for stay-green present in any one line. This suggests that marker-assisted breeding could improve the selection and pyramiding of favorable alleles for stay-green in future sorghum breeding lines and hybrids.

CHAPTER VII

CHARACTERIZATION AND UTILIZATION OF NEAR ISOGENIC SORGHUM LINES EXPRESSING STAY-GREEN[1]

Introduction

Four stay-green QTL (X-Stg1-4) spanning ~10-20cM were identified using a recombinant inbred population derived from BTx642 and RTx7000 (Xu *et al.* 2000). In a population, the influence of a single QTL on phenotype is often difficult to quantify because other loci influence the same trait due to epistatic interactions (Tuinistra *et al.* 1997). Near isogenic lines (NILs) can help clarify complex genetic interactions and phenotypes such as those associated with the stay-green trait. NILs are constructed by continually backcrossing the progeny of a cross to the parental line that lacks the phenotype (recurrent parent) with continual selection for the trait and/or region of the genome corresponding to the target QTL. NILs provide an excellent starting point for the isolation of genes by map-based cloning. For example, in rice 14 QTL that regulate flowering time were mapped in a cross of *O. sativa japonica* and *O. sativa indica.* Three flowering time QTL, Hd1, Hd3a, and Hd6 were fine-mapped using NIL-derived material and isolated by a map-based cloning approach (Paran and Zamir 2003).

Darrell Rosenow constructed 34 RTx7000 NILs that contained segments of DNA from BTx642 spanning one or more of the stay-green QTL. NILs containing only X-Stg1, X-Stg2, X-Stg3, or X-Stg4 were identified and were found to have an enhanced

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[1] Reprinted with permission from "Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence" by Harris et al., 2007. Journal of Experimental Botany, **58**, 327-338. Copyright 2007 by Oxford University Press.

stay-green phenotype relative to RTx7000. The stay-green NIL X-Stg1 provided the starting material for the initial steps towards map-based cloning the gene or genes that modulate this QTL. Because all stay-green QTL studies in this chapter refer only to those identified by Xu *et al.* (2000), I will therefore, for this chapter only, remove the authors last name initial from all stay-green QTL.

Results

Construction and analysis of near isogenic stay-green lines

Stay-green NILs possessing one or more stay-green QTL were created by D. Rosenow and his team by backcrossing progeny from a BTx642/RTx7000 cross four (#6000 series) or six times (#2000 series) to the senescent parent RTx7000 (Figure 7.1). With each backcross generation, marker-assisted selection was used to identify plants that contain BTx642 DNA spanning one or a combination of the stay-green QTL mapped in this population. Progeny derived from each backcross were screened for one or more of the stay-green loci derived from BTx642 using DNA markers that mapped within or near each locus (Fig. 7.2, DNA markers with arrows). For example, progeny containing BTx642 DNA spanning Stg1 were identified using the markers NPI414, Xtxs1114, and BNL15.20 (Fig. 7.2, markers in bold with arrows to the right). As a consequence several RTx7000 NILs were generated that contain a block of BTx642 DNA spanning Stg1 (Fig. 7.2, NILs 6078-1, 6086-3, 6102-23, 6100-7). Similarly, NILs containing BTx642 DNA spanning all or a portion of Stg2, Stg3 and/or Stg4 were generated using Xtxs584, RZ323, CSU58, A12-420 (Stg2), Xtxs1307, Xtxs1111, UMC5

(Stg3) and Xtxs713 (Stg4) (Figure 7.2). Selection was continued until the BC4 or BC6 generation where the lines were selfed to create BC4F2-4 or BC6F2-4 lines.

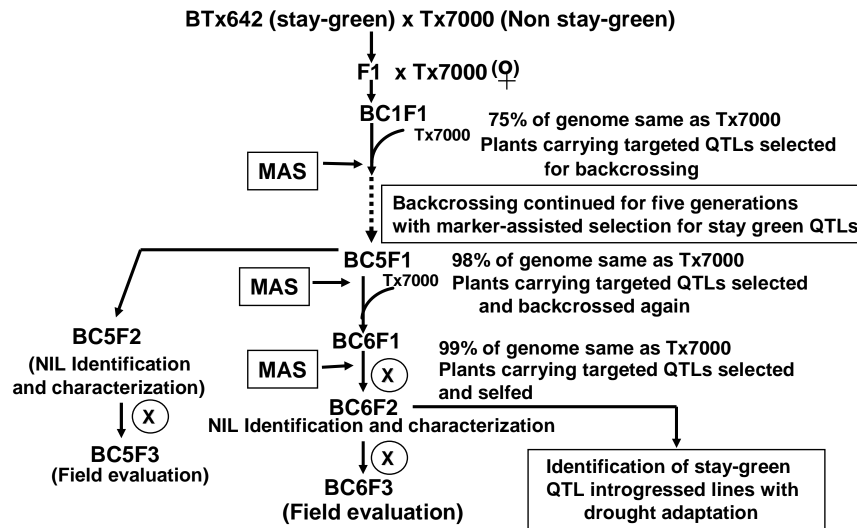


Figure 7.1 Scheme for developing near-isogenic lines (NILs) for stay-green QTL using marker-assisted selection (MAS) (modified from Subudhi *et al.* 2000).

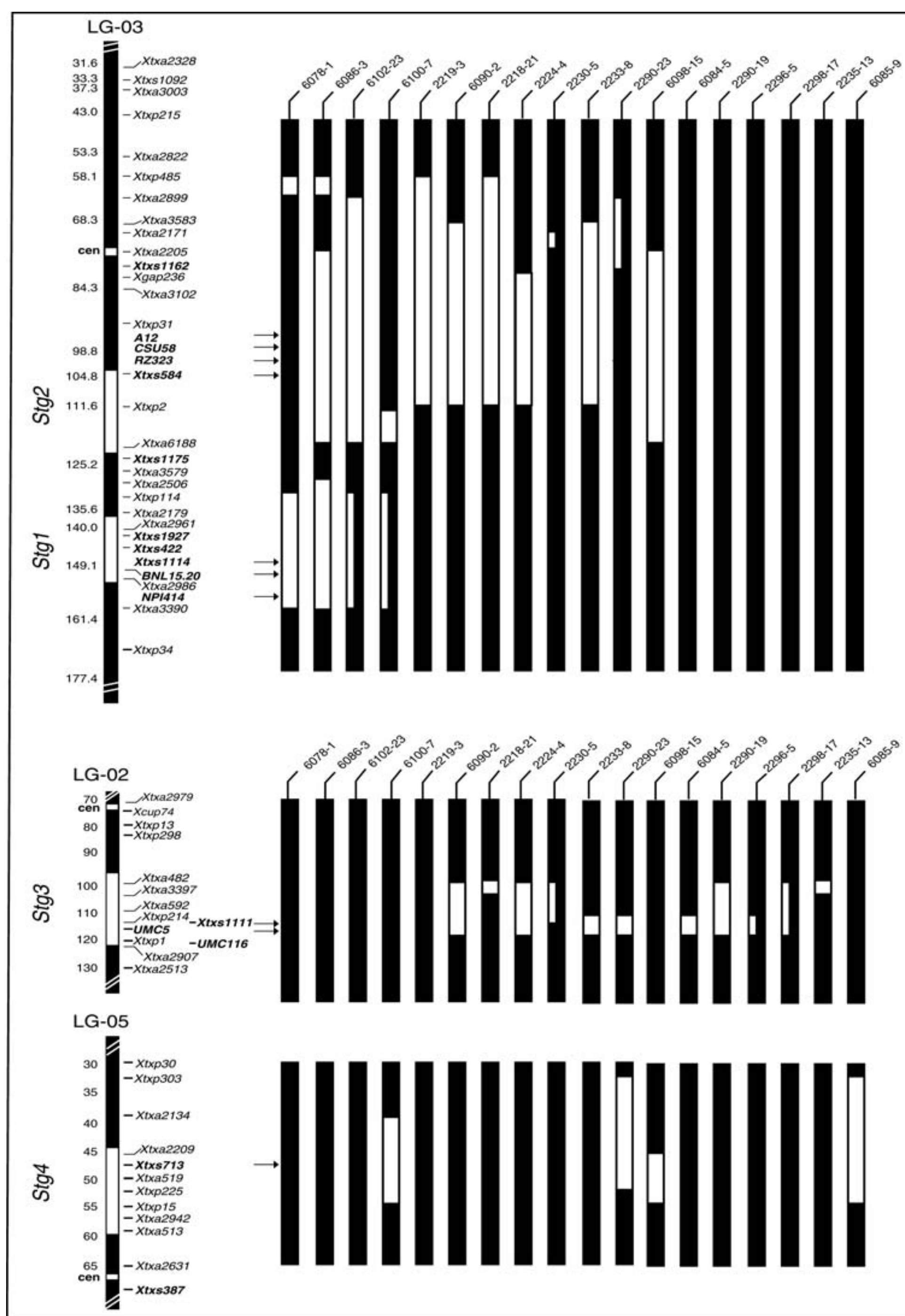


Figure 7.2 Location of BTx642 DNA spanning regions of LG-03, LG-02, and LG-05 in 18 of the RTx7000 NILs. White bars correspond to a region of BTx642 DNA while black represents RTx7000 DNA. The reference chromosomes (far left) are shaded in white to indicate the location of each stay-green QTL as determined by Xu *et al*. (2000). White bars that are half the width of each chromosome represent heterozygous DNA. NILs containing identical regions introgressed from BTx642 are shown only once. For example, NILs 2209-4, 2208-12, 2219-8 and 2219-3 contain the same introgressed region of BTx642. Similarly, 2293-12, 2289-19 are similar to 6084-5, and 6083-1 and 2229-5 is similar to 2290-19. Markers in bold are RFLP markers used by Xu *et al*. (2000) that were located on the TAMU-ARS genome map. Markers with arrows to their right were used to select the desired region from BTx642. Marker-assisted selection was used for each of the six backcross generations. Markers in bold were located in the figure using the genetic map constructed by Subudhi *et al.* (2000) map as a reference. Marker txs1307 (not shown) was also used for marker-assisted selection and was placed in the same location as txs1111.

In the present study, each NIL was genotyped by using 113 AFLP and SSR markers. Sixty-two of the DNA markers used in the analysis were located either within or adjacent to each stay-green locus, to determine the size and location of BTx642 DNA in each line. Figure 7.2 shows genotypes of 18 of the original 34 NILs. The reference chromosome, far left, displays markers used for genotyping, marker assisted selection (markers in bold with arrows), and markers used to align the genetic maps constructed by Xu *et al.* (2000) with the TAMU-ARS genome map (bold markers). The size and location of the Stg1-4 regions were determined using markers shared by the two maps. The stay-green QTL regions are shaded in white on the reference chromosome. The genotypes of each of the NILs are represented by black and white shading where black indicates a chromosomal area with a RTx7000 allele and white represents a chromosomal area with a BTx642 allele. Stg1 NIL 6078-1 contains an introgression from BTx642 that encompasses the entire Stg1 region. Stg2 NIL 2219-3 has a BTx642 introgression that spans most of the Stg2 locus. Stg3 NIL 2290-19 and Stg4 NIL 6085-9 also contain BTx642 DNA that spans most of their respective QTL.

NILs possessing only one stay-green QTL were physiologically characterized by Dr. Andrew Borrell in Australia to determine if each favorable allele located in each QTL could contribute to the stay-green phenotype independently. Dr. Andrew Borrell physiologically characterized the NILs at the Hermitage Research Station (Queensland, Australia) during the 2004 (Experiment 1) and 2005 (Experiment 2) seasons. Plants were grown under a rain-out shelter at either high density (20 plants per m^2, HD) or low density (10 plants per m^2, LD) with four replications. Irrigation was applied until 16-24

days before anthesis at which time plants underwent a terminal water deficit. Drought was more severe in Experiment 1 than 2 due to earlier planting in the 2004 season.

Three physiological parameters were measured for each NIL and RTx7000: absolute rate of leaf senescence, relative rate of leaf senescence, and green leaf area at maturity. The absolute rate of leaf senescence is the total loss of greenness (chlorophyll content) from anthesis to maturity while the relative rate of leaf senescence is the loss of greenness per day after anthesis. The green leaf area at maturity was measured on the leaf below the flag leaf (FL-1) using a chlorophyll meter.

The rate of leaf senescence was significantly lower in Experiment 1 and 2 for the Stg2 and Stg3 NILs as compared to RTx7000 while the Stg1 and Stg4 NILs had a lower rate of leaf senescence relative to RTx7000 only in Experiment 2 (Table 7.1). The relative rate of leaf senescence was significantly less in the Stg2 NIL compared to RTx7000 in both experiments. Green leaf area at maturity was at least twice as high in the Stg2 NIL as compared to RTx7000 in Experiments 1 and 2 while the Stg1 NIL was significantly higher than RTx7000 in Experiment 2. In Experiment 2 at maturity, Stg1 and Stg2 NILs had a higher green leaf area as compared to RTx7000 under low density.

Table 7.1 Absolute rate of leaf senescence, relative rate of leaf senescence and green leaf area at maturity were measured in the stay-green NILs and their recurrent parent RTx7000 at two plant densities during a terminal post-anthesis water deficit in 2004 and 2005.

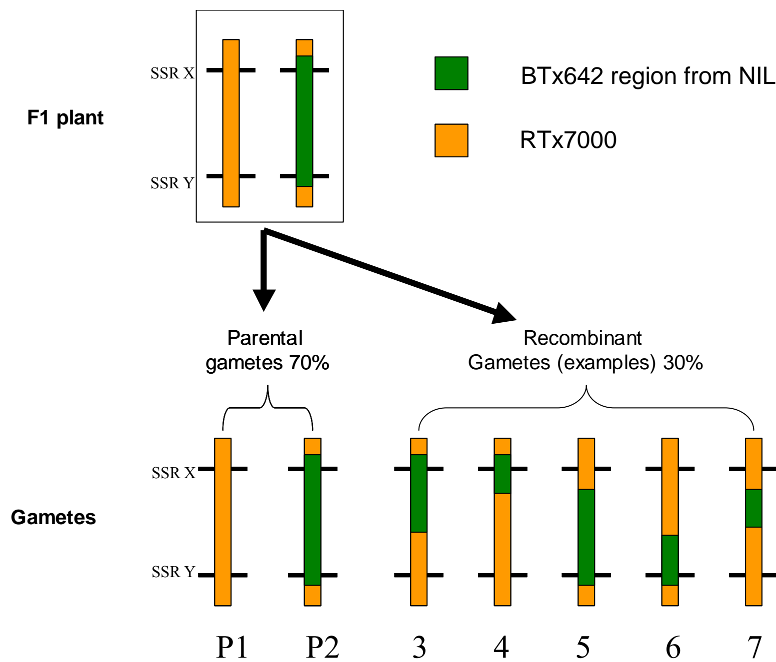
		Main Effects			Genotype x Treatment Interactions					
Stg region	Genotype	Absolute rate of leaf senescence (cm^2 m^{-2}d^{-1})	Relative rate of leaf senescence (%loss LAI d^{-1})	Green leaf area at maturity (cm^2 m^{-2})	Absolute rate of leaf senescence (cm^2 m^{-2} d^{-1})		Relative rate of leaf senescence (%loss LAI d^{-1})		Green leaf area at maturity (cm^2 m^{-2})	
Experiment 1 (2004 Season)										
					High density	Low density	High density	Low density	High density	Low density
Stg1	6078-1	619 bc [a]	2.58 b	2125 a	778 ab	459 ab	2.63 b	2.53 b	2106 a	2145 a
Stg2	2219-3	**518 a** [b]	**2.01 a**	**6644 b**	**681 a**	**354 a**	**2.24 a**	**1.79 a**	**6445 b**	**6842 b**
Stg3	2290-19	**578 ab**	2.55 b	2112 a	**722 a**	433 ab	2.66 b	2.43 b	1878 a	2346 a
none	RTx7000	715 c	2.69 b	1292 a	866 b	564 b	2.78 b	2.60 b	859 a	1725 a
LSD (*P*=0.05)		99	0.22	1456	140	140	0.31	0.31	2060	2060
P-value		0.003	<0.001	<0.001	0.068	0.068	0.13	0.13	0.243	0.243
Experiment 2 (2005 Season)										
					High density	Low density	High density	Low density	High density	Low density
Stg1	6078-1	**461 a**	**1.23 a**	**5901 bc**	478 ab	**444 a**	1.32 ab	**1.14 a**	3363 ab	**8440 b**
Stg2	2219-3	**509 a**	**1.22 a**	**6561 c**	537 ab	**480 a**	1.26 a	**1.18 a**	5765 b	**7356 b**
Stg3	2290-19	**509 a**	1.34 bc	2900 a	549 ab	**470 a**	1.38 b	1.29 b	2047 a	3753 a
Stg4	6085-9	**495 a**	**1.28 ab**	4420 ab	**455 a**	535 ab	1.26 a	1.30 b	4652 b	4188 a
none	RTx7000	612 b	1.35 c	3128 a	601 b	624 b	1.33 ab	1.36 b	3732 ab	2524 a
LSD (*P*=0.05)		92	0.06	1719	130	130	0.08	0.08	2431	2431
P-value		0.016	<0.001	<0.001	0.423	0.423	0.003	0.003	0.006	0.006

The abbreviations a, b, and c indicate if values are significantly different from one another for each parameter measured. For example if two NILs have an "a" after their values these values are not significantly different from one another. Values in bold indicate the results obtained from a NIL were significantly different from results obtained on the recurrent parental line RTx7000.

Utilization of 6078-1, a Stg1 NIL, for fine-mapping

The overall plan for fine-mapping Stg1 using the Stg1 NIL 6078-1 is shown in Figure 7.3 (figure adapted from David Jordan). The procedure starts with Stg1 NIL 6078-1 that contains a segment of BTx642 DNA spanning Stg1 from ~125cM to ~161cM. NIL 6078-1 was crossed to RTx7000 in order to generate recombinant lines that contain various sub-portions of the region of BTx642 DNA present in NIL 6078-1. The stay-green phenotype cannot be accurately characterized using single plant assays. Therefore, F2 progeny from the cross of 6078-1/Tx7000 were selfed and homozygous recombinant F3 lines containing sub-portions of Stg1 from BTx642 were identified using DNA markers. Homozygous F3 lines containing sub-portions of the region spanning Stg1 were phenotyped for expression of stay-green in Australia by Dr. Andy Borrell. Overall, 34 homozygous plants with different sub-portions of Stg1 were identified. The size and location of BTx642 DNA in each of these lines was analyzed using a series of DNA markers spanning the locus. The results showed that a series of recombinant lines had been recovered that contain different portions of the original segment of BTx642 DNA present in the Stg1 NIL 6078-1 (Table 7.2). These lines were phenotyped for expression of the stay-green trait by Dr. Andrew Borrell in Australia. This analysis revealed that two sub-regions of Stg1, 5 and 6cM regions, respectively, contain genes that can contribute to the stay-green trait.

A)



B)

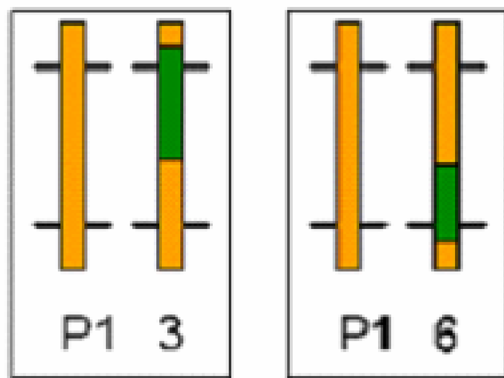
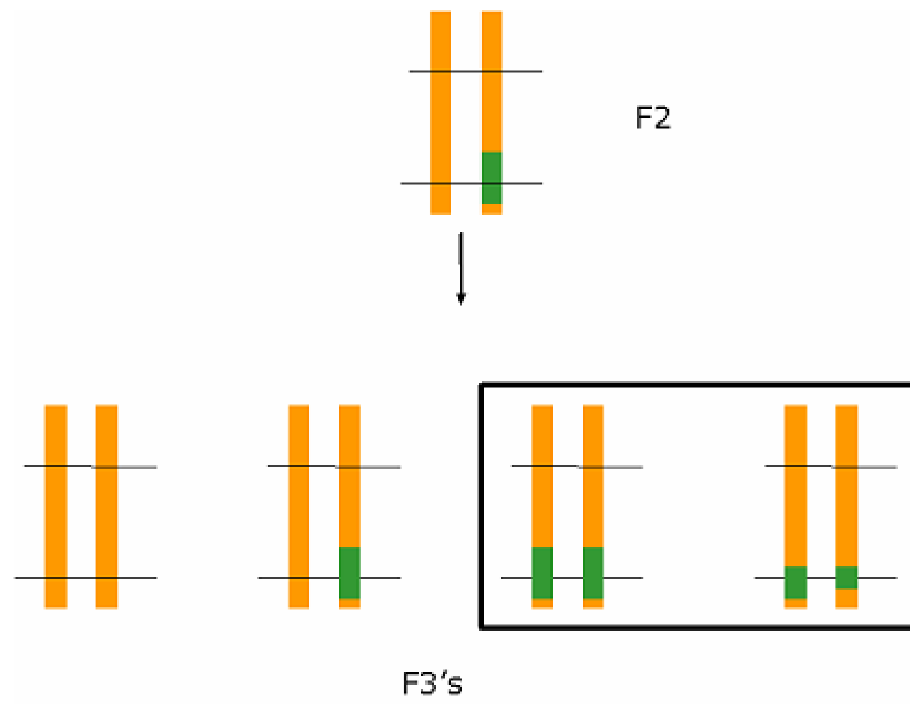


Figure 7.3 Fine-mapping strategy (adapted from David Jordan). A) Possible gametes from an F1 plant. B) Example of F2 plants formed from one recombinant Stg1 NIL gamete. C) Possible F3 plants from plants with one recombinant Stg1 NIL gamete.

Figure 7.3, continued

C)



Marker	cM	6078-1	RTx7000	52-82	53-81	48-89	48-88	51-100	45-96	45-98	45-99	52-88	44-100	47-90	53-83	Dominant
txa2171	74.4	T	T	T	T	T	T	T	T	T	T	T	T	T	T	CODOM
txa2233	125.2-127.5	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
txa2506	125.2-127.5	T	T	T	T	T	T	T	B	B	B	B	T	B	B	T
txp114	134.7-135.6	BB	TT	TT	TT	TT	TT	TT	BB	BB	BB	BB	BB	BB	BB	CODOM
txa2179	135.6-140	B	T	T	T	T	T	T	B	B	B	B	T	B	B	T
txa2243	140-142.5	B	T	B	T	T	T	T	B	B	B	B	T	B	T	T
txp439	145.5-147.3	BB	TT	BB	TT	TT	TT	BB	BB	BB	BB	TT	TT	BB	TT	CODOM
txa2986	150.3	B	T	B	T	T	T	B	B	B	B	T	T	T	T	B
txp442	151.9-156.2	BB	TT	BB	BB	TT	TT	BB	BB	BB	BB	T	TT	T	TT	CODOM
txp38	158.3-161.4	BB	TT	BB	BB	BB	BB	BB	TT	TT	TT	TT	TT	TT	TT	CODOM
txp421	158.3-161.4	BB	TT	BB	BB	BB	BB	BB	T	T	TT	TT	TT	TT	TT	CODOM
txa3390	158.3-161.4	B	T	B	B	B	B	B	T	T	T	T	T	T	T	B
txp420	161.4-165.1	BB	TT	TT	TT	TT	TT		T	T	TT	TT	TT	TT	TT	CODOM
txa2275	166.9-169	T	T	T	T	T	T	T	T	T	T	T	T	T	T	B
txa2232	184.6-190.0	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T

Table 7.2 Genotypes of F3 Stg1 homozygous crossover plants that were phenotyped by Andrew Borrell in low and high density plant growth conditions in Australia. Marker names, location of each marker on LG-03, and dominance of each marker is shown where 'B' indicates that the BTx642 alllele is dominant and 'T' indicates the RTx7000 allele is dominant. Each column represents a Stg1 homozygous crossover plant and the size and location of each BTx642 crossover is highlighted in green. A 'BB' or 'TT' for a marker in the Stg1 homozygous crossover plants indicates that a codominant marker was used and that plant has both alleles from one parent.

Discussion

Analysis of the stay-green trait is complex because this trait can be modulated by allelic variation in a large number of genes and because expression of the trait is dependent on environmental conditions. The need for analysis of genotypes expressing the stay-green trait in multiple locations and in rows of plants meant that phenotyping could only be done on homozygous recombinant lines. This situation made map-based cloning genes for stay-green very challenging. As a first step toward map-based gene isolation, eighteen different RTx7000 NILs were identified that contained regions of BTx642 DNA spanning all or a portion of the regions spanning Stg1-4. Four NILs were identified that contained BTx642 DNA spanning only Stg1, Stg2, Stg3, or Stg4. These NILs were physiologically characterized for expression of the stay-green trait under terminal post-anthesis water deficit in Australia over a two year period and each showed a stay-green phenotype. The Stg2 NIL exhibited the strongest stay-green phenotype with a lower absolute rate of leaf senescence and relative rate of leaf senescence and a higher green leaf area at maturity than RTx7000 in both years examined. Measurements of leaf greenness with a chlorophyll meter showed a trend of delayed onset of senescence and a reduced rate of leaf senescence in the Stg2 NIL compared to RTx7000 (Harris *et al.* 2007). These results support the finding that Stg2 has a greater effect on the expression of the stay-green phenotype than the other three QTL identified in the BTx642/RTx7000 RI population (Xu *et al.* 2000; Subudhi *et al.* 2000).

The Stg3 NIL had a lower absolute rate of leaf senescence in both years of the study. In addition, the stay-green phenotype exhibited by the Stg1 and Stg4 NILs was more strongly expressed in less severe drought conditions.

The physiological analysis of the stay-green NILs indicated that each of the four stay-green loci analyzed could contribute to the stay-green phenotype in the absence of the other stay-green loci. Therefore, the stay-green NILs for each locus could be used as the starting point for map-based cloning and the initial steps towards cloning the gene for Stg1 were carried out. The Stg1 NIL 6078-1 was crossed to RTx7000 (non-stay green recurrent parent), and homozygous F3 lines containing recombinant chromosomes were identified. DNA markers spanning Stg1 were used to identify F3 lines containing various segments of BTx642 DNA, a subset of which was expected to confer the stay-green trait. Phenotypic analysis of these lines by Dr. Andy Borrell over a two year period showed that two regions of the Stg1 locus spanning 5cM and 6cM, respectively, each contained favorable alleles for stay-green. This 11cM region contains an estimated 5 million base pairs and if one assumes there is one gene for every 10 kbp, then this region would include approximately 500 genes. Further fine-mapping and analysis of candidate genes is underway to identify the genes involved in this response.

CHAPTER VIII

CONCLUSIONS

Diversity of the sorghum race Bicolor

A previous study analyzed the genetic relationships among 50 *Sorghum bicolor* subspecies *bicolor* inbreds including many genotypes that are progenitors of current material used in U.S. sorghum breeding programs (Menz *et al.* 2004). This analysis identified clusters of genotypes representing the *S. bicolor* subspecies *bicolor* races Kafir, Durra, Caudatum, and at least one representative of the race Guinea. Genotypes of the race Bicolor were not well represented in the prior study of sorghum diversity.

The race Bicolor is thought to be the progenitor of the other *S. bicolor* subsp. *Bicolor* cultivated races. Therefore the diversity of seven sorghum accessions of the race Bicolor were analyzed using AFLP markers and compared to the 50 cultivated inbred lines and a few additional sorghum genotypes. The sorghum genotypes generally clustered based on their race or working group as previously reported with the exception of genotypes of the race Bicolor which failed to form a group (Menz *et al.* 2004; Cui *et al.* 1995; Deu *et al.* 1994; Ahnert *et al*. 1996; Casa *et al.* 2005). The race Bicolor contained many alleles that were not present in representatives of the other *S. bicolor* races analyzed, consistent with the hypothesis that the race Bicolor is the ancestor of the other cultivated races of *S. bicolor* subsp. *bicolor*. Accessions of the race Bicolor may contain novel alleles not present in other sorghum races that are useful for sorghum breeding.

Diversity of sorghum genotypes that express stay-green

The stay-green trait for post-flowering drought tolerance was discovered in sorghum by Dr. Darrell Rosenow in the late 1970's. Since that initial discovery, eight main sources of stay-green have been identified in sorghum germplasm. Seven of the genetic sources of stay-green originate from an overlapping region of Sudan and Ethiopia, one genotype originates from Nigeria, and no stay-green genotypes originate from southern Africa. The eight sources of stay-green are not closely related overall. These genotypes cluster based on genetic similarity into five different groups based on race or working group, or they did not cluster (IS17459C). This suggests that favorable alleles for stay-green were selected from diversity present in several different races in the drought prone environment present in Sudan, Ethiopia, and Nigeria. This is consistent with other results in this study, showing that different sources of stay-green modulate this trait using a range of alleles and genes.

BTx642/RTx7000 genetic map construction

BTx642 is one of the most important sources of stay-green for research and sorghum breeding. During the course of the current research, the BTx642/RTx7000 genetic map was refined through the use of AFLP and SSR markers. These markers allowed the genetic map based on BTx642/RTx7000 to be expanded and cross-referenced to the integrated genetic, physical, and cytogenetic map based on BTx623/IS3620C (Menz *et al.* 2002; Kim *et al.* 2005b). The updated genetic map based on BTx642/RTx7000 spanned 1255.2cM, ~1.5x longer than the previous map based on this population generated using a limited number of RFLP markers (Xu *et al.* 2000).

Segregation distortion was observed on LG-03, LG-10, and in several other regions of the genetic map. This may be due to selection against lines that flowered either very early or very late during construction of the RIL population derived from BTx642 and RTx7000. Several of the linkage groups of the BTx642/RTx7000 genetic map were >50% smaller than the corresponding linkage groups of the genetic map based on BTx623/IS3620C. Genetic markers mapping across these linkage groups showed that there was little or no genetic difference between BTx642 and RTx7000 across a substantial portion of LG-09 and LG-07 explaining why these linkage groups are relatively short. DNA marker analysis of the progenitors of BTx642 revealed that the regions of low genetic diversity between BTx642 and RTx7000 correspond to regions of BTx642 derived from BTx406. Low genetic diversity in these regions reflected the fact that RTx7000 and BTx406 are both Kafir-Milo genotypes with common ancestry.

Complexity, origin, and utilization of stay-green QTL

The genetic basis of stay-green has been analyzed in six populations utilizing four different sources of stay-green. These studies identified 39 stay-green QTL of which 23 were identified in more than one environment (or year). The 23 stay-green QTL were located in 12 regions of the BTx623/IS3620C genetic map indicating that allelic variation in at least twelve genes can modulate quantitative variation in the stay-green trait. Analysis of diversity in regions spanning stay-green QTL indicated that sorghum germplasm contains several favorable alleles for many of the QTL.

BTx642 is a major source of stay-green and as a consequence this genotype has been used for genetic analysis of stay-green in several studies. These studies identified

four to seven stay-green QTL in each population and only a subset of the QTL were identified in more than one population. Haplotype analysis of the parents of each cross and their progenitors suggested several reasons for this result. In several cases, there was little genetic variation among the parental lines in the region spanning a stay-green QTL identified in another population. In other cases, the favorable allele for stay-green was not derived from BTx642. For example, favorable alleles for stay-green in two different QTL originated from RTx430 in the population derived from BTx642/RTx430. In addition, X-Stg2 and X-Stg4 were not mapped in QL41/QL39 because QL41 did not inherit the favorable alleles for these QTL from BTx642. Furthermore, favorable alleles for several stay-green QTL originated from BTx406, a non stay-green parental line. Transgressive segregation is often observed in QTL studies where a parent that does not express the phenotype contributes favorable alleles for the phenotype being assayed (Young *et al.* 1996). Reasons for transgressive segregation in plants consistently point to the action of complementary genes, where one allele produces an observable phenotype only in conjunction with a second allele from a different parent. In some cases overdominance may also be involved, where the impact of a heterozygote on phenotype is greater than that of either homozygote (Rieseberg *et al.* 1999). Thus, it seems likely that several alleles of BTx406 produce a stay-green phenotype through interaction with alleles present in RTx430 or BTx642.

BTx642 has been used extensively in sorghum breeding programs as a source of stay-green. Haplotype analysis showed that sorghum breeding lines derived from BTx642 lack favorable alleles corresponding to X-Stg2, a locus that contributes

significantly to the stay-green phenotype in mapping populations. An allele for lemon yellow seed color was linked to the favorable allele for X-Stg2. Selection against lemon yellow seed color may explain why favorable alleles for X-Stg2 were not present in stay-green breeding lines derived from BTx642. In contrast, favorable alleles from BTx642 for X-Stg3 were found in 10 of the 13 stay-green sorghum-breeding lines analyzed. This suggests that BTx642 derived alleles for X-Stg3 may be quite useful for the production of sorghum breeding lines with stay-green. This study also showed that further progress in breeding for stay-green should be possible through the use of marker-assisted breeding for additional alleles for stay-green.

Characterization of stay-green NILs and fine-mapping X-Stg1

RTx7000 near isogenic lines containing one or more regions of BTx642 spanning X-Stg1, X-Stg2, X-Stg3, and X-Stg4 were created by Dr. Darrell Rosenow and Dr. Henry Nguyen. In the current work, thirty four of the NILs were genotyped across the regions conferring stay-green to determine the number and size of the introgressions from BTx642 in these lines. This analysis identified RTx7000 NILs that contained BTx642 DNA spanning only one of the four stay-green QTL. Each of these NILs showed a stay-green phenotype indicating that favorable alleles for each of the four stay-green QTL derived from BTx642 could modulate this trait independently. Moreover, the RTx7000 X-Stg2 NIL showed the strongest stay-green phenotype among the NILs analyzed consistent with prior results based on QTL analysis. (Subudhi *et al.* 2000).

The RTx7000 NIL for X-Stg1 (6078-1) was used to fine-map this locus as a first step towards map-based cloning. The NIL 6078-1 was crossed to RTx7000 and F2

progeny with recombinant chromosomes in the interval spanning X-Stg1 were identified using DNA markers. The F2 lines were selfed and F3 progeny screened to identify lines that were homozygous for sub-portions of X-Stg1 derived from BTx642. Approximately 30 homozygous recombinant lines were identified and phenotyped over two years for stay-green in Australia. This allowed X-Stg1 to be fine-mapped to a region <10 cM. This process will be continued until the gene or genes for X-Stg1 are identified.

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APPENDIX A

Table A-1. List of numbers with their corresponding genotypes as shown in Figure 4.1.

Number	Genotype	Origin
1	03BRON299	
2	B.HF8	
3	B01084	
4	B03MN952	
5	B03MN954	
6	B03MN960	
7	B2-2	
8	B402	
9	B403	
10	B923171	
11	B923296	
12	BOK11	
13	BTx3042	
14	BTx3197	
15	BTx378	
16	BTx398	
17	BTx399	
18	BTx406	
19	BTx623	
20	BTx625	
21	BTx626	
22	BTx631	
23	BTx635	
24	BTx642	Ethiopia
25	BTxARG-1	
26	E36-1	Ethiopia
27	ICSV400	Mali
28	IS12555C	Ethiopia
29	IS12608C	Ethiopia
30	IS12610C	Ethiopia
31	IS12646C	Ethiopia
32	IS12661C	Ethiopia
33	IS12666C	Ethiopia
34	IS17459C	Sudan
35	IS22380/PI569826	Sudan
36	IS2508C	Sudan
37	IS2816C	Zimbabwe
38	IS2856C	South Africa
39	IS3552C	Sudan

Table A-1, continued

Number	Genotype	Origin
40	IS3620C	Nigeria
41	IS6705C	B. Faso
42	IS7452C	Nigeria
43	IS9290C	Sudan
44	KS19	
45	MACIA	Zimbabwe
46	MP531	
47	Short Kaura/PI285193	Nigeria
48	QL12	
49	QL33	
50	QL39	
51	QL41	
52	ROOMN7645	
53	R931945-2-2	
54	R974443-1-2	
55	RO4044	
56	RO4047	
57	RO4050	
58	RTx2858	
59	RTx414	
60	RTx430	
61	RTx432	
62	RTx433	
63	RTx436	
64	SC326-6	Ethiopia
65	TAM2566	Ethiopia
66	TAM428	Ethiopia
67	Tx2536	
68	Tx2737	
69	Tx2783	
70	Tx2817	Ethiopia
71	Tx2862	
72	Tx2880	
73	Tx2903	
74	Tx2908	
75	Tx7000	
76	Tx7078	
77	IS12553C/SC33	Ethiopia
78	IS12568C/SC56-14E	Sudan
79	IS12661C/SC170-6	

The pedigree information for the 50 cultivated lines used in the diversity study can be found in Menz *et al.* (2004). The pedigrees of the additional 28 lines added to the original 50 cultivated lines are listed in Table 3.3.

APPENDIX B

Table B-1 BTx642/RTx7000 LG-01 markers.

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txa15403	**LG-01**	**20.61**	**framework**	**0**	-	-		P-CTC M-CGG
txa15478	LG-01	28.82	unique	0-3.3	>2.0	txa15403		E-TAC M-CAC
txa6791	LG-01	21.64	unique	0-3.3	>2.0	txa15403		P-CTC M-CGG
txa4829	LG-01	26.15	unique	0-3.3	>2.0	txa15403		P-CGT M-CCG
txa2723	**LG-01**	**anchor**	**framework**	**3.3**	-	-		E-ACC M-CAC
txa74	**LG-01**	**21.36**	**framework**	**6.3**	-	-		E-CAA M-CAT
txa3050	**LG-01**	**anchor**	**framework**	**8.7**	-	-		E-ACC M-CGC
txa6374	**LG-01**	**anchor**	**framework**	**16.4**	-	-		P-CTC M-CAC
txa15095	LG-01	25.85	unique	16.4-18.1	>2.0	txa6374		E-TGA M-CAA
Xcup6	**LG-01**	**22.78**	**framework**	**18.1**	-	-	SD	SSR
txa15063	**LG-01**	**10.57**	**framework**	**27.9**	-	-	SD	E-CAA M-CTC
txa15049	**LG-01**	**21.64**	**framework**	**68.1**	-	-		E-AGT M-CGG
txa15068	LG-01	6.1	region	68.1-87.2	>2.0	txa15049		E-CAA M-CCC
txa3298	**LG-01**	**21.92**	**framework**	**87.2**	-	-		E-AGT M-CGG
txa15469	LG-01	25.22	region	87.2-89.9	>2.0	txa3298		E-ACC M-CAT
txa15450	LG-01	28.52	region	87.2-89.9	>2.0	txa3298		E-AGT M-CCC
txa15338	LG-01	27.04	region	87.2-89.9	>2.0	txa3298		E-CAA M-CCC
txa15230	LG-01	25.22	region	87.2-89.9	>2.0	txa3298		E-AGT M-CAT
txa913	LG-01	24.93	region	87.2-89.9	>2.0	txa3298		E-CAA M-CCC
txa2770	LG-01	anchor	unique	87.2-89.9	>2.0	txa3298		E-ACC M-CAA
txa3292	LG-01	24.93	region	87.2-93.8	<2.0	txa3298		E-AGT M-CGG
txa2748	LG-01	22.78	region	87.2-89.9	>2.0	txa3299		E-ACC M-CAA
txa80	**LG-01**	**anchor**	**framework**	**89.9**	-	-		E-CAA M-CAT
txa15429	LG-01	25.22	unique	89.9-93.8	>2.0	txa80		P-CTC M-CAC
txa6779	LG-01	29.42	unique	89.9-93.8	>2.0	txa80		P-CTC M-CGG
txa15192	LG-01	24.64	unique	89.9-93.8	>2.0	txa80		P-CGT M-CAA
txa15200	LG-01	22.2	unique	89.9-93.8	>2.0	txa80		P-CGT M-CTA
txa15176	LG-01	29.42	region	89.9-93.8	>2.0	txa80		P-CGT M-CCG
txp418b	LG-01	26.15	region	89.9-93.8	>2.0	txa80		SSR
txa15414	LG-01	25.56	region	89.9-93.8	>2.0	txa80		E-GAA M-CGT
txa2738	**LG-01**	**17.98**	**framework**	**93.8**	-	-		E-ACC M-CAG
txa15358	LG-01	26.74	region	93.8-95.9	>2.0	txa2738		E-TGA M-CAA
txa1899	LG-01	26.44	unique	93.8-95.9	>2.0	txa2738		E-ACC M-CAC
txa6361	LG-01	28.52	unique	93.8-95.9	>2.0	txa2738		P-AGA M-CGG
txa2270	**LG-01**	**22.2**	**framework**	**95.9**	-	-		E-GAA M-CGT
txa15368	LG-01	19.39	unique	95.9-109.9	>2.0	txa2270		E-TGA M-CTA
txa15190	LG-01	16.91	region	95.9-109.9	>2.0	txa2270		P-CGT M-CAA
txa15421	LG-01	9.18	unique	95.9-109.9	>2.0	txa2270		P-CTC M-CTA
txa283	**LG-01**	**21.92**	**framework**	**109.9**	-	-		E-TGA M-CAC
txa284	**LG-01**	**21.36**	**framework**	**112**	-	-		E-TGA M-CAC
txa15386	LG-01	26.44	region	112-114.1	>2.0	txa284		E-AGT M-CGG
txa15189	**LG-01**	**22.2**	**framework**	**114.1**	-	-		P-CGT M-CAA

Table B-1, continued

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txp335	**LG-01**	**anchor**	**framework**	**120**	-	-		SSR
txa2658	**LG-01**	**17.17**	**framework**	**124.6**	-	-		E-GGA M-CTT
txa15162	**LG-01**	**11.55**	**framework**	**133.7**	-	-		P-CTC M-CGT
txp58	**LG-01**	**anchor**	**framework**	**144.7**	-	-		SSR
Y1 gene	LG-01	16.91	unique	144.7-157.9	>2.0	txp58		SSR
txa15397	LG-01	10.35	unique	144.7-157.9	>2.0	txp58	SD	P-AGA M-CGG
txa15458	**LG-01**	**25.85**	**framework**	**157.9**	-	-		E-CAA M-CAC
txa15408	LG-01	22.2	region	157.9-161	>2.0	txa15458		E-ACC M-CTA
txa15234	**LG-01**	**7.32**	**framework**	**161**	-	-		E-ACC M-CAC
txa15045	**LG-01**	**21.08**	**framework**	**163.4**	-	-		E-GGA M-CCA
txa15363	LG-01	29.12	unique	163.4-167.3	>2.0	txa15045		E-TGA M-CAA
txa15362	**LG-01**	**13.53**	**framework**	**167.3**	-	-		E-TGA M-CAA
txp319	**LG-01**	**anchor**	**framework**	**177.2**	-	-		SSR
txa15035	**LG-01**	**20.61**	**framework**	**188.7**	-	-		E-GGA M-CTT
txa15398	**LG-01**	**21.92**	**framework**	**190.8**	-	-		P-AGA M-CGG
txa15402	**LG-01**	**23.35**	**framework**	**192.9**	-	-		P-CTC M-CGG
txa15480	LG-01	7.14	unique	192.9-201.4	>2.0	txa15402		E-GGA M-CTA
txa15077	**LG-01**	**12.34**	**framework**	**201.4**	-	-		E-CTG M-CAA

Markers in bold are framework markers and non bold markers are placed markers. Placed markers are categorized as unique or region depending on if the marker could be placed only between two markers (unique) or between more than two markers (region). Marker names, LOD score of the assignments of each marker to LG-01 is shown, cM positions on LG-01, LOD score of the placed markers assignment to each bin, leftmost marker, and the primer combination used to amplify each AFLP marker. ‘Txa’ markers are markers that are AFLPs and ‘txp’ markers are markers that are SSRs. SD signifies that the marker exhibited segregation distortion in the population. Placed markers shown in red have a LOD score less than two when they were placed between framework markers. ‘Anchor’ markers are those markers used to link those markers to specific chromosomes. ‘Txi’ markers are those markers that contain insertions and deletions. The Y1 gene amplifies a region that contains a transposon insertion in some lines. ‘Cup’ markers are SSR markers created by Cornell University scientists (Schloss *et al.* 2002).

Table B-2 BTx642/RTx7000 LG-02 markers.

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txp96	**LG-02**	**anchor**	**framework**	**0**	**-**	-		SSR
txa15303	**LG-02**	**18.57**	**framework**	**7.1**	-	-		E-CAA M-CAT
txa15194	LG-02	11.32	unique	7.1-10.2	>2.0	txa15303		P-CGT M-CAA
txa904	**LG-02**	**24.64**	**framework**	**10.2**	-	-		E-CAA M-CCC
txa15452	LG-02	29.42	unique	10.2-12.3	>2.0	txa904		E-AGT M-CCC
txa15265	**LG-02**	**19.39**	**framework**	**12.3**	-	-		E-GGA M-CTT
txa15331	**LG-02**	**14.27**	**framework**	**18.7**	-	-		E-GGA M-CGT
txa15431	LG-02	20.61	unique	18.7-35.9	>2.0	txa15331		P-CTC M-CAC
txp304	**LG-02**	**anchor**	**framework**	**35.9**	-	-		SSR
txa15435	LG-02	13.28	unique	35.9-82.7	>2.0	txp304		E-GAA M-CCA
txa15043	**LG-02**	**19.66**	**framework**	**82.7**	-	-	SD	E-AGT M-CAG
txa15474	LG-02	29.42	region	82.7-86	>2.0	txa15043		E-TAC M-CAC
txa15425	LG-02	29.12	region	82.7-86	>2.0	txa15043		P-CTC M-CAT
txa15336	LG-02	28.82	region	82.7-86	>2.0	txa15043		E-CAA M-CCC
txa15323	LG-02	29.12	region	82.7-86	>2.0	txa15043		E-TGA M-CAC
txa15260	LG-02	24.64	region	82.7-86	>2.0	txa15043		E-GGA M-CTT
txa607	LG-02	29.42	region	82.7-86	>2.0	txa15043		E-TGA M-CTA
txa15231	LG-02	29.12	region	82.7-86	>2.0	txa15043		E-ACC M-CAA
txa3183	LG-02	29.42	region	82.7-86	>2.0	txa15043		E-AGT M-CAT
txa3223	LG-02	anchor	region	82.7-86	>2.0	txa15043		E-AGT M-CCG
txa15214	LG-02	28.82	region	82.7-86	>2.0	txa15043		P-CTC M-CAA
txa2125	LG-02	26.74	region	82.7-86	>2.0	txa15043		E-GAA M-CAA
txa15199	LG-02	29.42	region	82.7-86	>2.0	txa15043		P-CGT M-CTA
txa15017	LG-02	28.82	region	82.7-86	>2.0	txa15043		E-CAA M-CAG
txa15099	LG-02	24.93	region	82.7-86	>2.0	txa15043		E-TGA M-CAC
txa15067	LG-02	29.12	region	82.7-86	>2.0	txa15043		E-CAA M-CCC
txa2659	LG-02	29.42	unique	82.7-86	>2.0	txa15043		E-GGA M-CTT
Xcup74	LG-02	anchor	region	82.7-86	>2.0	txa15043		SSR
txa274	LG-02	29.42	region	82.7-86	>2.0	txa15043		E-TGA M-CAA
txa2592	LG-02	26.74	region	82.7-86	>2.0	txa15043		E-GGA M-CTA
txa3215	LG-02	29.12	region	82.7-86	>2.0	txa15043		E-AGT M-CCC
txa6231	LG-02	24.05	region	82.7-86	>2.0	txa15043		P-CTC M-CGT
txa3283	LG-02	26.44	unique	82.7-86	>2.0	txa15043		E-AGT M-CGG
txa3182	LG-02	29.12	unique	82.7-86	>2.0	txa15043		E-AGT M-CAT
txa3289	LG-02	26.44	region	82.7-86	>2.0	txa15043	SD	E-AGT M-CGG
txa2889	LG-02	28.52	region	82.7-86	>2.0	txa15043		E-ACC M-CGC
txa15273	LG-02	29.12	unique	82.7-86	>2.0	txa15043		E-CAA M-CAG
txa15400	LG-02	29.42	unique	82.7-86	>2.0	txa15043		P-CTC M-CGG
txa2035	LG-02	29.42	unique	82.7-86	>2.0	txa15043		E-GGA M-CCA
txa15409	LG-02	26.44	region	82.7-86	>2.0	txa15043		E-ACC M-CTA
txa15224	LG-02	29.42	unique	82.7-86	>2.0	txa15043		P-CTC M-CTA
txa2724	**LG-02**	**28.82**	**framework**	**86**	-	-		E-ACC M-CAC

Table B-2, continued

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txa15434	LG-02	28.22	unique	86-88.7	>2.0	txa2724		E-GAA M-CCA
txa15419	LG-02	28.82	region	86-88.7	>2.0	txa2724		E-ACC M-CAA
txa15175	LG-02	24.93	unique	86-88.7	>2.0	txa2724		P-CGT M-CCG
txa15054	LG-02	24.35	unique	86-88.7	>2.0	txa2724		E-CAA M-CAT
txa1921	LG-02	28.52	unique	86-88.7	>2.0	txa2724		E-ACC M-CAG
txa2040	LG-02	26.74	unique	86-88.7	>2.0	txa2724		E-GGA M-CCA
txa2715	**LG-02**	**20.05**	**framework**	**88.7**	-	-		E-ACC M-CAC
txa3778	**LG-02**	**17.18**	**framework**	**92.6**	-	-		E-TAC M-CTC
txa15191	LG-02	18.57	region	92.6-106.9	>2.0	txa3778		P-CGT M-CAA
txp464b	**LG-02**	**11.34**	**framework**	**106.9**	-	-	SD	SSR
txp430	**LG-02**	**anchor**	**framework**	**114.2**	-	-		SSR
txa15328	LG-02	29.12	unique	114.2-117.6	>2.0	txp430		E-GGA M-CGT
txa15233	LG-02	24.96	region	114.2-117.6	>2.0	txp430		E-ACC M-CAC
txa15262	LG-02	26.74	unique	114.2-117.6	>2.0	txp430		E-GGA M-CTT
txa592	LG-02	28.82	unique	114.2-117.6	>2.0	txp430		E-TGA M-CTA
txa2593	LG-02	27.92	unique	114.2-117.6	>2.0	txp430		E-GGA M-CTA
txa3779	**LG-02**	**18.84**	**framework**	**117.6**	-	-		E-TAC M-CTC
txa15305	LG-02	26.44	region		>2.0	txa3779		E-CAA M-CAT
txa614	LG-02	28.52	region		>2.0	txa3779		E-TGA M-CAA
txa15070	LG-02	20.61	unique		>2.0	txa3779		E-CAA M-CCC
txa2938	LG-02	7.54	region		>2.0	txa3779		E-ACC M-CTA
txa2570	**LG-02**	**28.82**	**framework**	**130.3**	-	-		E-GGA M-CGT
txa15286	LG-02	24.93	region		>2.0	txa2570		E-CTG M-CTA
txa6242	LG-02	26.74	unique			txa2570		P-CTC M-CGT
txa15460	LG-02	28.52	unique		>2.0	txa2570		E-CAA M-CAC
txa634	**LG-02**	**26.74**	**framework**	**135.6**	-	-		E-TGA M-CAA
txa15001	LG-02	23.76	unique		>2.0	txa634		E-ACC M-CAA
txp7	**LG-02**	**8.13**	**framework**	**141.9**	-	-	SD	SSR
txa15393	LG-02	22.78	region		>2.0	txp7		P-AGA M-CGG
txa15394	LG-02	23.47	region		>2.0	txp7		P-AGA M-CGG
txa15143	**LG-02**	**6.59**	**framework**	**157.9**	-	-	SD	E-GAA M-CGT
txa15132	**LG-02**	**9.84**	**framework**	**168.6**	-	-		E-ACC M-CTA

Markers in bold are framework markers and non bold markers are placed markers. Placed markers are categorized as unique or region depending on if the marker could be placed only between two markers (unique) or between more than two markers (region).Marker names, LOD score of the assignments of each marker to LG-02 is shown, cM positions on LG-02, LOD score of the placed markers assignment to each bin, leftmost marker, and the primer combination used to amplify each AFLP marker. 'Txa' markers are markers that are AFLPs and 'txp' markers are markers that are SSRs. SD signifies that the marker exhibited segregation distortion in the population. Placed markers shown in red have a LOD score less than two when they were placed between framework markers. 'Anchor' markers are those markers used to link those markers to specific chromosomes. 'Cup' markers are SSR markers created by Cornell University scientists (Schloss *et al.* 2002).

Table B-3 BTx642/RTx7000 LG-03 markers.

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txp496	**LG-03**	**8.13**	**framework**	**0**	-	-		SSR
txa15013	**LG-03**	**10.57**	**framework**	**12.9**	-	-		E-AGT M-CAG
txa5895	**LG-03**	**23.06**	**framework**	**22.4**	-	-		E-TAC M-CAT
txa6263	LG-03	23.06	unique	22.4-25.7	>2.0	txa5895		P-CTC M-CTA
txa261	**LG-03**	**anchor**	**framework**	**25.7**	-	-		E-TGA M-CAA
txa15445	LG-03	23.06	unique	25.7-31	>2.0	txa261		E-AGT M-CCG
txa15047	LG-03	18.24	unique	25.7-31	>2.0	txa261		E-AGT M-CGG
txa15048	**LG-03**	**24.93**	**framework**	**31.2**	-	-		E-AGT M-CGG
txa15448	**LG-03**	**20.33**	**framework**	**34.1**	-	-		E-ACC M-CGC
txa15446	LG-03	29.12	unique	34.1-39.2	>2.0	txa15448		E-ACC M-CGC
txa15106	LG-03	24.93	unique	34.1-39.2	>2.0	txa15448		E-TGA M-CTA
txa2734	**LG-03**	**8.96**	**framework**	**39.2**	-	-		E-ACC M-CAG
txa15447	LG-03	26.74	unique	39.2-41.3	>2.0	txa2734		E-ACC M-CGC
txa2598	**LG-03**	**21.92**	**framework**	**41.3**				E-GGA M-CTA
txa15024	LG-03	23.35	unique	41.3-49.7	>2.0	txa2598		E-GGA M-CGT
txa15287	LG-03	21.92	unique	41.3-49.7	>2.0	txa2598		E-CTG M-CTA
txa15475	**LG-03**	**24.93**	**framework**	**49.7**	-	-		E-TAC M-CAC
txa15113	LG-03	20.05	region	41.3-50.9	<2.0	txa15475	SD	E-TAC M-CTC
txa15187	**LG-03**	**24.64**	**framework**	**50.9**	-	-		P-CGT M-CGT
txa6085	LG-03	11.03	unique	50.9-54.6	>2.0	txa15187		P-CTC M-CAT
txa15071	LG-03	26.15	unique	50.9-54.6	>2.0	txa15187		E-CAA M-CCC
txa3552	**LG-03**	**19.39**	**framework**	**54.6**	-	-		E-CTG M-CTA
txa2122	LG-03	28.22	region	54.6-57.5	>2.0	txa3552		E-GAA M-CAA
txa15051	LG-03	26.15	region	54.6-57.5	>2.0	txa3552		E-CAA M-CAT
txa6809	**LG-03**	**22.2**	**framework**	**57.5**	-	-	SD	P-CTC M-CGT
txp485	**LG-03**	**14.18**	**framework**	**62.7**	-	-	SD	SSR
txa15010	LG-03	9.68	unique	62.7-69.5	>2.0	txp485		E-ACC M-CAC
txa15468	LG-03	24.93	region	62.7-69.5	>2.0	txp485	SD	E-ACC M-CAT
txa15467	LG-03	21.92	region	62.7-69.5	>2.0	txp485		E-ACC M-CAT
txa15160	**LG-03**	**21.64**	**framework**	**69.5**	-	-		P-CTC M-CGT
txa6027	LG-03	11.39	unique	69.5-72.2	>2.0	txa15160	SD	P-AGA M-CAC
txa15412	**LG-03**	**26.74**	**framework**	**72.2**	-	-		P-CTC M-CAA
txa15436	**LG-03**	**18.3**	**framework**	**74.9**	-	-	SD	E-GAA M-CCA
txa15479	**LG-03**	**18.24**	**framework**	**80.6**	-	-	SD	E-GGA M-CAT
txa15289	LG-03	23.35	region	80.6-85.1	>2.0	txa15479	SD	E-CTG M-CAA
txa15112	LG-03	28.82	region	80.6-85.1	>2.0	txa15479	SD	E-TAC M-CTC
txa918	LG-03	27.04	region	80.6-85.1	>2.0	txa15479	SD	E-GGA M-CAT
txa15000	LG-03	28.82	unique	80.6-85.1	>2.0	txa15479	SD	E-ACC M-CAA
txa6161	LG-03	27.04	region	80.6-85.1	>2.0	txa15479	SD	P-CTC M-CCG
txa205	LG-03	29.42	unique	80.6-85.1	>2.0	txa15479	SD	E-CAA M-CTC
txa2030	**LG-03**	**11.03**	**framework**	**85.1**	-	-	SD	E-GGA M-CCA
txa15018	LG-03	28.82	unique	85.1-88.7	>2.0	txa2030	SD	E-CAA M-CAG
txa15136	LG-03	29.12	region	85.1-88.7	>2.0	txa2030	SD	E-GAA M-CAA
txa15456	LG-03	28.52	region	85.1-88.7	>2.0	txa2030	SD	E-CAA M-CAC
txa15138	LG-03	29.12	region	85.1-88.7	>2.0	txa2030	SD	E-GAA M-CAA
txa15100	LG-03	13.4	region	80.6-88.7	<2.0	txa2030		E-TGA M-CAC
txa15066	LG-03	17.98	region	85.1-88.7	>2.0	txa2030	SD	E-CAA M-CCC

Figure B-3, continued

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txa15041	LG-03	29.42	region	85.1-88.7	>2.0	txa2030	SD	E-AGT M-CAT
txa5349	LG-03	29.42	region	85.1-88.7	>2.0	txa2030	SD	E-CTG M-CTA
txa6364	LG-03	29.12	region	85.1-88.7	>2.0	txa2030	SD	P-AGA M-CGG
txa3637	LG-03	27.04	region	85.1-88.7	>2.0	txa2030	SD	E-TAC M-CAC
txa15033	**LG-03**	**22.2**	**framework**	**88.7**	-	-	SD	E-GGA M-CTA
Sb5_236	LG-03	17.44	unique	88.7-91.7	>2.0	txa15033	SD	SSR
txa4147	**LG-03**	**23.47**	**framework**	**91.7**	-	-	SD	P-CGT M-CTA
txa15432	LG-03	26.74	region	91.7-97.3	>2.0	txa4147		P-CTC M-CAC
txp31	**LG-03**	**anchor**	**framework**	**97.3**	-	-	SD	SSR
txa3294	**LG-03**	**24.64**	**framework**	**98.4**	-			E-AGT M-CGG
txa15167	LG-03	21.64	unique	98.4-101.3	>2.0	txa3294		P-CTC M-CGT
Seed Colo	**LG-03**	**20.33**	**framework**	**101.3**	-	-		morphological
txa15185	LG-03	28.82	unique	101.3-105.5	>2.0	Seed Color		P-CGT M-CGT
txp2	**LG-03**	**13.78**	**framework**	**105.5**	-	-		SSR
txa15308	LG-03	29.42	unique	105.5-112.5	>2.0	txp2		E-CAA M-CTC
txa15227	**LG-03**	**20.89**	**framework**	**112.5**	-	-		E-AGT M-CAG
txa255	**LG-03**	**16.64**	**framework**	**115.2**	-	-		E-TGA M-CAA
txa2120	LG-03	29.12	unique	115.2-120.3	>2.0	txa255		E-GAA M-CAA
txa3296	**LG-03**	**23.06**	**framework**	**120.3**	-	-		E-AGT M-CGG
txa15006	LG-03	25.85	unique	120.3-122.2	>2.0	txa3296		E-ACC M-CAA
txp114	**LG-03**	**anchor**	**framework**	**122.2**	-	-		SSR
txa15257	LG-03	25.85	region	122.2-126.4	>2.0	txp114		E-GGA M-CTA
txa2961	LG-03	18.3	unique	122.2-126.4	>2.0	txp114		E-ACC M-CTA
txp439	**LG-03**	**21.08**	**framework**	**126.4**	-	-		SSR
txa4063	LG-03	20.33	unique	126.4-128.8	>2.0	txp439		P-CGT M-CGT
txp446	**LG-03**	**25.82**	**framework**	**128.8**	-	-		SSR
txa4727	LG-03	18.51	region	128.8-131.7	>2.0	txp446		P-CGT M-CAA
txa15418	LG-03	27.04	unique	128.8-131.7	>2.0	txp446		E-ACC M-CAG
txp442	LG-03	16.41	unique	128.8-131.7	>2.0	txp446		SSR
txa15371	**LG-03**	**21.73**	**framework**	**131.7**	-	-		E-TGA M-CTA
txa15325	LG-03	29.12	unique	131.7-136.2	>2.0	txa15371		E-TGA M-CAC
txa15196	LG-03	27.62	unique	131.7-136.2	>2.0	txa15371		P-CGT M-CAA
txp447	LG-03	27.02	unique	131.7-136.2	>2.0	txa15371		SSR
txp285	**LG-03**	**anchor**	**framework**	**136.2**	-	-		SSR
txa1717	LG-03	26.74	unique	136.2-138.3	>2.0	txp285		E-GGA M-CTT
txp448	LG-03	24.08	unique	136.2-138.3	>2.0	txp285		SSR
txp38	LG-03	anchor	unique	136.2-138.3	>2.0	txp285		SSR
txp449	**LG-03**	**21.42**	**framework**	**138.3**	-	-		SSR
txa4964	**LG-03**	**21.92**	**framework**	**140.4**	-	-		P-CGT M-CTA
txp34	**LG-03**	**anchor**	**framework**	**144.3**	-	-		SSR
txa15215	**LG-03**	**18.24**	**framework**	**151.3**	-	-		P-CTC M-CAA
txa15149	**LG-03**	**12.57**	**framework**	**155.2**			SD	P-CTC M-CCG
txa15390	**LG-03**	**9.62**	**framework**	**165.9**	-	-	SD	E-GGA M-CCA
txa15253	LG-03	7.17	unique	off-end	>2.0	txa15390	SD	E-GGA M-CTA
txa15040	LG-03	6.62	region	off-end	>2.0	txa15390		E-GGA M-CTT

Markers in bold are framework markers and non bold markers are placed markers. Placed markers are categorized as unique or region depending on if the marker could be placed only between two markers (unique) or between more than two markers (region). Marker names, LOD score of the assignments of each marker to LG-03 is shown, cM positions on LG-03, LOD score of the placed markers assignment to each bin, leftmost marker, and the primer combination used to amplify each AFLP marker. 'Txa' markers are markers that are AFLPs and 'txp' markers are markers that are SSRs. SD signifies that the marker exhibited segregation distortion in the population. Placed markers shown in red have a LOD score less than two when they were placed between framework markers. 'Anchor' markers are those markers used to link those markers to specific chromosomes. 'Seed Color' is a morphological marker as BTx642 as lemon yellow seeds and RTx7000 has seed red in color.

Table B-4 BTx642/RTx7000 LG-04 markers.

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txp504	**LG-04**	**anchor**	**framework**	**0**	-	-	SD	SSR
txa15459	LG-04	18.84	unique	0-6.2	>2.0	txp504		E-CAA M-CAC
txa2121	**LG-04**	**13.43**	**framework**	**6.2**	-	-		E-GAA M-CAA
txa1895	LG-04	7.72	region	6.2-27.4	<2.0	txa2121		E-ACC M-CAC
txa15369	LG-04	29.12	unique	6.2-27.4	>2.0	txa2121		E-TGA M-CTA
txa15415	LG-04	25.22	unique	6.2-27.4	>2.0	txa2121		P-CTC M-CAA
txa2669	**LG-04**	**anchor**	**framework**	**27.4**	-	-		E-GGA M-CTT
txa15366	LG-04	23.06	unique	27.4-30.1	>2.0	txa2669		E-TGA M-CAA
txa2966	**LG-04**	**20.33**	**framework**	**30.1**	-	-		E-ACC M-CTA
txa15225	LG-04	24.64	region	30.1-33.4	>2.0	txa2966		P-CTC M-CTA
txa2676	**LG-04**	**19.12**	**framework**	**33.4**	-	-		E-GGA M-CTT
txa15211	LG-04	24.64	region	33.4-50.1	>2.0	txa2676		P-CTC M-CAA
txa15072	LG-04	19.66	region	33.4-50.1	>2.0	txa2676		E-CAA M-CCC
txa6561	**LG-04**	**24.64**	**framework**	**50.1**	-	-		P-CTC M-CAA
txa15374	LG-04	23.64	region	50.1-52.9	>2.0	txa6561		P-CTC M-CAA
txa2680	**LG-04**	**26.74**	**framework**	**52.9**	-	-		E-GGA M-CTT
txa15015	LG-04	28.52	region	52.9-54.5	>2.0	txa2680		E-AGT M-CAT
txa158	LG-04	29.42	region	52.9-54.5	>2.0	txa2680		E-CAA M-CCC
txa15140	LG-04	29.12	region	52.9-54.5	>2.0	txa2680		E-GAA M-CAA
txa15123	LG-04	29.42	region	52.9-54.5	>2.0	txa2680		E-ACC M-CAG
txa15056	LG-04	28.52	region	52.9-54.5	>2.0	txa2680		E-CAA M-CAT
txa15022	LG-04	29.12	region	52.9-54.5	>2.0	txa2680		E-GGA M-CGT
txa2567	LG-04	29.12	region	52.9-54.5	>2.0	txa2680		E-GGA M-CGT
txa2029	LG-04	29.12	region	52.9-54.5	>2.0	txa2680		E-GGA M-CAT
txa287	LG-04	25.85	region	52.9-54.5	>2.0	txa2680		E-TGA M-CAC
txa2713	LG-04	anchor	region	52.9-54.5	>2.0	txa2680		E-ACC M-CAC
txp12	**LG-04**	**anchor**	**framework**	**54.5**	-	-		SSR
txa15206	LG-04	24.64	unique	54.5-72.4	>2.0	txp12		P-CTC M-CTA
txa15373	LG-04	16.15	region	54.5-72.4	>2.0	txp12		P-CTC M-CAA
txa15110	LG-04	8.97	unique	54.5-72.4	>2.0	txp12		E-TAC M-CTC
txa15037	LG-04	7.93	region	54.5-72.4	>2.0	txp12	SD	E-GGA M-CTT
txa15062	**LG-04**	**27.04**	**framework**	**72.4**	-	-		E-CAA M-CTC
txa27	LG-04	25.85	region	72.4-80.2	>2.0	txa15062		E-CAA M-CAC
txa15121	LG-04	14.02	region	72.4-80.2	>2.0	txa15062		E-ACC M-CAG
txa2186	LG-04	27.92	unique	72.4-80.2	>2.0	txa15062		E-GAA M-CCA
txa6091	LG-04	26.74	region	72.4-80.2	>2.0	txa15062		P-CTC M-CAT
txa2017	**LG-04**	**6.59**	**framework**	**80.2**	-	-		E-GGA M-CAT
txa15360	LG-04	26.44	unique	80.2-94.3	>2.0	txa2017		E-TGA M-CAA
txp327	**LG-04**	**anchor**	**framework**	**94.3**	-	-		SSR
txp60	LG-04	22.89	unique	94.3-95.9	>2.0	txp327		SSR
txa250	**LG-04**	**24.96**	**framework**	**95.9**	-	-		E-TGA M-CAA
txp51	**LG-04**	**anchor**	**framework**	**99.2**	-	-		SSR
txa15285	**LG-04**	**14.93**	**framework**	**104.9**	-	-		E-CTG M-CTA
txa15251	**LG-04**	**13.4**	**framework**	**111.3**	-	-		E-GGA M-CTA
txa15249	**LG-04**	**6.25**	**framework**	**118.3**	-	-		E-GGA M-CTA
txa15019	**LG-04**	**16.64**	**framework**	**123.2**	-	-		E-CAA M-CAG
txa15183	**LG-04**	**21.36**	**framework**	**125.5**	-	-		P-CGT M-CGT
txa15404	LG-04	21.64	unique	125.5-131.3	>2.0	txa15183		P-CTC M-CGG
txa15304	**LG-04**	**7.34**	**framework**	**131.3**	-	-		E-CAA M-CAT

Markers in bold are framework markers and non bold markers are placed markers. Placed markers are categorized as unique or region depending on if the marker could be placed only between two markers (unique) or between more than two markers (region). Marker names, LOD score of the assignments of each marker to LG-04 is shown, cM positions on LG-04, LOD score of the placed markers assignment to each bin, leftmost marker, and the primer combination used to amplify each AFLP marker. 'Txa' markers are markers that are AFLPs and 'txp' markers are markers that are SSRs. SD signifies that the marker exhibited segregation distortion in the population. Placed markers shown in red have a LOD score less than two when they were placed between framework markers. 'Anchor' markers are those markers used to link those markers to specific chromosomes.

Table B-5 BTx642/RTx7000 LG-05 markers.

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txa6833	LG-05	22.31	region	off-end	>2.0	-		P-CTC M-CTA
Y12464	**LG-05**	**13.18**	**framework**	**0**				SSR
txa6019	**LG-05**	**anchor**	**framework**	**6.4**				P-AGA M-CAC
txa2022	LG-05	26.44	region	6.4-22.5	>2.0	txa6019		E-GGA M-CAT
txp30	**LG-05**	**anchor**	**framework**	**22.5**				SSR
txa3659	**LG-05**	**16.91**	**framework**	**27**				E-TAC M-CAT
txa15365	**LG-05**	**23.64**	**framework**	**28.6**				E-TGA M-CAA
txa15159	LG-05	16.64	region	28.6-38.5	>2.0	txa15365		P-CTC M-CGT
txa15254	**LG-05**	**9.62**	**framework**	**38.5**				E-GGA M-CTA
txa15451	**LG-05**	**18.51**	**framework**	**45.5**				E-AGT M-CCC
txa15424	LG-05	17.17	region	45.5-52.5	>2.0	txa15451		P-CTC M-CAT
txa15291	LG-05	20.61	unique	45.5-52.5	>2.0	txa15451		E-CTG M-CAA
txa15188	LG-05	15.89	unique	45.5-52.5	>2.0	txa15451		P-CGT M-CGT
txa15346	**LG-05**	**20.89**	**framework**	**52.5**				E-TGA M-CAA
txa15440	LG-05	26.44	region	52.5-57.2	>2.0	txa15346		E-GGA M-CAT
txa15324	LG-05	23.06	unique	52.5-57.2	>2.0	txa15346		E-TGA M-CAC
txp225	**LG-05**	**24.35**	**framework**	**57.2**				SSR
txa15407	LG-05	17.98	region	57.2-62.6	>2.0	txp225		E-TAC M-CTC
txa6270	LG-05	24.35	region	57.2-62.6	>2.0	txp225		P-CTC M-CTA
txa597	LG-05	14.93	region	57.2-62.6	>2.0	txp225		E-TGA M-CTA
txa2038	**LG-05**	**24.05**	**framework**	**62.6**				E-GGA M-CCA
txa2677	LG-05	29.42	unique	62.6-63.6	>2.0	txa2038		E-GGA M-CTT
txa15355	LG-05	29.12	unique	62.6-63.6	>2.0	txa2038		E-GAA M-CAA
txa15430	LG-05	29.42	unique	62.6-63.6	>2.0	txa2038		P-CTC M-CAC
txa15337	LG-05	29.42	unique	62.6-63.6	>2.0	txa2038		E-CAA M-CCC
txa15270	LG-05	29.12	unique	62.6-63.6	>2.0	txa2038		E-CAA M-CAG
txa15198	LG-05	29.12	unique	62.6-63.6	>2.0	txa2038		P-CGT M-CTA
txa2894	LG-05	27.92	unique	62.6-63.6	>2.0	txa2038		E-ACC M-CGC
txp15	**LG-05**	**anchor**	**framework**	**63.6**				SSR
txa15036	LG-05	29.12	unique	63.6-66.9	>2.0	txp15		E-GGA M-CTT
txa15131	LG-05	29.42	unique	63.6-66.9	>2.0	txp15		E-ACC M-CTA
txa15462	LG-05	28.82	unique	63.6-66.9	>2.0	txp15		E-CAA M-CAC
txa15327	LG-05	27.92	unique	63.6-66.9	>2.0	txp15		E-GGA M-CGT
txa15406	LG-05	26.74	region	63.6-66.9	>2.0	txp15		E-CAA M-CTC
txa15274	LG-05	26.44	region	63.6-66.9	>2.0	txp15		E-CAA M-CAG
txa15229	LG-05	29.42	unique	63.6-66.9	>2.0	txp15		E-AGT M-CAT
txa6484	LG-05	24.64	region	63.6-66.9	>2.0	txp15		E-TAC M-CTC
txa15079	LG-05	24.64	region	63.6-66.9	>2.0	txp15		E-CTG M-CAA
txa910	LG-05	27.04	region	63.6-66.9	>2.0	txp15		E-CAA M-CCC
txa15030	LG-05	27.92	unique	63.6-66.9	>2.0	txp15		E-GGA M-CTA
txa15417	LG-05	25.56	region	63.6-66.9	>2.0	txp15		P-CGT M-CCG

Table B-5, continued

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txa15179	**LG-05**	**19.39**	**framework**	**66.9**				P-CGT M-CGT
txa15178	LG-05	10.53	region	66.9-71.4	>2.0	txa15179	SD	P-CGT M-CGT
txa290	**LG-05**	**15.19**	**framework**	**71.4**				E-TGA M-CAC
txa1637	**LG-05**	**23.06**	**framework**	**73**				E-GGA M-CTA
txa15416	LG-05	29.12	unique	73-76.9	>2.0	txa1637		E-GGA M-CTA
txa15410	LG-05	8.97	unique	73-76.9	>2.0	txa1637		E-TGA M-CTA
txa15032	**LG-05**	**29.12**	**framework**	**76.9**				E-GGA M-CTA
txa15122	LG-05	29.42	unique	76.9-86.8	>2.0	txa15032		E-ACC M-CAG
txa36	LG-05	24.35	region	76.9-86.8	>2.0	txa15032		E-CAA M-CAC
txa15064	**LG-05**	**12.81**	**framework**	**86.8**				E-CAA M-CTC
txa15039	**LG-05**	**22.2**	**framework**	**94.5**				E-GGA M-CTT
txa15217	LG-05	25.22	unique	94.5-96.6	>2.0	txa15039		P-CTC M-CTA
txa15016	LG-05	29.12	unique	94.5-96.6	>2.0	txa15039		E-AGT M-CAT
txa6924	**LG-05**	**17.71**	**framework**	**96.6**				E-AGT M-CAG
txa15320	LG-05	20.05	region	96.6-100.5	>2.0	txa6924		E-TGA M-CAC
txp123	LG-05	anchor	region	96.6-100.5	>2.0	txa6924		SSR
txp262	**LG-05**	**13.16**	**framework**	**100.5**				SSR
txa15354	**LG-05**	**14.02**	**framework**	**107.6**				E-GAA M-CAA
txa5690	**LG-05**	**15.86**	**framework**	**117.5**				P-AGA M-CGG
txa15401	**LG-05**	**24.64**	**framework**	**121.4**				P-CTC M-CGG
txa15236	LG-05	6.59	unique	121.4-134.7	>2.0	txa15401		E-ACC M-CAC
txa15476	LG-05	28.82	unique	121.4-134.7	>2.0	txa15401		E-TAC M-CAC
txa15387	LG-05	27.04	unique	121.4-134.7	>2.0	txa15401		E-AGT M-CGG
txa15267	LG-05	23.64	unique	121.4-134.7	>2.0	txa15401		E-GGA M-CTT
txa15242	LG-05	16.91	unique	121.4-134.7	>2.0	txa15401		E-ACC M-CAG
txa15237	LG-05	26.44	region	121.4-134.7	>2.0	txa15401		E-ACC M-CAC
txa2045	LG-05		unique			txa15401		E-GGA M-CCA
txa15341	**LG-05**	**7.34**	**framework**	**134.7**			SD	E-TAC M-CAT

Markers in bold are framework markers and non bold markers are placed markers. Placed markers are categorized as unique or region depending on if the marker could be placed only between two markers (unique) or between more than two markers (region). Marker names, LOD score of the assignments of each marker to LG-05 is shown, cM positions on LG-05, LOD score of the placed markers assignment to each bin, leftmost marker, and the primer combination used to amplify each AFLP marker. 'Txa' markers are markers that are AFLPs and 'txp' markers are markers that are SSRs. SD signifies that the marker exhibited segregation distortion in the population. Placed markers shown in red have a LOD score less than two when they were placed between framework markers. 'Anchor' markers are those markers used to link those markers to specific chromosomes. Y12464 is the Genbank accession for the probe HHUK05-H (Bowers *et al.* 2003).

Table B-6 BTx642/RTx7000 LG-06 markers.

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txa5056	LG-06	26.74	region	off-end	>2.0	-		E-CTG M-CAA
txa15282	LG-06	29.12	region	off-end	>2.0			E-CTG M-CTA
txa15177	LG-06	26.74	region	off-end	>2.0	-		P-CGT M-CGT
txa15031	LG-06	21.92	region	off-end	>2.0	-		E-CAA M-CAT
txa15021	**LG-06**	**8.54**	**framework**	**0**	-	-		E-CTG M-CTA
txa15439	LG-06	14.02	unique	0-12.3	>2.0	txa15021		E-GGA M-CAT
txa7	LG-06	24.93	region	0-12.3	>2.0	txa15021		E-CAA M-CAG
txa3550	**LG-06**	**anchor**	**framework**	**12.3**	-	-		E-CTG M-CTA
txa4001	**LG-06**	**19.39**	**framework**	**15.6**	-	-		P-CGT M-CCG
txa15255	LG-06	7.74	unique	15.6-41.8	>2.0	txa4001		E-GGA M-CTA
txa15276	LG-06	15.19	unique	15.6-41.8	>2.0	txa4001		E-CTG M-CTA
txa279	LG-06	7.34	region	15.6-41.8	>2.0	txa4001	SD	E-TGA M-CAC
txa15411	LG-06	9.4	unique	15.6-41.8	>2.0	txa4001	SD	E-GGA M-CTT
txp145	**LG-06**	**anchor**	**framework**	**41.8**	-	-		SSR
txa3213	**LG-06**	**24.64**	**framework**	**44**	-	-		E-AGT M-CCC
txa15221	LG-06	24.93	unique	44-45.6	>2.0	txa3213		P-CTC M-CTA
txp274	LG-06	anchor	region	44-45.6	>2.0	txa3213		SSR
txp97	**LG-06**	**anchor**	**framework**	**45.6**	-	-		SSR
txa15443	**LG-06**	**23.64**	**framework**	**47.2**	-	-		E-GGA M-CAT
txa278	**LG-06**	**20.33**	**framework**	**49**	-	-		E-TGA M-CAC
txa15044	LG-06	28.82	unique	49-52.1	>2.0	txa278		E-GGA M-CGT
txa15318	LG-06	17.71	region	49-52.1	>2.0	txa278		E-TAC M-CTC
txa6162	LG-06	26.74	region	49-52.1	>2.0	txa278		P-CTC M-CCG
txa15481	LG-06	28.82	unique	49-52.1	>2.0	txa278		E-AGT M-CAG
txa3282	**LG-06**	**anchor**	**framework**	**52.1**	-	-		E-AGT M-CGG
txa2963	**LG-06**	**24.64**	**framework**	**55.4**	-	-		E-ACC M-CTA
txa15145	LG-06	29.12	unique	55.4-60.5	>2.0	txa2963		E-GAA M-CGT
txa15025	LG-06	16.91	unique	55.4-60.5	>2.0	txa2963		E-GGA M-CGT
txa15042	**LG-06**	**18.24**	**framework**	**60.5**	-	-		E-AGT M-CAT
txa15204	**LG-06**	**22.2**	**framework**	**62.6**	-	-		P-CGT M-CTA
txa1502	LG-06	27.04	region	62.6-71.7	>2.0	txa15204		E-GGA M-CCA
txa2039	**LG-06**	**18.24**	**framework**	**71.7**	-	-		E-GGA M-CCA
txa15259	LG-06	27.04	region	71.7-74.4	>2.0	txa2039		E-GGA M-CTT
txa15181	LG-06	27.04	region	71.7-74.4	>2.0	txa2039		P-CGT M-CGT
txa4062	**LG-06**	**23.35**	**framework**	**74.4**	-	-		P-CGT M-CGT
txp17	**LG-06**	**anchor**	**framework**	**76.2**	-	-		SSR
txa15454	LG-06	24.93	unique	76.2-79.3	>2.0	txp17		E-AGT M-CCC
txa15244	LG-06	25.22	region	76.2-79.3	>2.0	txp17		E-ACC M-CTA
txa2741	LG-06	26.74	region	76.2-79.3	-	txp17		E-ACC M-CAG
txa15442	**LG-06**	**20.33**	**framework**	**79.3**	-	-		E-GGA M-CAT

Markers in bold are framework markers and non bold markers are placed markers. Placed markers are categorized as unique or region depending on if the marker could be placed only between two markers (unique) or between more than two markers (region). Marker names, LOD score of the assignments of each marker to LG-06 is shown, cM positions on LG-06, LOD score of the placed markers assignment to each bin, leftmost marker, and the primer combination used to amplify each AFLP marker. 'Txa' markers are markers that are AFLPs and 'txp' markers are markers that are SSRs. SD signifies that the marker exhibited segregation distortion in the population. Placed markers shown in red have a LOD score less than two when they were placed between framework markers. 'Anchor' markers are those markers used to link those markers to specific chromosomes.

Table B-7 BTx642/RTx7000 LG-07 markers.

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txa600	LG-07	23.06	unique	off-end	>2.0	-		E-TGA M-CTA
txa3165	**LG-07**	**24.93**	**framework**	**0**	**-**	**-**		E-AGT M-CAG
txp36	LG-07	29.12	unique	0-1.6	>2.0	txa3165		SSR
txa15352	LG-07	26.74	region	0-1.6	>2.0	txa3165		E-GAA M-CAA
txp40	**LG-07**	**anchor**	**framework**	**1.6**	**-**	**-**		SSR
txa15438	LG-07	23.18	unique	1.6-6.7	>2.0	txp40		E-GGA M-CAT
txa23	LG-07	28.82	unique	1.6-6.7	>2.0	txp40		E-CAA M-CAC
txa3211	LG-07	23.35	unique	1.6-6.7	>2.0	txp40		E-AGT M-CCC
txp417	**LG-07**	**anchor**	**framework**	**6.7**	**-**	**-**		SSR
txa15137	**LG-07**	**16.12**	**framework**	**11.2**	**-**	**-**		E-GAA M-CAA
txa15004	**LG-07**	**15.86**	**framework**	**25**	**-**	**-**		E-ACC M-CAA
txp481	**LG-07**	**13.04**	**framework**	**29.6**	**-**	**-**		SSR
txa15247	LG-07	17.76	region	29.6-32.9	>2.0	txp481		E-ACC M-CTA
txa15364	**LG-07**	**23.06**	**framework**	**32.9**	**-**	**-**		E-TGA M-CAA
txp159	**LG-07**	**anchor**	**framework**	**37**	**-**	**-**		SSR
txp312	**LG-07**	**anchor**	**framework**	**41.8**	**-**	**-**		SSR
txa16	**LG-07**	**8.13**	**framework**	**54.1**	**-**	**-**		E-CAA M-CAG
txa15422	**LG-07**	**22.78**	**framework**	**55.7**	**-**	**-**		P-CTC M-CAT
txa15055	LG-07	24.35	region	55.7-64.8	>2.0	txa15422		E-CAA M-CAT
txa3624	LG-07	25.22	region	55.7-64.8	>2.0	txa15422		E-TAC M-CAC
txa262	**LG-07**	**10.09**	**framework**	**64.8**	**-**	**-**		E-TGA M-CAA
txa2578	LG-07	29.12	unique	64.8-69.9	>2.0	txa262		E-GGA M-CGT
txa3530	**LG-07**	**16.41**	**framework**	**69.9**	**-**	**-**		E-CTG M-CTA
txa15342	LG-07	29.42	unique	69.9-71.5	>2.0	txa3530		E-GAA M-CGT
txa2278	LG-07	29.42	unique	69.9-71.5	>2.0	txa3530		E-GAA M-CGT
txa15332	LG-07	29.12	unique	69.9-71.5	>2.0	txa3530		E-CAA M-CCC
txa15284	LG-07	27.04	unique	69.9-71.5	>2.0	txa3530		E-CTG M-CTA
txa15444	**LG-07**	**24.35**	**framework**	**71.5**	**-**	**-**		E-AGT M-CCG
txa15156	**LG-07**	**17.17**	**framework**	**75.4**	**-**	**-**		P-CTC M-CGT
txa15375	LG-07	26.44	region	off-end	>2.0	txa15156		P-CGT M-CAA

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Table B-8 BTx642/RTx7000 LG-08 markers.

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txp273	**LG-08**	**anchor**	**framework**	**0**	-	-		SSR
txa15399	LG-08	27.04	region	0-7.9	>2.0	txp273		P-CTC M-CGG
txa15367	LG-08	26.74	region	0-7.9	>2.0	txp273		E-TGA M-CTA
txa15130	LG-08	24.64	region	0-7.9	>2.0	txp273	SD	E-ACC M-CTA
txa2034	**LG-08**	**12.34**	**framework**	**7.9**	-	-		E-GGA M-CCA
txa15351	**LG-08**	**21.92**	**framework**	**15.4**	-	-		E-GAA M-CAA
txa15133	LG-08	24.64	region	15.4-17.7	>2.0	txa15351		E-GAA M-CAA
txa2594	**LG-08**	**12.57**	**framework**	**17.7**	-			E-GGA M-CTA
txa15014	LG-08	17.98	region	17.7-21.6	>2.0	txa2594		E-AGT M-CAG
txa15076	**LG-08**	**21.92**	**framework**	**21.6**	-	-		E-CAA M-CCC
Xcup47	**LG-08**	**anchor**	**framework**	**41.8**	-	-		SSR
txa15405	LG-08	28.52	unique	41.8-59.8	>2.0	Xcup47		E-ACC M-CAC
txa15427	LG-08	28.82	unique	41.8-59.8	>2.0	Xcup47		P-AGA M-CAC
txa15455	LG-08	29.42	unique	41.8-59.8	>2.0	Xcup47		E-AGT M-CCC
txa15309	LG-08	27.04	unique	41.8-59.8	>2.0	Xcup47		E-CAA M-CTC
txa6094	LG-08	29.12	unique	41.8-59.8	>2.0	Xcup47		P-CTC M-CAT
txa2602	LG-08	12.34	region	41.8-59.8	>2.0	Xcup47		E-GGA M-CTT
txa15232	LG-08	13.53	region	41.8-59.8	>2.0	Xcup47		E-ACC M-CAA
txa15053	LG-08	28.52	unique	41.8-59.8	>2.0	Xcup47		E-CAA M-CAT
txa3377	LG-08	29.12	unique	41.8-59.8	>2.0	Xcup47		E-CTG M-CAA
txa3546	**LG-08**	**29.42**	**framework**	**59.8**	-	-		E-CTG M-CTA
txa15453	LG-08	29.42	region	59.8-65.5	>2.0	txa3546		E-AGT M-CCC
txa15361	LG-08	24.05	region	59.8-65.5	>2.0	txa3546	SD	E-TGA M-CAA
txa15359	LG-08	29.12	region	59.8-65.5	>2.0	txa3546		E-TGA M-CAA
txa2024	LG-08	29.42	region	59.8-65.5	>2.0	txa3546		E-GGA M-CAT
txa15344	LG-08	29.42	region	59.8-65.5	>2.0	txa3546		E-GAA M-CGT
txa15340	LG-08	28.82	region	59.8-65.5	>2.0	txa3546		E-TAC M-CAT
txa15312	LG-08	25.22	unique	59.8-65.5	>2.0	txa3546		E-CAA M-CTC
txa15300	LG-08	28.52	unique	59.8-65.5	>2.0	txa3546	SD	E-CAA M-CAT
txa15243	LG-08	28.82	region	59.8-65.5	>2.0	txa3546		E-ACC M-CAG
txa15238	LG-08	24.35	region	59.8-65.5	>2.0	txa3546		E-ACC M-CAC
txa6206	LG-08	29.42	region	59.8-65.5	>2.0	txa3546		P-CTC M-CGG
txa150	LG-08	29.42	region	59.8-65.5	>2.0	txa3546		E-CAA M-CCC
txa15057	**LG-08**	**7.92**	**framework**	**65.5**	-	-	SD	E-CAA M-CAT
txa15256	**LG-08**	**18.57**	**framework**	**68.8**	-	-		E-GGA M-CTA
txp354	LG-08	14.93	region	68.8-78.5	>2.0	txa15256	SD	SSR
txa2582	LG-08	28.52	unique	68.8-78.5	>2.0	txa15256		E-GGA M-CGT
txp321	**LG-08**	**anchor**	**framework**	**78.5**	-	-		SSR
txp18	LG-08	anchor	unique	78.5-81.3	>2.0	txp321		SSR
txa606	**LG-08**	**21.36**	**framework**	**81.3**	-	-		E-TGA M-CTA
txa15011	**LG-08**	**19.12**	**framework**	**84.6**	-	-		E-ACC M-CAC
txa15151	**LG-08**	**10.57**	**framework**	**93.8**	-	-		P-CTC M-CCG
txa15186	**LG-08**	**18.24**	**framework**	**97.7**	-	-		P-CGT M-CGT
txa2189	**LG-08**	**12.68**	**framework**	**105**	-	-		E-GAA M-CCA
txa15216	**LG-08**	**6.1**	**framework**	**117.2**	-	-		P-CTC M-CTA

Markers in bold are framework markers and non bold markers are placed markers. Placed markers are categorized as unique or region depending on if the marker could be placed only between two markers (unique) or between more than two markers (region). Marker names, LOD score of the assignments of each marker to LG-08 is shown, cM positions on LG-08, LOD score of the placed markers assignment to each bin, leftmost marker, and the primer combination used to amplify each AFLP marker. 'Txa' markers are markers that are AFLPs and 'txp' markers are markers that are SSRs. SD signifies that the marker exhibited segregation distortion in the population. 'Anchor' markers are those markers used to link those markers to specific chromosomes. Placed markers shown in red have a LOD score less than two when they were placed between framework markers.

Table B-9 BTx642/RTx7000 LG-09 markers.

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txa15174	LG-09	26.74	region	off-end	>2.0	-		P-CGT M-CCG
txp289	**LG-09**	**12.1**	**framework**	**0**				SSR
txa1920	**LG-09**	**26.15**	**framework**	**0.5**				E-ACC M-CAG
txa15322	LG-09	24.93	unique	.5-7.5	>2.0	txa1920		E-TGA M-CAC
txa15310	LG-09	22.2	region	.5-7.5	>2.0	txa1920		E-CAA M-CTC
txp358	**LG-09**	**anchor**	**framework**	**7.5**				SSR
txp459	**LG-09**	**anchor**	**framework**	**10.9**				SSR
txp410	**LG-09**	**20.05**	**framework**	**13.7**				SSR
txa15353	**LG-09**	**14.68**	**framework**	**19.7**				E-GAA M-CAA
txp412	**LG-09**	**anchor**	**framework**	**26.7**				SSR
txa15261	**LG-09**	**19.5**	**framework**	**33.1**				E-GGA M-CTT
txa15356	LG-09	27.62	region	33.1-40.1	>2.0	txa15261		E-GAA M-CAA
Sb6_42	LG-09	16.12	unique	33.1-40.1	>2.0	txa15261		SSR
txa15126	LG-09	24.93	region	33.1-40.1	>2.0	txa15261		E-ACC M-CAG
txp230	**LG-09**	**anchor**	**framework**	**40.1**				SSR
txa15105	LG-09	26.74	unique	40.1-43.4	>2.0	txp230		E-TGA M-CTA
txa15420	**LG-09**	**19.39**	**framework**	**43.4**				E-TGA M-CTA
txa15128	LG-09	26.74	region	off-end	>2.0	txa15420		E-ACC M-CTA

Markers in bold are framework markers and non bold markers are placed markers. Placed markers are categorized as unique or region depending on if the marker could be placed only between two markers (unique) or between more than two markers (region). Marker names, LOD score of the assignments of each marker to LG-09 is shown, cM positions on LG-09, LOD score of the placed markers assignment to each bin, leftmost marker, and the primer combination used to amplify each AFLP marker. 'Txa' markers are markers that are AFLPs and 'txp' markers are markers that are SSRs. Placed markers shown in red have a LOD score less than two when they were placed between framework markers. 'Anchor' markers are those markers used to link those markers to specific chromosomes.

Table B-10 BTx642/RTx7000 LG-10 markers.

Name	Chrom	LOD	Mapping	cM	LOD to Bin	Locus	Notes	Primer Combin.
txa15384	LG-10	21.92	region	off-end	>2.0	-		E-AGT M-CGG
txa2678	**LG-10**	**anchor**	**framework**	**0**				E-GGA M-CTT
txa3281	LG-10	26.74	region	0-33.3	>2.0	txa2678		E-AGT M-CGG
txa2730	**LG-10**	**24.64**	**framework**	**33.3**			SD	E-ACC M-CAG
txa15008	LG-10	25.85	region	33.3-41.1	>2.0	txa2730	SD	E-ACC M-CAC
txa2750	LG-10	anchor	region	33.3-41.1	>2.0	txa2730	SD	E-ACC M-CAA
txa2737	**LG-10**	**13.4**	**framework**	**41.1**			SD	E-ACC M-CAG
txa212	LG-10	27.04	region	41.1-45	>2.0	txa2737	SD	E-CAA M-CTC
txa15165	**LG-10**	**19.39**	**framework**	**45**			SD	P-CTC M-CGT
txa15423	LG-10	23.35	unique	45-52	>2.0	txa15165	SD	P-CTC M-CAT
txa15147	LG-10	29.12	unique	45-52	>2.0	txa15165	SD	E-GAA M-CGT
txa15307	LG-10	23.64	unique	45-52	>2.0	txa15165		E-CAA M-CTC
txa15026	LG-10	8.96	unique	45-52	>2.0	txa15165	SD	E-GGA M-CGT
txa15096	**LG-10**	**11.03**	**framework**	**52**			SD	E-TGA M-CAA
txa15180	LG-10	18.51	unique	52-70.9	>2.0	txa15096	SD	P-CGT M-CGT
txa15348	**LG-10**	**19.66**	**framework**	**70.9**			SD	E-GAA M-CGT
txa15157	LG-10	23.76	region	70.9-75.1	>2.0	txa15348	SD	P-CTC M-CGT
txa2128	LG-10	23.35	region	70.9-75.1	>2.0	txa15348	SD	E-GAA M-CAA
txa15218	**LG-10**	**26.44**	**framework**	**75.1**			SD	P-CTC M-CTA
txa15038	LG-10	25.22	region	75.1-78.6	>2.0	txa15218	SD	E-GGA M-CTT
txa15457	LG-10	28.82	region	75.1-78.6	>2.0	txa15218	SD	E-CAA M-CAC
txa15202	LG-10	29.42	region	75.1-78.6	>2.0	txa15218	SD	P-CGT M-CTA
txa15316	LG-10	24.64	region	75.1-78.6	>2.0	txa15218	SD	E-TAC M-CTC
txa15239	LG-10	29.42	region	75.1-78.6	>2.0	txa15218	SD	E-ACC M-CAG
txa15350	LG-10	24.93	region	75.1-78.6	>2.0	rxa15218	SD	E-GAA M-CAA
txa15343	LG-10	29.12	region	75.1-78.6	>2.0	txa15218	SD	E-GAA M-CGT
txa15241	LG-10	29.42	region	75.1-78.6	>2.0	txa15218	SD	E-ACC M-CAG
txa15321	LG-10	29.12	region	75.1-78.6	>2.0	txa15218	SD	E-TGA M-CAC
txa15299	LG-10	21.36	unique	75.1-78.6	>2.0	txa15218	SD	E-CAA M-CAT
txa15271	LG-10	28.52	region	75.1-78.6	>2.0	txa15218	SD	E-CAA M-CAG
txa15245	LG-10	29.42	region	75.1-78.6	>2.0	txa15218	SD	E-ACC M-CTA
txa15163	LG-10	29.12	region	75.1-78.6	>2.0	txa15218	SD	P-CTC M-CGT
txa15080	LG-10	29.12	region	75.1-78.6	>2.0	txa15218	SD	E-CTG M-CAA
txp217	LG-10	anchor	region	75.1-78.6	>2.0	txa15218	SD	SSR
txa6039	LG-10	anchor	region	75.1-78.6	>2.0	txa15218	SD	P-CTC M-CAA
txa156	LG-10	27.04	region	75.1-78.6	>2.0	txa15218	SD	E-CAA M-CCC
txa2048	LG-10	25.85	region	75.1-78.6	>2.0	txa15218	SD	E-GGA M-CCA
txp130	LG-10	26.12	region	75.1-78.6	>2.0	txa15218	SD	SSR
txa2947	LG-10	27.62	region	75.1-78.6	>2.0	txa15218	SD	E-ACC M-CTA
txa2281	LG-10	28.82	region	75.1-78.6	>2.0	txa15218	SD	E-GAA M-CGT
txa15258	**LG-10**	**22.2**	**framework**	**78.6**			SD	E-GGA M-CTT
txa3787	**LG-10**	**17.44**	**framework**	**83.1**			SD	E-TAC M-CTC
txa15219	LG-10	20.61	region	83.1-91.1		txa3787	SD	P-CTC M-CTA
txa15272	**LG-10**	**24.35**	**framework**	**91.1**			SD	E-CAA M-CAG
txa15220	**LG-10**	**17.17**	**framework**	**92.7**			SD	P-CTC M-CTA
Sb1_1	**LG-10**	**anchor**	**framework**	**106**				SSR
txa6280	**LG-10**	**14.93**	**framework**	**132.2**				P-CTC M-CTA
txa15050	LG-10	22.78	unique	132.2-138		txa6280		E-CAA M-CAT
Xcup7	**LG-10**	**anchor**	**framework**	**138**				SSR

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VITA

Karen Ruth Harris received a Bachelor of Science degree with a dual major in Biochemistry, Cellular, and Molecular Biology and Ecology and Evolution from the University of Tennessee at Knoxville in 2001. She entered the Biochemistry and Biophysics program at Texas A&M University in August 2001, and received her Doctor of Philosophy degree in May 2007. Her research interests include plant molecular biology, genetics, and genomics.

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