GENETIC CONTROL OF SALT TOLERANCE TRAITS IN SEASHORE PASPALUM (*PASPALUM VAGINATUM* SW.)

by

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(Under the Direction of Katrien Devos)

ABSTRACT

Seashore paspalum (*Paspalum vaginatum* Swartz) is an exceptionally salt tolerant grass species which inhabits warm, coastal areas around the world. Due to its growth habit and its ability to tolerate high levels of salt, it has been used as a turfgrass in locations near the sea and in other areas with water quality issues. Seashore paspalum's ability to thrive in saline environments makes it an important resource that could contribute as a model to the study of salt tolerance in the grasses, but also as a potential source of salt tolerance genes that could be used for increasing the salt tolerance of other species. In general, the germplasm resources of crop species, including their wild-relatives, lack significant salt tolerance. For this reason, it has been difficult to develop salt tolerant cultivars in most crops. Despite this limitation, salinity is an increasingly important contributor to yield-limiting stress worldwide and the amount of arable land which is salt-affected is increasing annually. If the salt tolerance genes of seashore paspalum could be leveraged to increase salt tolerance in other crops, that could have broad implications for agriculture. With this in mind, we conducted a series of studies on seashore paspalum with the goal of identifying the genes which condition its high level of salt tolerance. In general, we elected to employ a genetic mapping approach. To identify a pair of paspalum

lines for use as parents for a mapping population, we developed microsatellite markers and used them to complete a genetic diversity study on a panel of 90 diploid accessions. Two genetically diverse parents which were also identified as differing in salt tolerance were used to create an F1 mapping population. Using a genotyping-by-sequencing approach, we developed several thousand single nucleotide polymorphism markers and used them to construct a pair of genetic maps which were compared to the *Sorghum bicolor* and *Setaria italica* reference genomes. We screened the mapping population for a number of salt tolerance traits including sodium ion and potassium ion accumulation in leaves and the maintenance of high K^+/Na^+ ratios in leaves. We consistently identified QTL for each of these traits and used our comparative genomic analysis to identify seven total candidate genes in the two orthologous regions of sorghum. A survey of the reported functions of the closest characterized homologs of these candidates in Swiss-Prot suggested a possible role in the potassium accumulation and ion ratio traits for a high-affinity potassium transporter (HKT), while each of the positional candidates in the region associated with sodium accumulation seemed unlikely to confer the trait. A preliminary analysis of an RNA-Seq dataset generated from the parental lines confirmed expression of the HKT gene in leaves but not roots and also identified a metallothionein gene as a strong candidate for the sodium accumulation trait. Metallothionein transcripts are among the most highly expressed in both roots and leaves, show increased expression under salt, and exhibits higher expression in the paternal than maternal parent. Finally, in sorghum, a fragment with homology to the gene localizes to the region in paspalum which contains the sodium abundance QTL.

INDEX WORDS: Seashore paspalum, *Paspalum vaginatum*, halophyte, salt tolerance, genetic maps, QTL mapping, comparative genomics

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BS, Truman State University, 2008

BA, Truman State University, 2008

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2016

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May 2016

PROLOGUE

"…rivers [turned] into a desert,

flowing springs into thirsty ground,

and fruitful land into a salt waste,

because of the wickedness of those who lived there."

Psalm 107:33-34

"What's past is prologue"

The Tempest: Act 2, Scene1

ACKNOWLEDGEMENTS

I would like express my deepest gratitude to my advisor Dr. Katrien Devos, whose commitment and support made this project possible. Her encouragement and patience taught me about science but also about myself – to focus, and to "take the initiative," as she likes to say.

I would also like to thank my committee members Drs. Paul Raymer, Lisa Donovan, John Burke and Xiaoyu Zhang for their guidance in this project and the innumerable conversations we had about various and sundry other topics. I have been very fortunate to have them on my committee and on my side throughout this process.

Last but not least, I would like to thank Michael Boyd and the rest of the greenhouse staff (past and present) for looking after my plant babies and putting up with me in general.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Soil salinity is a significant issue threatening agricultural productivity around the world. Though natural geology is often the cause, saline soils resulting from irrigation are increasingly affecting farming systems dependent upon artificial water supplementation. As irrigated agriculture provides one-third of the world's food on only 15% of its cultivated land, maintaining productivity in these fields will be essential for ensuring food security for a growing global population (Flowers 2004; Munns and Tester 2008). Though enhancing salt tolerance is recognized as an important goal in plant breeding, conventional breeding programs have met with very limited success in advancing toward salt-tolerant cultivars (Flowers 2004; Jenks, Hasegawa et al. 2007; Munns, James et al. 2012). This is attributed to the complex nature of salt tolerance in crop species and the fact that substantial tolerance is rarely found in the germplasm of cultivated species (Flowers 2004, Colmer, Munns et al. 2005, Munns 2005). As such, expanding our understanding of some of the loci responsible for imparting this trait in an extremely salt-tolerant species that is closely related to many agronomically important grasses would serve to identify specific mechanisms of salt tolerance that may be relevant as well as the genes that are functionally responsible. Elucidating these relationships could move us closer to the goal of being able to breed crops with greater salt tolerance (Denby and Gehring 2005, Munns, James et al. 2012). Accomplishing these breeding goals would help to maintain food production in the face of the ever increasing amount of arable land that is becoming salt-affected.

Seashore paspalum, (*Paspalum vaginatum* Swartz) is the species we have chosen as our study system to explore salt tolerance in the grasses. Seashore paspalum is an exceptionally salt-tolerant species that has become a popular warm season turf species (Duncan and Carrow 2000). The *Paspalum* genus is in the Paspaleae, a tribe which contains the former x=10 members of the Paniceae (Morrone, Aagesen et al. 2012). It is phylogenetically closely related to both *Sorghum* and *Setaria*, so reference genomes of species in those groups should have utility for comparative mapping in *Paspalum*. Additionally, we hope that the close genetic relationship between *Paspalum* and the many grain and forage producing members of the Panicoideae increases the likelihood that the genetic loci in seashore paspalum that confer salt tolerance can be leveraged in these important relatives in the future. Our overarching goal in undertaking this research was to expand our understanding of seashore paspalum in order to identify salt tolerance QTL and potentially candidate genes that could be put forward for further characterization.

In order to achieve this aim, we surveyed the available seashore paspalum germplasm for genetic diversity and salt tolerance level in order to select parents for a mapping population. We developed and utilized 43 microsatellite markers in a panel of 90 diploid accessions from the University of Georgia collection and Germplasm Resource Information Network (GRIN). An F1 mapping population was generated and 181 individual progeny genotyped with a modified genotyping-by-sequencing approach which generated 2748 sequence tags containing SNPs. A pseudo testcross mapping strategy was used to construct a pair of parental linkage maps. The maternal map included 1262 SNP markers while the paternal map included 1092 SNP markers. In both maps, ten linkage groups were unambiguously resolved and the average spacing between markers was 1.90 cM. The genetic markers comprising these maps were compared to the *Sorghum bicolor* and *Setaria italica* genomes with BLAST. The identification of orthologous

positions allowed for an evaluation of genome structure in *Paspalum vaginatum* Sw. compared to these references. Traits related to salt tolerance were screened in the progeny array while exposed to a seawater strength irrigation solution. The traits of focus in this screen included organ-specific biomass fractions, total biomass, as well as sodium ion and potassium ion accumulation in green leaf fractions as well as potassium/sodium ion ratio (Munns and James 2003). With these data, QTL for sodium ion abundance, potassium ion abundance, and ion ratios were detected in the genomic contribution of the paternal parent. Finally, transcriptome data for roots and leaves of the parental lines under salt and freshwater conditions were evaluated. General expression patterns were consistent with organ-specificity as well as the existence of distinct transcriptional patterns in organ material from lines exposed to high salt (versus freshwater) for long durations. Finally, the expression patterns of candidate genes identified in the QTL study were evaluated as well as a survey of genes that were upregulated in response to salt exposure. An HKT6-like gene was located within the QTL region for potassium abundance and K^+/Na^+ ratio, but expression data did not identify significant differences between the parents. A group of metallothionein transcripts were among the most highly transcribed, and comparative information in sorghum indicates that one of these may map close to, if not in the QTL interval, identified for sodium abundance.

Paspalum History and Biology

Composed of about 350 species, *Paspalum* L. is one of the most species-rich genera in the grass family (Denham 2005, Zuloaga and Morrone 2005). Its members inhabit a broad range of habitat types across the Americas, but its center of diversity is the vast tropical savannah stretching through the interior of Brazil and including parts of Uruguay and Northern Argentina (Zuloaga and Morrone 2005, Rua, Speranza et al. 2010). Extending out from this region of

greatest species richness, members of *Paspalum* dominate the grassland ecosystems across the continent (Rua et al. 2010) and are common in many areas of North America as well (Chase 1929). Several species of *Paspalum* have been selected for agronomic applications including as a grain crop: *P. scrobiculatum* (kodo millet), an orphan cereal grown in south Asia; forage: *P. notatum* (bahiagrass) and *P. dilatatum* (dallisgrass), both introduced to and common in the southeastern United States (Bennett 1962); and turf: *P. vaginatum* (seashore paspalum), of note for its salt tolerance (Duncan and Carrow 2000). Traditionally allied with the x=10 Paniceae, *Paspalum* and several smaller genera have recently been assigned to the resurrected tribe Paspaleae, of the *Poaceae* sub-family Panicoideae (Morrone, Aagesen et al. 2012) (Figure 1.1). Seashore paspalum is a sexual diploid with a base chromosome number of 10.

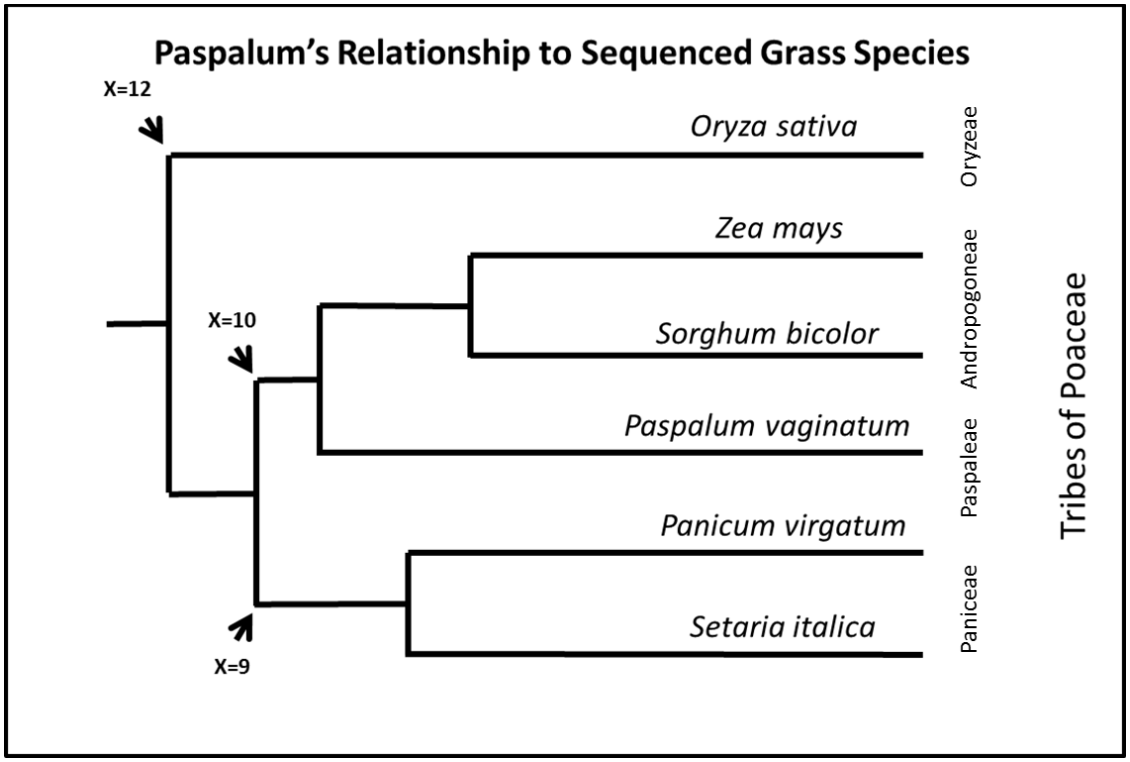

Figure 1.1 Dendrogram showing *Paspalum vaginatum* and several sequenced grasses

The model system rice and members of the Panicoideae shown; lineage-specific base chromosome numbers indicated, as are tribe names, following Morrone et al. (2012).

Seashore paspalum is self-incompatible so individuals are obligate out-crossers and highly heterozygous (Duncan and Carrow 2000). It is very closely related to *Paspalum distichum* Linnaeus which is distinguished from *P. vaginatum* by a number of morphotypical characters including pubescent glumes on the former and glabrous glumes on the latter (Echarte and Clausen 1993). This pair of species comprises the Disticha subgroup within the *Paspalum* genus (Rua, Speranza et al. 2010). By flow cytometry, the haploid genome size of *Paspalum vaginatum* has been estimated to 1pg/1C or around 960 Mb (Liu, Jarret et al. 1995). This genome estimate suggests that the genome of diploid (of seashore paspalum) is roughly equivalent to that of *Sorghum bicolor* (Laurie and Bennett 1985)

In the United States, interest in employing seashore paspalum as a turf dates back to at least the mid-1920s (Duncan and Carrow 2000). Some of the early source materials originated from Sea Island, GA where some ecotypes were already established; it is presumed that introduction came from Africa by way of the Atlantic slave trade (Duncan and Carrow, 2000). The use of seashore paspalum as a turf expanded and successful clones were moved around the world. More and more work was being published on seashore paspalum by the 1980s, and in 1993 the first breeding program was established at the University of Georgia, Griffin Campus (Duncan and Carrow, 2000).

Interest continues to expand today as this halophytic species garners increased attention as a commercially successful warm-season turfgrass (Duncan and Carrow, 2000). It has become very popular in coastal areas and regions that have limited access to freshwater. There is variation for salt tolerance within the species (P. Raymer, personal communication) with some accessions having been shown to survive for more than a year when irrigated with a seawater strength salt solution (Endo et al., 2005). This level of salt tolerance is ten times higher than

that of rice and three times higher than that of wheat (Marcum and Murdoch 1994, Colmer, Munns et al. 2005). A phylogenetic study of the genus *Paspalum* has placed *P. vaginatum* into a small cluster which contains only a few close relatives, all of which lack salt tolerance (Jarret et al., 1998). More recently published phylogenies confirm this placement (Giussani et al., 2001; Rua et al., 2010; Morrone et al. 2012).

Grasses as a Single Genetic System

Early work in grass comparative genomics confirmed that the genomes of related grass species contained conserved genic content (Hulbert, Richter et al. 1990). More than genic sequence alone, gene orders themselves were determined to be conserved among grasses: maize and rice (Ahn and Tanksley 1993) and wheat and rice as well as between wheat and maize (Moore, Devos et al. 1995). This led to the general understanding that grass genomes have conserved genomic structure and content and that in general, the grasses could be thought of as a single genetic system (Bennetzen and Freeling 1993, Freeling 2001).

Due to this relationship, comparative linkage maps offer insights into chromosome evolution as well as provide a framework for understanding the genetics in divergent species (Ahn and Tanksley 1993, Gale and Devos 1998). Grasses, taken together, basically represent "different manifestations of one tractable genome" (Bennetzen and Freeling 1993). It was posited that differences between grass species would mostly be conditioned by allelic variation at conserved loci or as a consequence of the neofunctionalization of genes (Bennetzen and Freeling 1993). As a result, the grass family can be thought of as a single system containing a veritable menagerie of phenotypic diversity which will more than likely be conditioned by conserved genes which perform identical or related functions in distinct grass taxa (Parvathaneni, Jakkula et al. 2013, Zhang, Kong et al. 2015). And as a consequence, an understanding of the genetic

components which underlay the diversity of form and ability present in any of the thousands of species of grasses have the potential to be leveraged in genomes of other grasses. This concept has been referred to as "The Principle of Pairs" and as early as October of 1997, salinity tolerance was offered as an example of a trait that could be targeted in this manner (Mike Freeling, personal communication: http://freelinglab.berkeley.edu/historical/opinions/pairs.html).

Salinity: The Problem

Soil salinity is a major cause of abiotic stress to plants and limits agricultural productivity around the world. It is estimated that, worldwide, 800 million hectares of land are affected by salinity (FAO 2008). Salinity in the soil can occur for a number of reasons, both natural and human-induced: 1) as the result of naturally occurring minerals present in the parent rock of an area, 2) as the result of seawater intrusion in coastal areas, 3) from the accumulation of salt from irrigation, 4) as a result of land use changes which affect the water-table level (FAO). The vast majority of salt-affected land has become salinized by natural means (Rengasamy 2002), but irrigation and land clearing are important contributors to the "secondary" salinization of arable lands (Munns and Tester 2008). In total, 2% of land farmed by dryland agriculture has become affected by salinity as a result of land use, while 20% of irrigated agricultural land is now salt-affected (FAO 2008). Irrigated agricultural land is in general at least twice as productive as rainfed land and produces one third of the world's food on only 15% of its cultivated land (Munns and Tester 2008). Because irrigation is so important for food production but also contributes to the salinization of arable land, if irrigation in vulnerable areas is to remain sustainable, then the salinity tolerance of crops will need to be increased (Flowers 2004). Salinity levels themselves are communicated in several ways including as solute concentrations (grams/liter) as well as by the electrical conductivity (EC) of their solution. The latter is often

expressed in millimho/cm or dS/m and is used to refer to the salinity of a saturated soil paste or of an irrigation/hydroponic solution (Munns and Tester 2008). FAO recognizes four categories of salinity with regard to soils and their conductivity: non-saline (0-4.5 dS/m), slightly saline (4.5-9 dS/m), medium saline (9-18 dS/m) and highly saline (>18 dS/m). The most highly salt-tolerant crops are generally considered to be able to withstand salinities of ~9 dS/m, while more sensitive crops can be affected by salt levels which give conductivities of ~4 dS/m (FAO). The salinity of seawater is generally cited within the range 45-55 dS/m.

Effect of Salinity on Plants

Salinity effects on plants can be generalized to occur in two distinct phases: an osmotic phase, which begins when the plant is first exposed to salt and an ionic phase, which occurs after exposure has occurred long enough for ions to begin accumulating in organs (Munns and Tester 2008).

The osmotic effect is immediate and initially reduces shoot growth substantially; this effect is not ion specific, rather solute abundance outside the roots impedes the ability of the plant to maintain water uptake (Munns and Tester 2008). The osmotic effects of salt exposure occur as a direct result of the decrease in soil water potential associated with increases in solute concentration and functionally are similar to dehydration stress. As such, conserved mechanisms exist in plants to deal with this water-limited stress (Munns and Tester 2008). When osmotic stress occurs, leaf cells initially lose turgor, but this effect is short-lived if osmotic adjustment occurs, however cell elongation rates in leaves remain reduced (Fricke and Peters 2002). Root growth is less affected than leaf growth and root elongation recovers after salt exposure (Munns 2002). For leaves, the adjustment in growth patterns results in the slower appearance of new leaves as well as cell dimension changes which result in smaller, thicker leaves (Munns and

Tester 2008). These leaf anatomical changes result in a higher chloroplast density, which has the potential to keep photosynthetic rates (per unit leaf area) unchanged, even though stomatal conductance may be reduced (James, Rivelli et al. 2002).

If the photosynthetic rate slows, then the plant's primary source of both energy and structural material is affected. Additionally, this also causes oxidative stress as reactive oxygen species (ROS) are produced. Along with this comes an increase in the enzymes associated with detoxification (Apel and Hirt 2004) but these different metabolites seem to play much more complex, and still not completely understood, roles in controlling growth, development, and activating other stress responses (Bailey-Serres and Mittler 2006).

The ionic effects of salt exposure take longer to manifest as they occur in areas where and as a consequence of ions having accumulated to toxic levels. The primary site of sodium toxicity in plants is in the leaf blade, where these ions accumulate as a consequence of their presence in the transpiration stream (Munns 2002). Because sodium movement is almost completely unidirectional *in planta*, sodium that has reached the leaf will remain there, accumulating as more arrives if excretion mechanisms are not leveraged (Tester and Davenport 2003). Sodium toxicity occurs when Na^+ outcompetes K^+ for binding sites in enzymes that require K^+; there are at least fifty enzymes with such sites where sodium can bind, but Na^+ binding does not promote the proper enzyme activity (Bhandal and Malik 1988). Protein synthesis itself requires K^+; it is essential for the binding of tRNA to ribosomes (Blaha, Stelzl et al. 2000) as well as probably other aspects of ribosome function (Wyn Jones, Brady et al. 1979).

Mechanism of Salt Tolerance in Plants

The mechanisms of tolerance to salinity relate to the two phases mentioned above: the osmotic and ionic phases (Munns and Tester 2008). Mechanisms to deal with the osmotic phase involve

osmoregulation via the production of compatible organic solutes including sugars, proline, and glycine betaine (Stewart 1981, McCue and Hanson 1990). These compounds are generally recognized to reduce the osmotic gradient across membranes and prevent the dehydrating action of such gradients (Munns and Tester 2008). Proline's role as primarily an osmoregulator is not straightforward however, as proline does not accumulate rapidly in response to dehydration stress, but later in the time course as a result of injury (Hanson, Nelsen et al. 1977, Moftah and Michel 1987). Biochemical evidence indicates that proline suppresses rubisco activity by dissociating small subunits from the holoenzyme (Sivakumar, Sharmila et al. 1998, Sivakumar, Sharmila et al. 2001). This evidence suggests that proline's role is likely more than just an osmoregulator, and may function as an emergency brake of sorts, rapidly shutting down rubisco activity to help maintain water status.

The ionic phase, in contrast to the osmotic phase, begins later in the exposure time course and is a response to the accumulation of ions, particularly sodium, in cells/organs. Mechanistic approaches that provide tolerance to sodium accumulation include: 1) sodium exclusion from shoots, 2) tissue tolerance via compartmentalization, and 3) sodium excretion. It should be noted that these phases are not disjoint, rather the osmotic phase continues for the duration of exposure.

Sodium exclusion is an important salt tolerance mechanism. Its purpose is to filter out or prevent the passage of (and thus delay) the accumulation of sodium to toxic levels (Tester and Davenport 2003). Exclusion itself can occur in a number of specific ways, but generally it is summarized as a process which controls shoot access by limiting delivery to the root xylem (Munns and Tester 2008). Munns and Tester (2008) classified movement into the xylem in four distinct components: 1. Influx into cells in the outer half of root, 2. Efflux out of these cells into the soil solution, 3. Efflux from cells in the inner half of the root to the xylem and 4. Influx back

into these cells from the xylem before the transpiration moves Na^+ to the leaf. For all of these components, ion transporters including those from the HKT family as well as Na^+/H^+ antiporters are implicated (Munns and Tester 2008). These types of exclusion mechanisms are implicated in salt tolerance identified in rice as well as several wheats (Lindsay, Lagudah et al. 2004, Huang, Spielmeyer et al. 2006, Byrt, Platten et al. 2007, James, Blake et al. 2011, Almeida, Katschnig et al. 2013). Another approach to achieving sodium exclusion involves the sequestration of metal ions by chelation; this can be accomplished by members of the metallothionein protein family (Grennan 2011). Though this approach to exclusion is much less discussed in the literature, it has been implicated to be involved in both salinity and heavy metal tolerance mechanisms (Endo, Yoshida et al. 2005, R Cozza 2013).

Intracellular compartmentalization of toxic ions into vacuoles occurs to keep concentrations low in the cytoplasm, perhaps as low as 10-30mM (Carden, Walker et al. 2003). In this way, leaves or other organs can maintain normal function despite high levels of sodium having accumulated there (Munns and Tester 2008). Increased rates of intracellular compartmentalization can be conferred by differences in expression level of vacuolar Na^+/H^+ antiporters such as NHX1-like genes or vacuolar H^+-translocating pyrophosphatase (AVP-like genes) (Jha, Shirley et al. 2010). It is important to note that in general, the enzymes of salt-tolerant plants are not more tolerant than those from salt sensitive plants, so maintaining spatial separation when Na^+ levels increase is essential to preventing injury/toxicity (Munns and Tester 2008).

Sodium excretion at the leaf blade or in the stem is achieved through the use of salt glands (Thomson 1975). Species that achieve salt tolerance by this mechanism include some mangrove species (Drennan and Pammenter 1982) as well as some Chloridoid grasses (Marcum

1999). The absence of salt glands and excretion mechanisms has been documented in seashore paspalum (Marcum and Murdoch, 1994).

Salt Tolerance in Paspalum

Inquiries into the mechanisms of salt tolerance utilized by this paspalum species have been ongoing for over twenty years and with the exceptions of Endo et al. (2005) and Liu, Du et al. (2012) have focused almost exclusively on physiology (Marcum and Murdoch 1994, Lee 2000, Lee, Carrow et al. 2005, Lee, Carrow et al. 2008, Liu, Du et al. 2011). As we move forward in understanding salt tolerance in paspalum, it would behoove us to leverage what is already known about paspalum stress physiology and explore these processes with molecular approaches involving genetics, genomics, and transcriptomics.

A phylogenetic study of the genus has placed *P. vaginatum* into a small cluster which contains only a few close relatives, all of which lack salt tolerance (Jarret, Liu et al. 1998). Previous attempts to study the salt tolerance mechanism in seashore paspalum have failed to arrive at a single satisfactory conclusion (Marcum and Murdoch 1994, Endo, Yoshida et al. 2005, Lee, Duncan et al. 2007, Lee, Carrow et al. 2008). Marcum and Murdoch (1994) carried out salt screens and tissue sampling of a single paspalum genotype and implicated the restriction of Na^+ and Cl^- ions from the shoot as well as vacuolar compartmentalization and the production of compatible osmolytes (glycinebetaine and proline, but not trigonelline) as modes of action. Endo, Yoshida et al. (2005) mined the transcriptome of seashore paspalum for genes that are differentially expressed in response to saltwater irrigation versus freshwater control. The study followed up on two genes: a metallothionein and a UDP-galactose (glucose)-4-epimerase (*UGE1*). When the metallothionein gene was transformed into rice and backcrossed (BC_1F_1) into a salt-sensitive background, the expression of metallothionein was shown to increase the

survival rate at moderate salt levels compared to the metallothionein-negative control and the salt sensitive parent (Koshihikari). Additionally, though the survival rate of Nipponbare control plants was higher under salt, the metallothionein containing transgenic lines set seed whereas Nipponbare did not. Similar results were found for transformants containing the *UGE1* gene, namely that increased survivial rates of the transformants under salt as well as increased seed production. Additionally, biochemical detection protocols show that the compatible osmolytes glycinebetaine and proline are produced in paspalum responding to saline conditions, but that proline accumulation happens only after long-term exposure to high levels of salt (Marcum and Murdoch 1994, Lee, Duncan et al. 2007, Lee, Carrow et al. 2008). Confirming the roles of the genes mentioned above as well as identifying others that may play a role in the observed responses to salt and the high salt tolerance in *P. vaginatum* in general would provide insights into a mechanism/s which may be transferable to close relatives; if successful, this would have broad impacts for agriculture.

References

Ahn, S. and S. D. Tanksley (1993). "Comparative linkage maps of the rice and maize genomes." Proceedings of the National Academy of Sciences 90(17): 7980-7984.

Almeida, P., D. Katschnig and A. H. de Boer (2013). "HKT transporters—state of the art." International Journal of Molecular Sciences 14(10): 20359-20385.

Apel, K. and H. Hirt (2004). "Reactive oxygen species: metabolism, oxidative stress, and signal transduction." Annu Rev Plant Biol 55: 373-399.

Bailey-Serres, J. and R. Mittler (2006). "The roles of reactive oxygen species in plant cells." Plant Physiology 141(2): 311.

Bennett, H. (1962). Dallisgrass, bahiagrass, and vaseygrass. Forages, Volume II, The Science of Grassland Agriculture. R.F. Barnes, C.J. Nelson, K.J. Moore, M. Collins. Ames, The Iowa State University Press**:** 281–285.

Bennetzen, J. L. and M. Freeling (1993). "Grasses as a single genetic system: genome composition, collinearity and compatibility." Trends in Genetics **9**(8): 259-261.

Bhandal, I. and C. Malik (1988). "Potassium estimation, uptake, and its role in the physiology and metabolism of flowering plants." International Review of Cytology-a Survey of Cell Biology 110: 205-254.

Blaha, G., U. Stelzl, C. M. Spahn, R. K. Agrawal, J. Frank and K. H. Nierhaus (2000). "Preparation of functional ribosomal complexes and effect of buffer conditions on tRNA positions observed by cryoelectron microscopy." Methods Enzymol 317: 292-309.

Byrt, C. S., J. D. Platten, W. Spielmeyer, R. A. James, E. S. Lagudah, E. S. Dennis, M. Tester and R. Munns (2007). "HKT1;5-like cation transporters linked to Na(+) exclusion loci in wheat, Nax2 and Kna1." Plant Physiology 143(4): 1918-1928.

Carden, D. E., D. J. Walker, T. J. Flowers and A. J. Miller (2003). "Single-cell measurements of the contributions of cytosolic Na+ and K+ to salt tolerance." Plant Physiology 131(2): 676-683.

Chase, A. (1929). The North American species of *Paspalum*. Washington, G.P.O.

Colmer, T., R. Munns and T. Flowers (2005). " Improving salt tolerance of crop plants." Australian Journal of Experimental Agriculture 45: 1425-1443.

Denby, K. and C. Gehring (2005). "Engineering drought and salinity tolerance in plants: lessons from genome-wide expression profiling in *Arabidopsis*." Trends in Biotechnology 23(11): 547-552.

Denham, S. (2005). "Revisio´n sistema´tica del subge´nero Harpostachys de Paspalum (Poaceae, Panicoideae, Paniceae)." Annals of the Missouri Botanical Garden 92: 463–532.

Drennan, P. and N. W. Pammenter (1982). "Physiology of salt excretion in the mangrove *Avicennia marina* (Forsk.) Vierh." New Phytologist 91(4): 597-606.

Duncan, R. and R. Carrow (2000). Seashore Paspalum: the Environmental Turfgrass, Sleeping Bear Press.

Echarte, A. and A. Clausen (1993). "Morphological affinities between *Paspalum distichum* senso lato and *Paspalum vaginatum*." Boletin de la Sociedad Argentina de Botanica 29(3-4): 143-152.

Endo, N., K. Yoshida, M. Akiyoshi, Y. Yoshida and N. Hayashi (2005). "Putative UDP-galactose epimerase and metallothioneine of *Paspalum vaginatum* enhanced the salt tolerance of rice, *Oryza sativa* L. from transplanting to harvest stages." Breeding Science 55(2): 163-173.

FAO. (2008). "FAO Land and Plant Nutrition Management Service." from http://www.fao.org/ag/agl/agll/spush.

Flowers, T. J. (2004). "Improving crop salt tolerance." Journal of Experimental Botany 55(396).

Freeling, M. (2001). "Grasses as a single genetic system. Reassessment 2001." Plant Physiology 125(3): 1191-1197.

Fricke, W. and W. S. Peters (2002). "The biophysics of leaf growth in salt-stressed barley. A study at the cell level." Plant Physiol 129(1): 374-388.

Gale, M. D. and K. M. Devos (1998). "Plant comparative genetics after 10 years." Science 282(5389): 656-659.

Grennan, A. K. (2011). "Metallothioneins, a diverse protein family." Plant Physiology 155(4): 1750-1751.

Hanson, A. D., C. E. Nelsen and E. H. Everson (1977). "Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars." Crop Science 17(5): 720-726.

Huang, S., W. Spielmeyer, E. S. Lagudah, R. A. James, J. D. Platten, E. S. Dennis and R. Munns (2006). "A sodium transporter (HKT7) is a candidate for Nax1, a gene for salt tolerance in durum wheat." Plant Physiology 142(4): 1718-1727.

Hulbert, S. H., T. E. Richter, J. D. Axtell and J. L. Bennetzen (1990). "Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes." Proceedings of the National Academy of Sciences 87(11): 4251-4255.

James, R., A. Rivelli, R. Munns and S. v. Caemmerer (2002). "Factors affecting CO_2 assimilation, leaf injury and growth in salt-stressed durum wheat." Functional Plant Biology 29(12): 1393-1403.

James, R. A., C. Blake, C. S. Byrt and R. Munns (2011). "Major genes for Na+ exclusion, Nax1 and Nax2 (wheat HKT1;4 and HKT1;5), decrease Na+ accumulation in bread wheat leaves under saline and waterlogged conditions." Journal of Experimental Botany 62(8): 2939-2947.

Jarret, R. L., Z. W. W. Liu and R. W. Webster (1998). "Genetic diversity among *Paspalum* spp. as determined by RFLPs." Euphytica 104(2): 119-125.

Jenks, M., P. Hasegawa and S. Jain (2007). Advances in Molecular Breeding Toward Drought and Salt-tolerant Crops. Dordtrecht, Netherlands., Springer.

Jha, D., N. Shirley, M. Tester and S. J. Roy (2010). "Variation in salinity tolerance and shoot sodium accumulation in *Arabidopsis* ecotypes linked to differences in the natural expression levels of transporters involved in sodium transport." Plant, Cell & Environment 33(5): 793-804.

Laurie, D. and M. Bennett (1985). "Nuclear DNA content in the genera *Zea* and *Sorghum*-Intergeneric, interspecific and intraspecific variation." Heredity 55: 307-313.

Lee, G. (2000). Comparative Salinity Tolerance and Salt Tolerance Mechanisms of Seashore Paspalum Ecotypes. Athens, GA, University of Georgia.

Lee, G., R. Carrow, R. Duncan, M. Eiteman and M. Rieger (2008). "Synthesis of organic osmolytes and salt tolerance mechanisms in *Paspalum vaginatum*." Environ Exp Bot 63: 19-27.

Lee, G., R. N. Carrow and R. R. Duncan (2005). "Growth and water relation responses to salinity stress in halophytic seashore paspalum ecotypes." Scientia Horticulturae 104(2): 221-236.

Lee, G. J., R. R. Duncan and R. N. Carrow (2007). "Nutrient uptake responses and inorganic ion contribution to solute potential under salinity stress in halophytic seashore paspalums." Crop Science 47(6): 2504-2512.

Lindsay, M. P., E. S. Lagudah, R. A. Hare and R. Munns (2004). "A locus for sodium exclusion (Nax1), a trait for salt tolerance, mapped in durum wheat." Functional Plant Biology 31(11): 1105-1114.

Liu, Y., H. Du, X. He, B. Huang and Z. Wang (2012). "Identification of differentially expressed salt-responsive proteins in roots of two perennial grass species contrasting in salinity tolerance." J Plant Physiol 169(2): 117-126.

Liu, Y., H. Du, K. Wang, B. Huang and Z. Wang (2011). "Differential photosynthetic responses to salinity stress between two perennial grass species contrasting in salinity tolerance." HortScience 46(2): 311-316.

Liu, Z. W., R. L. Jarret, S. Kresovich and R. R. Duncan (1995). "Characterization and analysis of simple sequence repeat (SSR) loci in seashore paspalum (*Paspalum vaginatum* Swartz)." Theoretical and Applied Genetics 91(1): 47-52.

Marcum, K. (1999). "Salinity tolerance mechanism of grasses in the sub-family Chloridoideae." Crop Sci 39(4): 1153 - 1160.

Marcum, K. and C. Murdoch (1994). "Salinity tolerance mechanisms of six C4 turfgrasses." J Am Soc Hort Sci 119: 779 - 784.

McCue, K. F. and A. D. Hanson (1990). "Drought and salt tolerance: towards understanding and application." Trends in Biotechnology 8: 358-362.

Moftah, A. E. and B. E. Michel (1987). "The effect of sodium chloride on solute potential and proline accumulation in soybean leaves." Plant Physiol 83(2): 238-240.

Moore, G., K. M. Devos, Z. Wang and M. D. Gale (1995). "Cereal genome evolution: grasses, line up and form a circle." Current Biology 5(7): 737-739.

Morrone, O., L. Aagesen, M. A. Scataglini, D. L. Salariato, S. S. Denham, M. A. Chemisquy, S. M. Sede, L. M. Giussani, E. A. Kellogg and F. O. Zuloaga (2012). "Phylogeny of the Paniceae (Poaceae: Panicoideae): integrating plastid DNA sequences and morphology into a new classification." Cladistics 28(4): 333-356.

Munns, R. (2002). "Comparative physiology of salt and water stress." Plant Cell Environ 25: 239-250.

Munns, R. (2005). "Genes and salt tolerance: bringing them together." New Phytologist 167: 645-663.

Munns, R. and R. A. James (2003). "Screening methods for salinity tolerance: a case study with tetraploid wheat." Plant and Soil 253: 201-218.

Munns, R., R. A. James, B. Xu, A. Athman, S. J. Conn, C. Jordans, C. S. Byrt, R. A. Hare, S. D. Tyerman, M. Tester, D. Plett and M. Gilliham (2012). "Wheat grain yield on saline soils is improved by an ancestral Na+ transporter gene." Nat Biotech 30(4): 360-364.

Munns, R. and M. Tester (2008). "Mechanisms of salinity tolerance." Annu Rev Plant Biol 59: 651-681.

Parvathaneni, R. K., V. Jakkula, F. K. Padi, S. Faure, N. Nagarajappa, A. C. Pontaroli, X. Wu, J. L. Bennetzen and K. M. Devos (2013). "Fine-mapping and identification of a candidate gene underlying the d2 dwarfing phenotype in pearl millet, *Cenchrus americanus* (L.) Morrone." G3 (Bethesda) 3(3): 563-572.

R Cozza, L. B., MB Bitonti (2013). "Expression pattern of a type-2 metallothionein gene in a wild population of the psammophyte *Silene nicaeensis* " Protoplasma 250: 381-389.

Rengasamy, P. (2002). "Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: an overview." Australian Journal of Experimental Agriculture 42: 351-361.

Rua, G. H., P. R. Speranza, M. Vaio and M. Arakaki (2010). "A phylogenetic analysis of the genus *Paspalum* (Poaceae) based on cpDNA and morphology." Plant Systematics and Evolution 288(3-4): 227-243.

Sivakumar, P., P. Sharmila and P. P. Saradhi (1998). "Proline suppresses rubisco activity in higher plants." Biochem Biophys Res Commun 252(2): 428-432.

Sivakumar, P., P. Sharmila and P. P. Saradhi (2001). "Proline suppresses rubisco activity by dissociating small subunits from holoenzyme." Biochem Biophys Res Commun 282(1): 236-241.

Stewart, C.R. (1981). "Proline accumulation: physiological aspects." The Physiology and Biochemistry of Drought Resistance in Plants. Aspinall, D. and L.G. Paleg. Sydney, Academic Press: 243-259.

Tester, M. and R. Davenport (2003). "Na^+ tolerance and Na^+ transport in higher plants." Ann Bot 91(5): 503-527.

Thomson, W. W. (1975). The structure and function of salt glands. Plants in Saline Environments. A. Poljakoff-Mayber and J. Gale. Berlin, Heidelberg, Springer Berlin Heidelberg: 118-146.

Wyn Jones, R., C. Brady and J. Spears (1979). Ionic and osmotic relations in plant cells. Recent advances in the biochemistry of cereals. D. Laidman and R. Wyn Jones. London, Academic Press: 63–103.

Zhang, D., W. Kong, J. Robertson, V. H. Goff, E. Epps, A. Kerr, G. Mills, J. Cromwell, Y. Lugin, C. Phillips and A. H. Paterson (2015). "Genetic analysis of inflorescence and plant height components in sorghum (Panicoidae) and comparative genetics with rice (Oryzoidae)." BMC Plant Biology 15(1): 1-15.

Zuloaga, F. O. and O. Morrone (2005). Revisión de las especies de Paspalum para América del Sur austral: (Argentina, Bolivia, sur del Brasil, Chile, Paraguay y Uruguay), Missouri Botanical Garden Press.

CHAPTER 2

CHARACTERIZATION OF PLOIDY LEVEL AND GENETIC DIVERSITY IN A PANEL OF SEASHORE PASPALUM (*PASPALUM VAGINATUM* SW.) ACCESSIONS AND A CLOSE RELATIVE WITH FLOW CYTOMETRY AND A NOVEL SET OF MICROSATELLITE MARKERS[1]

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Abstract

Characterizing the genetic diversity and ploidy level in members of a panel of *Paspalum vaginatum* Sw. and *Paspalum distichum* L. accessions was completed in order to select two diverse diploid lines for use as parents for mapping population development. To achieve this DNA content was measured by flow cytometry with chromosome counts used to confirm the presence of both diploid and polyploid lines in the panel. Previously reported DNA content estimations from *P. distichum*, *P. notatum*, and *P. lividum* were used to establish a haploid genome size estimate of 0.61pg for *P. vaginatum*. A set of 43 microsatellite markers were developed and used to genotype the diploid accessions assembled here. Population structure analysis and principal coordinates analysis grouped the germplasm into four subpopulations. One subpopulation was comprised of four accessions that had character traits consistent with *Paspalum distichum* L., while the other three subpopulations contained only members resembling *Paspalum vaginatum* Sw. accessions. Two individuals (HI33 and 509022) were subsequently selected as parents for mapping population development.

Introduction

It has been hypothesized that the *Paspalum* genus originated on the South American continent during the climatic repatterning of the Miocene-Pliocene boundary (Rua, Speranza et al. 2010). This supposition is based on the observation that the genus' basal members tend to be shade-tolerant species, and as such, likely predate the grassland expansions of the South American continent (Rua, Speranza et al. 2010). Since then, the genus has undergone extensive diversification. *Paspalum* contains between 330 and 400 members and is one of the most species-rich genera among the grasses (Chase 1929, Zuloaga and Morrone 2005). The genus

Paspalum was formerly a member of the Paniceae, however the tribe Paspaleae has recently been reinstated and now contains the x=10 Paniceae *s.l.*, which includes the *Paspalum* genus (Morrone, Aagesen et al. 2012). Though the genus is of New World origin, many of its species now range beyond South America (Chase 1929). Some members have achieved a much broader distribution as a result of having been adopted as forages and/or turf (*P. notatum* Fluegge, *P. dilitatum* Poir., and *P. vaginatum* Sw.) (Gates, Quarin et al. 2004). Though the genus contains a large number of species, relatively few have received focused scholarly attention. In general, the genus is a fixture in the apomixis literature (the phenomenon is common in the genus), as well as in the forage breeding literatures (Quarin and Burson 1991, Quarin, Espinoza et al. 2001). The species *Paspalum simplex* Morong ex Britton has been studied in the context of cytogeographic variation (Urbani, Quarin et al. 2002, Brugnoli, Urbani et al. 2013). The members of *Paspalum* occupy important niches in the tropical and sub-tropical regions of the world.

The focus of this work is primarily the species *Paspalum vaginatum* Swartz., also known as seashore paspalum. This species is a salt-tolerant stoloniferous perennial that has successfully colonized brackish and coastal environments in tropical and sub-tropical areas around the world (Duncan and Carrow 2000). Owing to its creeping habit, ease of propagation, and its salt tolerance, it has become a popular turf grass in warmer climes, particularly in coastal areas as well as those with water use restrictions or water quality issues (Duncan and Carrow 2000). A close relative, *Paspalum distichum* Linnaeus (formerly *Paspalum paspaloides* (Michx.) Lams.-Scribn), is also widely distributed in warmer regions of the world, but is considered to exhibit significantly lower levels of salt-tolerance (Skerman and Riveros 1990). As a result, the ranges of *P. vaginatum* and *P. distichum* do not overlap, and *P. distichum* has a broader distribution range than *P. vaginatum* (Skerman and Riveros 1990). This pair of species wholly comprise the

subgroup ‘Disticha’ within the *Paspalum* genus and as almost all members of *Paspalum*, exhibit a base chromosome number of x = 10 (Zuloaga and Morrone 2005).

Though some early diversity studies have grouped other species as sister to these two, more recent phylogenetic analyses support a close genetic relationship between *P. vaginatum* Sw. and *P. distichum* L. and indicate that they form a single clade devoid of other taxa (Jarret, Liu et al. 1998, Souza-Chies, Essi et al. 2006, Rua, Speranza et al. 2010). *Paspalum vaginatum* has typically been described as a sexual diploid (Echarte and Clausen 1993, Duncan and Carrow 2000), but at least once has it been identified as occurring in a tetraploid state (Hojsgaard, Honfi et al. 2009). *Paspalum distichum*, on the other hand, is identified almost exclusively as a polyploid with cytotypes ranging from tetraploid to hexaploid, including pentaploids and hyperpentaploids (2n=52,54,57, and 58) (Quarin and Burson 1991, Echarte, Clausen et al. 1992, Echarte and Clausen 1993, Hojsgaard, Honfi et al. 2009, Rua, Speranza et al. 2010). While all diploid paspalums are said to reproduce sexually, polyploid paspalums have been observed to exhibit apomixis; there is at least one report of hexaploid *P. distichum* exhibiting aposporous apomixis (Bashaw et al., 1970, Quarin and Hanna, 1980, Quarin, 1992, Quarin et al., 2001;).

Molecular genetic tools have the potential to augment studies of species relatedness which have traditionally relied on morphological observations such as chromosome counts, hybrid fertility, and anatomical descriptors. In *Paspalum*, cytological and comparative morphological studies have long been a mainstay for categorizing the relationships among the different member species (Banks, 1966, Bennett and Bashaw, 1966, Echarte et al., 1992, Echarte and Clausen, 1993, Aliscioni and Denham, 2008, Morrone et al., 2012). Work in the Disticha sub-group in particular has demonstrated the utility of utilizing cytotypical variation as well as a suite of morphotypical characters as distinguishing features between species. One study

identified some twenty morphotypical characters that were considered diagnostic, among them, glume pubescence in *P. distichum* L., and glabrous glumes in *P. vaginatum* Sw. (Echarte and Clausen 1993). Whether cytotype alone relates directly to the suite of discriminating features between these two species or whether perhaps they should be considered mono-specific has, as of yet, remained unresolved, but molecular genetic approaches have the potential to shed new light on the relationship between these species as well as regarding the genetic diversity present within them.

In the United States, seashore paspalum has been present as a salt-tolerant turfgrass in the coastal southeastern region since at least the 1920s (Duncan and Carrow 2000). There has long been speculation about how and when the species was introduced, but maritime trade, specifically involving slaves, has been the primary explanation (Duncan and Carrow 2000). Another hypothesis, not previously discussed in the literature (but the current theory held by this author), is that *P. vaginatum* and *P. distichum* were introduced from South America by the Spanish through the cattle trade. Regardless of how or when it arrived, *P. vaginatum* has been recognized over the past few decades as having potential as a turf. A seashore paspalum breeding program was launched at the University of Georgia, Griffin in 1993. In support of that endeavor, a germplasm collection was established at UGA. The UGA collection supplemented the materials held by the Germplasm Resource Information Network of the US Department of Agriculture (USDA-GRIN), which has maintained *Paspalum vaginatum* germplasm since at least 1961. Today, the available germplasm includes accessions from South America, the putative center of origin, as well as material from other regions where the species has become naturalized including Africa and North America.

Despite the availability of germplasm resources, there have only been a few reports regarding genetic diversity in the species (Liu et al. 1994, Liu et al. 1995, Brown et al. 1996, Chen et al. 2005, Harris-Shultz et al. 2013). The first genetic diversity study focusing specifically on *Paspalum vaginatum* analyzed a collection of 46 ecotypes with 145 random amplified polymorphic DNA (RAPD) markers (Liu, Jarret et al. 1994). Shortly thereafter, the same panel of 46 ecotypes was screened with five microsatellite markers developed from *P. vaginatum* cv. 'Excalibre' (Liu, Jarret et al. 1995). The same markers were used to genotype a semi-redundant collection of 10 seashore paspalum accessions of which five genotypes had been previously studied (Brown, Mitchell et al. 1996). Amplified fragment length polymorphisms (AFLPs) were used on 69 accessions of *P. vaginatum*, one accession each of *P. heironmi*, *P. lividum*, *P. unispicatum* and *P. remotum*, and one accession of an outgroup, *Anthoxathum odoratum* (Chen, Newman et al. 2005). Wang, Chen et al. (2006) characterized seventy-three accessions of seashore paspalum with forty microsatellite markers transferred from wheat, maize, and sorghum. Most recently, Harris-Shultz, Raymer et al. (2013) surveyed 17 *P. vaginatum* accessions with 79 microsatellite markers developed from line 'HI33' along with the 5 originally developed by Liu, Jarret et al. (1995). The microsatellites used in the diversity analysis reported by Harris-Shultz, Raymer et al. (2013) were selected for being located in or near genes documented in other species to be involved in salt tolerance, disease resistance or having an association with transposons (Harris-Shultz, Raymer et al. 2013).

Our study utilized a set of 43 microsatellite markers developed for this study from a *Pst*I-derived genomic library. These microsatellites were used to genotype a diversity panel containing 29 accessions of *P. vaginatum* and *P. distichum* sourced from GRIN, 60 lines from the UGA collection, and a single ecotype collected near Darien, GA. The diversity analysis was

combined with ploidy level estimations using flow cytometry to identify the genetic structure among the diploids in the collection so two diploid lines at the extremes of the available diversity could be used as parents for a mapping population.

Materials and Methods

Germplasm Screened

The seashore paspalum accessions comprising the diversity panel were acquired from GRIN, the UGA collection at Griffin, GA, or were collected from an estuarine area near Darien, GA (Table 2.1). The panel was assembled by collaborators (the paspalum germplasm manager at GRIN and the UGA paspalum breeder) to represent the paspalum diversity in their collections. Though some USDA accessions were originally collected as seed, all accessions used in this study, regardless of source, were obtained as vegetative cuttings and maintained as clonal propagules in greenhouses at the UGA-Athens campus, under natural light.

Flow Cytometry and DNA Content Estimation

Leaves were harvested and stored in sealed plastic bags on ice before processing for a maximum of 24 hours. The CyStain® PI Absolute P kit (Partec North America, Swedesboro, NJ) was used to extract and stain the nuclei; sample processing and data generation followed the manufacturer's instructions. Propidium iodide (PI)-stained trout erythrocytes (DNA Control PI, Partec North America, Swedesboro, NJ) were added to the samples as a standard. Samples were processed on a Partec PAS flow cytometer (Partec North America, Swedesboro, NJ). The proportion of the sample DNA value to the trout erythrocyte control was used to determine relative DNA content and assign a putative ploidy level to each sample. For samples run without the trout control, the mean of eighty control samples was used and the ratios calculated with that

mean as the control value. To estimate the genome size of diploid seashore paspalum, flow cytometer measurements for *P. distichum* (PI 284500), *P. notatum* (PI 508847), and *P. lividum* (PI 404874) were taken and a regression line calculated by comparing the values collected to those previously reported for *P. distichum* (PI 284500), *P. notatum* (PI 508847), and *P. lividum* accessions (PI 404637, PI 404638, PI 508668, and PI 508938) (Jarret, Ozias-Akins et al. 1995). Because the specific accession of *P. lividum* we screened was not among those referenced by Jarett et al., (1995), the mean of the values from those four other accessions was used. The regression line was used to estimate the DNA content of diploid *P. vaginatum* by utilizing the mean calculated from the 91 putative diploid accessions screened for DNA content.

Ploidy-level Confirmation by Chromosome Counts

Stolon cuttings were taken from plants selected for chromosome counts and placed on 1.5% w/v agar plates in a growth room with a light/dark cycle of 14hr/10hr, day/night temperatures of 30°C/24°C and a relative humidity ranging from 35% to 65%. After seven days, root tips from the roots which had developed from nodal meristems were harvested and processed to produce chromosome spreads following Kato (1999). Chromosome spreads were stained with DAPI and visualized on a Zeiss Axio 2 light microscope (Carl Zeiss Microscopy LLC, Thornwood, NY). Metaphase cells were photographed and chromosomes were counted.

Identifying Glabrous and Pubescent Glumes

A diagnostic feature cited as distinguishing *P. distichum* from *P. vaginatum* is the presence of pubescence on the glumes of the former and the lack thereof on the latter (Echarte and Clausen, 1993). Seed heads sampled from a selection of diversity panel members were examined under a stereo microscope to identify the glumes as pubescent or glabrous.

DNA Extraction

DNA was extracted from leaves using either the Qiagen DNeasy Plant DNA extraction kit following the manufacturer's protocol or using a 2% CTAB buffer-based phenol/chloroform extraction method modified from (Doyle and Doyle 1987). DNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop products, Wilmington, DE), assayed for integrity using a 2% agarose gel, and diluted to 10ng/µL for genotyping.

Microsatellite Marker Development

Microsatellite marker development followed the protocol of (Glenn and Schable 2005). In brief, genomic DNA from the seashore paspalum cv. 'Salam' was digested with the restriction endonuclease *Pst*I and ligated to oligonucleotide adapters (Ad1: 5'-CTCGTAGACTGCGTACATGCA and Ad2: 5'-TGTACGCAGTCTAC). Fragments in the range 500-1500 base pairs were gel extracted and purified using the QIAquick Gel Extraction Kit (Qiagen North America, Germantown, MD). *Pst*I-adaptor primers 5'-CGTAGACTGCGTACATGCAN were used to amplify the size-selected fragments using the polymerase chain reaction (PCR) with these conditions: [95° C, 2min; [95° C, 30sec; 56.3° C, 1min; 72° C, 1min 30sec] x 25; 72° C, 10min; hold 15° C]. The reaction mixture was composed of 1X GoTaq Buffer (Promega), 1X BSA (New England Biolabs), 2 mM MgCl2 (Promega), 150 µM of each dNTP, 0.5 µM adaptor primers, 1U GoTaq Flexi Taq Polymerase (Promega). To enrich for dinucleotide SSR containing fragments, a pool of 5'-biotinylated AC_{15} and AG_{15} oligonucleotides were mixed in equal proportions in 3x SSC buffer and hybridized to a genomic library constituting 1 µg of PCR amplified, cut and adaptor-ligated genomic DNA using a denaturing/annealing thermocycler program [95° C, 5min; 75° C, 5sec, -0.2° C per cycle, repeat 98 times; 55° C, 10min; 55° C, 5sec, -0.5° C per cycle, repeat 19 times; 45° C for 5sec; drop 1°

C per sec to 15° C; 15° C hold]. Streptavidin-coated magnetic beads Dynabeads® (Invitrogen) were used to capture the biotinylated probes and any associated DNA molecules. Captured DNA was amplified with PCR [95° C, 2min; [95° C, 30sec; 56.3° C, 1min; 72° C, 1min 30sec] x 35; 72° C, 30min; hold 15° C]. The reaction mixture was composed of 1X GoTaq Buffer (Promega), 1X BSA (New England Biolabs), 2 mM MgCl2 (Promega), 150 µM of each dNTP, 0.5 µM adaptor primers, 1U GoTaq Flexi Taq Polymerase (Promega). Amplicons were cloned into a pCR4-TOPO vector (Invitrogen) and transformed into *E. coli* strain DH5-alpha. Plasmid inserts were Sanger sequenced from both ends using T3 (5'- ATTAACCCTCACTAAAGGGA) and T7 (5'- TAATACGACTCACTATAGGG) universal primers. Sequences were assembled with Phrap and the resulting contigs and singletons were screened for the presence of SSRs with a minimum length of 7 repeat units for dinucleotide repeats, 6 for trinucleotide repeats and 5 for tetranucleotide repeats using a custom PERL script (courtesy of Dr. Philip San Miguel, Purdue University).

Table 2.1: Germplasm panel assembled for genetic diversity and/or ploidy screens

Accession ID	Source	Species (fr. USDA)	Glumes	Origin	sample DNA	control DNA	Ratio	Ploidy Estimate
PI 276245	USDA	*P. vaginatum*	glabrous	Uruguay	21.44	74.62	0.29	diploid
PI 284500*	USDA	*P.distichum*	pubescent	Chile/Ireland	42.92	73.30	0.59	tetraploid
PI 299042	USDA	*P. vaginatum*	glabrous	Zimbabwe	16.83	75.16	0.22	diploid
PI 364368	USDA	*P. vaginatum*	-	Mozambique	15.99	*76.45*	*0.21*	diploid
PI 364981*	USDA	*P. vaginatum*	-	South Africa	52.3	75.96	0.69	pentaploid
PI 377709	USDA	*P. vaginatum*	glabrous	South Africa	19.66	74.16	0.27	diploid
PI 403999	USDA	*P. distichum*	-	Australia	16.15	*76.45*	*0.21*	diploid
PI 508729	USDA	*P. distichum*	glabrous	Argentina	13.98	75.38	0.19	diploid
PI 508735	USDA	*P. distichum*	glabrous	Argentina	15.13	*76.45*	*0.20*	diploid
PI 508737	USDA	*P. distichum*	glabrous	Argentina	18.43	*76.45*	*0.24*	diploid
PI 543854 (Tropic Shore)	USDA	*P. vaginatum*	pubescent	Hawaii (USA)	17.93	78.47	0.23	diploid
PI 576134	USDA	*P. vaginatum*	glabrous	Brazil	18.36	76.72	0.24	diploid
PI 576138	USDA	*P. vaginatum*	glabrous	Brazil	15.97	76.83	0.21	diploid
PI 576140*	USDA	*P. vaginatum*	pubescent	Brazil	41.67	79.30	0.53	tetraploid
PI 612771	USDA	*P. vaginatum*	glabrous	Argentina/Peru	24.84	79.51	0.31	diploid
PI 614679	USDA	*P. vaginatum*	glabrous	Bahamas	17.79	*76.45*	*0.23*	diploid
PI 645598*	USDA	*P. vaginatum*	glabrous	Texas (USA)	31.34	69.12	0.45	triploid
PI 647885 (HYB5)	USDA	*P.* hybrid	glabrous		14.97	*76.45*	*0.20*	diploid
PI 647886 (HYB7)	USDA	*P.* hybrid	glabrous		20.73	*76.45*	*0.27*	diploid
PI 647888	USDA	*P.* sp.	glabrous	Isreal	19.36	72.81	0.27	diploid
PI 647891	USDA	*P. vaginatum*	glabrous	Isreal	19.82	79.50	0.25	diploid
PI 647893 (HI10)	USDA	*P. vaginatum*	glabrous	Hawaii (USA)	22.2	76.68	0.29	diploid
PI 647896 (HI32)	USDA	*P. vaginatum*	glabrous	Hawaii (USA)	19.3	76.98	0.25	diploid
PI 647900	USDA	*P. vaginatum*	glabrous	Thailand	19.62	75.11	0.26	diploid
PI 647901	USDA	*P. vaginatum*	-	Guam	19.13	79.97	0.24	diploid
PI 647908	USDA	*P. vaginatum*	glabrous	Georgia (USA)	17.6	76.50	0.23	diploid
PI 647913	USDA	*P. vaginatum*	glabrous	Argentina	17.91	75.41	0.24	diploid
PI 647914	USDA	*P. vaginatum*	glabrous	Argentina	14.52	74.30	0.20	diploid
PI 647915	USDA	*P. vaginatum*	-	Texas (USA)	20.21	*76.45*	*0.26*	diploid
PI 647917*	USDA	*P. vaginatum*	glabrous	Florida (USA)	33.31	80.36	0.41	triploid
PI 647918*	USDA	*P. vaginatum*	glabrous	Florida (USA)	33.23	77.85	0.43	triploid
PI 647919	USDA	*P. vaginatum*	-	Hawaii (USA)	16.57	76.79	0.22	diploid
PI 647920	USDA	*P. vaginatum*	glabrous	Louisiana (USA)	21.02	*76.45*	*0.27*	diploid
PI 647921	USDA	*P. vaginatum*	glabrous	Florida (USA)	21.82	*76.45*	*0.29*	diploid
PI 647922*	USDA	*P. vaginatum*	pubescent	Australia	48.78	76.07	0.64	tetraploid
PI 647923	USDA	*P. vaginatum*	glabrous	Australia	14.7	76.74	0.19	diploid
509020 (PI 509020)	UGA	*P. vaginatum*	glabrous	Argentina	18.19	73.93	0.25	diploid
509021 (PI 509021)	UGA	*P. vaginatum*	glabrous	Argentina	19.29	*76.45*	*0.25*	diploid
509022 (PI 509022)	UGA	*P. vaginatum*	glabrous	Argentina	15.48	*76.45*	*0.20*	diploid
509023 (PI 509023)	UGA	*P. vaginatum*	glabrous	Argentina	14.64	75.37	0.19	diploid
310-79 (PI 647906)	UGA	*P. vaginatum*	glabrous	Argentina	18.71	*76.45*	*0.24*	diploid
509018-2 (selection fr. PI 509018)	UGA	*P. vaginatum*	glabrous	Argentina	15.17	75.05	0.20	diploid
509018-3 (selection fr. PI 509018)	UGA	*P. vaginatum*	-	Argentina	20.14	75.72	0.27	diploid
561-79 (PI 647907)	UGA	*P. vaginatum*	glabrous	Argentina	16.3	72.36	0.23	diploid
Adalayd (PI 647902)	UGA	*P. vaginatum*	-	Australia	17.97	77.34	0.23	diploid
Aloha (PI 652948)	UGA	*P. vaginatum*	glabrous	Hawaii	24.65	77.15	0.32	diploid
Bahama	UGA	-	-		14.42	76.90	0.19	diploid
Belize	UGA	-	-		14.53	72.53	0.20	diploid
Cal	UGA	-	-		15.78	73.66	0.21	diploid
Cloister	UGA	-	glabrous		13.89	76.89	0.18	diploid
Collier	UGA	-	glabrous		14.24	79.36	0.18	diploid
Cuba 223	UGA	-	-		13.69	78.44	0.17	diploid
Darien	collection	-	-	Georgia (USA)	-	-	-	-
Durban (PI 614678)	UGA	*P. vaginatum*	glabrous		19.87	*76.45*	*0.26*	diploid
Excal (PI 647905)	UGA	*P. vaginatum*	-	Australia	14.62	78.92	0.19	diploid
FR-4	UGA	-	-		15.96	82.77	0.19	diploid
FSP1 (PI 647911)	UGA	*P. vaginatum*	-	Georgia (USA)	14.21	79.60	0.18	diploid
HH	UGA	-	glabrous		14.19	59.26	0.24	diploid
HI14 (PI 647894)	UGA	*P. vaginatum*	-	Hawaii (USA)	18.34	77.28	0.24	diploid
HI26 (PI 647895)	UGA	*P. vaginatum*	glabrous	Hawaii (USA)	20.72	78.21	0.26	diploid
HI33 (PI 647897)	UGA	*P. vaginatum*	-	Hawaii (USA)	17.72	77.06	0.23	diploid
HI36 (PI 647898)	UGA	*P. vaginatum*	glabrous	Hawaii (USA)	17.95	*76.45*	*0.23*	diploid
HI39 (PI 647899)	UGA	*P. vaginatum*	-	Hawaii (USA)	20.06	79.51	0.25	diploid
HI101	UGA	-	-		13.74	79.06	0.17	diploid

Table 2.1 (continued): Germplasm panel assembled for genetic diversity and/or ploidy screens

Note: Accession IDs marked with * were not included in the diversity screen; italicized control DNA and ratio values indicate no internal control was used with that sample, rather the mean value for all control DNAs (n=80) was used to calculate the ratio presented.

Accession ID	Source	Species (fr. USDA)	Glumes	Origin	sample DNA	control DNA	Ratio	Ploidy Estimate
Hignight S	UGA	-	glabrous		15.21	78.60	0.19	diploid
HYB5	UGA	-	-		19.04	*76.45*	*0.25*	diploid
HYB7	UGA	-	-		20	*76.45*	*0.26*	diploid
K8	UGA	-	-		15.35	78.23	0.20	diploid
Kai Luna	UGA	-	glabrous		21.89	*76.45*	*0.29*	diploid
KC9	UGA	-	-		20.39	78.39	0.26	diploid
Kim1	UGA	-	glabrous		16.08	70.71	0.23	diploid
Polo	UGA	-	glabrous		19.55	*76.45*	*0.26*	diploid
Prince	UGA	-	glabrous		20.85	*76.45*	*0.27*	diploid
Q36313	UGA	-	-		20.53	81.68	0.25	diploid
Q36315	UGA	-	-		18.25	81.99	0.22	diploid
Q37956	UGA	-	-		20.5	84.00	0.24	diploid
Salam	UGA	-	-		18.75	73.52	0.26	diploid
Sea Isle 1	UGA	-	-		17.16	78.00	0.22	diploid
Sea Isle 2000	UGA	-	-		19.17	76.39	0.25	diploid
Sea Isle Supreme	UGA	-	-		17.84	77.57	0.23	diploid
SeaDwarf	UGA	-	-		20.58	78.29	0.26	diploid
SIPV28-1 (PI 647912)	UGA	*P. vaginatum*	glabrous	Georgia (USA)	18.77	76.66	0.24	diploid
Spence	UGA	-	pubescent		23.05	70.82	0.33	diploid
Taliaferro	UGA	-	glabrous		14.42	75.88	0.19	diploid
Taylor 1 (PI 647903)	UGA	*P. vaginatum*	glabrous	North Carolina (USA)	16.89	75.83	0.22	diploid
Taylor 2 (PI 647904)	UGA	*P. vaginatum*	glabrous	North Carolina (USA)	15.83	75.01	0.21	diploid
TCR3	UGA	-	-		15.53	73.25	0.21	diploid
TCR6	UGA	-	-		15.87	76.87	0.21	diploid
Temple 1 (PI 647909)	UGA	*P. vaginatum*	glabrous	Texas (USA)	19.57	75.41	0.26	diploid
Temple 2 (PI 647910)	UGA	*P. vaginatum*	glabrous	Texas (USA)	15.22	74.85	0.20	diploid
TF P7-4	UGA	-	glabrous		17.8	77.27	0.23	diploid
TG Kona	UGA	-	glabrous		18.91	74.62	0.25	diploid
TOCGC	UGA	-	-		15.1	77.97	0.19	diploid
TRB2	UGA	-	-		16.27	77.01	0.21	diploid
Utah1	UGA	-	-		18.84	78.08	0.24	diploid
Vero Beach	UGA	-	glabrous		18.93	74.93	0.25	diploid
Wai Lua Kauai	UGA	-	glabrous		18.25	76.93	0.24	diploid
FR8*	UGA	-	-		18.39	75.75	0.24	diploid
PI 404874*	USDA	*P. lividum*	-	Uruguay	32.72	76.62	0.43	-
PI 422024*	USDA	*P. notatum*	-	Florida (USA)	14.55	75.50	0.19	-
PI 508847*	USDA	*P. notatum*	-		17.17	*76.45*	*0.22*	-
PI 364977*	USDA	*P. distichum*	-		58.33	*76.45*	*0.76*	hexaploid
PI 222796*	USDA	*P. distichum*	-		57.15	*76.45*	*0.75*	hexaploid
Sea Spray*	UGA	-	-		20.45	75.71	0.27	diploid

Primers were designed to the SSR flanking sequences with Primer3 (Rozen and Skaletsky 2000). A universal M13 tail (5'CGTTGTAAAACGACGGCCAGT) was added to the 5' end of all forward primers. Microsatellite primer pairs that successfully amplified from genomic DNA

of the source cultivar ‘Salam’ were selected to genotype the germplasm collection (Supplementary Table 2.1; Table 2.1).

Genotyping

A total of 90 paspalum accessions (Table 2.1) were genotyped with 43 microsatellite markers (Supplementary Table 2.1) in 14µL reaction volumes containing 1x GoTaq Flexi Buffer (Promega), 1.5 mM $MgCl_2$ (Promega), 300 µM of each dNTP, 0.33 µM fluorescently labeled (VIC, NED or FAM) universal M13 primer (5’CGTTGTAAAACGACGGCCAGT), 0.33 µM SSR-specific reverse primer (Supplemental Table 2.1), 0.06 µM tailed SSR-specific forward primer (Supplemental Table 2.1), 1.25 U of GoTaq (Promega) and 60 ng of genomic DNA. PCR conditions were as follows: [94°C, 4min; [94°C, 30sec; 65°C, 30sec -0.5°C per cycle, 72°C, 45 sec] x9; [94°C, 30sec; 59°C, 30sec; 72°C, 45sec]x31; 72°C, 20min; 10°C hold]. Three PCR products (3 µL each) labeled with one of the three different fluorochromes were pooled and diluted with 21 µL of H_2O. A 3 µL aliquot was added to 10 µL of a mixture of 1000 µL Hi-Di™ formamide (Applied Biosystems) and 33 µL GeneScan™ 500 ROX™ (Applied Biosystems), heat-denatured, flash-cooled, and run on an ABI 3730xl at the Georgia Genomics Facility.

Allele patterns were analyzed with GeneMarker software (Softgenetics) and allele calls were manually verified (Supplementary Table 2.2). The criteria for calling alleles were standardized for all genotypes regardless of ploidy; all peaks with heights ≥ 20% of the tallest peak were scored if the tallest peak was more than 5000 units tall; if the tallest peak was less than 5000 units tall, peaks ≥ 30% of the tallest peak were scored. This set of criteria was adopted after observing variation in peak intensities of specific SSR markers run multiple times on the same DNA source.

Detection of Clonality

Diploid accessions that had identical microsatellite patterns and hence were potentially clonal duplicates were identified using the 'Multilocus: Matching' tool in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). Missing data was ignored for the purpose of identity calling. Redundant diploid genotypes were removed from the dataset so that each multi-locus diploid genotype was represented only once in all further analyses (Table 2.3).

Population Structure Analysis

A population structure analysis was conducted with STRUCTURE v3.3.4 (Pritchard et al., 2000a) on the non-redundant diploid genotypes. To determine the number of putative subpopulations (K), K was set to vary from 1 to 10 with 20 iterative simulations per K. The simulations (burn in of 100,000; 1,000,000 Markov Chain Monte Carlo repetitions) used an admixture model and allele frequencies were set as independent. Structure Harvester (Earl and vonHoldt, 2012) was used to calculate the Delta K estimate of Evanno et al. (2005). The simulation with the highest log P(D) returned for the K identified as the most likely was selected as best representing the true population structure.

Genetic Distance and Principal Coordinates Analysis

Genetic distances between the non-redundant diploid genotypes were calculated using the codominant genotypic distance function in GenAlEx 6.5 and used to conduct a principal coordinates analysis, also in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). Summary statistics including the number of alleles per locus, the effective allele number per locus, observed heterozygosity (H_o), expected heterozygosity (H_e), and Shannon Information Index for each locus were calculated using GenAleEx6.5. Phylogenetic Information Content (PIC) values were calculated manually (Supplemental Table 2.1).

Results

Ploidy level Determination and Haploid Genome Size Estimation

The ratio of the DNA content measured in *P. vaginatum and P. distichum* accessions to the DNA content of trout erythrocyte control ranged from 0.17 to 0.76. However, the majority of accessions (91%) had ratios between 0.17 and 0.33 and were classified as diploid. Nine accessions (9%) had ratios between 0.41-0.76 and were classified as polyploid (Table 2.1 and Figure 2.1). Sixteen accessions were subjected to chromosome counts on root tip spreads to confirm chromosome numbers. Examples of chromosome counts are 20 in accessions 509022, HI33 and SeaDwarf, 30 in PI 645598 and ~50 in PI 364981 (Figure 2.2). The average flow cytometer output from the 91 putatively diploid accessions was 17.86. This value was used with a regression line calculated from DNA content estimates from other *Paspalum* species to estimate the *P. vaginatum* genome content at 1.21pg/2C or ~593Mb (Figure 2.3 and Table 2.2).

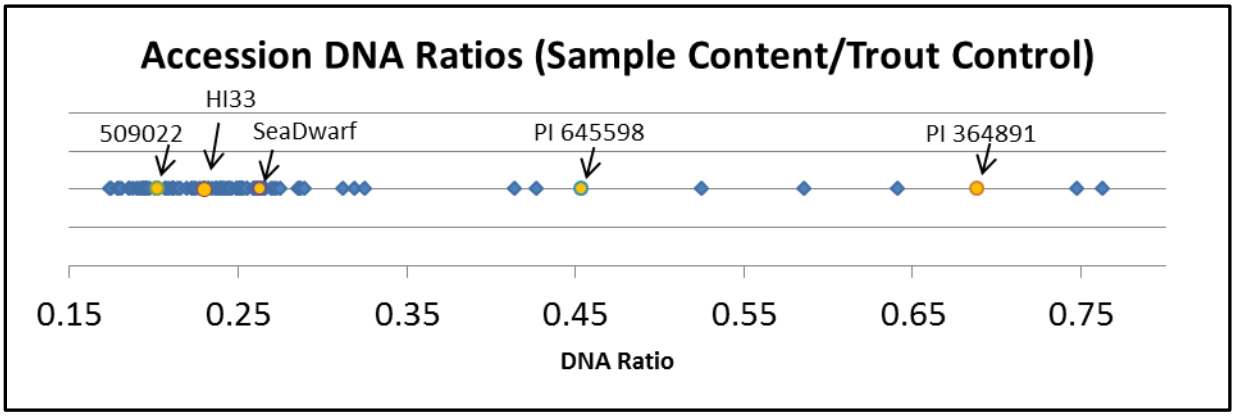

Figure 2.1: DNA ratio (DNA content/trout DNA control) for paspalum lines assayed by flow cytometry

Orange dots indicate the ratios of five lines for which chromosome counts were carried out.

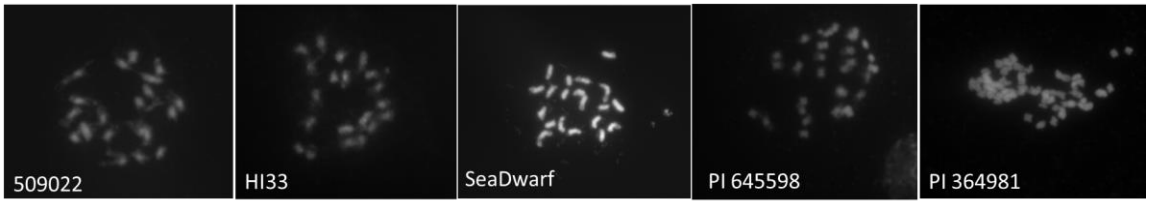

Figure 2.2: Chromosome spreads of select lines stained with DAPI

Spreads illustrate chromosome numbers in diploids (509022, HI33, and SeaDwarf) and polyploids (PI 645598, triploid; PI 364981, putatively pentaploid)

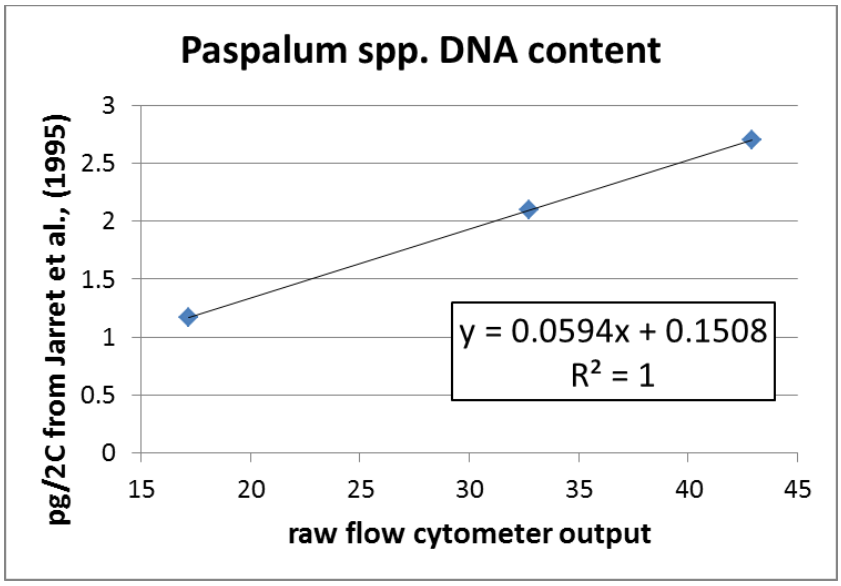

Figure 2.3: Relationship of DNA content for three Paspalum species

The regression line between DNA estimates for three of the *Paspalum* species reported by Jarret et al., (1995) and the same accessions (for *P. notatum* and *P. distichum*) and an available *P. lividum* accession (the PIs screened by Jarret et al., (1995) were not available for rerun). Note: These data are tabulated in Table 2.2.

Table 2.2: Summary of Paspalum spp. DNA Content Estimation

Results from Jarret et al., (1995) and values for the same accessions re-screened in this analysis (or an alternative accession, if the original PIs are no longer available); includes average content of 91 (diploid) *Paspalum vaginatum* lines and an estimate of the haploid genome size for the species calculated using this value and the regression equation.

Summary Table Comparing DNA Content Estimates					
values (pg/2C), from Jarret et al., (1995)			**values (raw), reported here**		
P. notatum	PI 508847	1.17	*P. notatum*	PI 508847	17.17
P. distichum	PI 284500	2.70	*P. distichum*	PI 284500	42.92
P. lividum	PI 404637	2.10	*P. lividum*	PI 404874	32.72
P. lividum	PI 404638	2.12			
P. lividum	PI 508668	2.08	***Paspalum vaginatum* genome size:**		
P. lividum	PI 508938	2.11	AVG of 91 putative diploids = 17.86		
*P. lividum**	mean of 4 PIs*	2.10*	Equates to:	**1.21 pg/2C**	
* *P. lividum* value derived from the mean of 4 PIs				**~593Mb per haploid**	

Categorizing Glumes as Pubescent or Glabrous

Seed heads from diploid and polyploid seashore paspalum lines were evaluated for the presence of glumes (Table 2.1). All putative diploid lines screened were glabrous and thus are *P. vaginatum* except two, Tropic Shore and Spence (Figure 2.4). Three polyploid lines, PI 647922 (Figure 2.4), PI 284500 and PI 576140 also had pubescent glumes. This indicates that these polyploid lines, as well as the diploids Tropic Shore and Spence, would be identified as *P. distichum* L. based on Echarte and Clausen (1993).

SSR Markers and Genotypic Analysis

From a total of 672 clones sequenced, 151 contained SSRs with a CA or GA motif repeat of 5 or greater (22.5% efficiency). Selection criteria for primer development required a minimum repeat length of seven for CA or GA motifs, six for 3 bp motifs and five for 4 bp motifs; 69 sequences

(55 with CA or GA motifs) remained after this filtering and all were used for primer design. Of those, forty-three (62%) gave good amplification when tested on genomic DNA from the cv. 'Salam.' These were used for genotypic characterization of ninety *P. vaginatum* and *P. distichum* accessions as well as one *P. notatum* accession and one *P. lividum* accession (Supplementary Table 2.2).

Amplification with the *P. vaginatum* SSR primer pairs was not reliably achieved in the accessions of *P. notatum* and *P. lividum*, thus multi-locus genotypes were not determined for these two species. The primer sequences, repeat motif, allele number, effective allele number, PIC score, and Shannon Information Index for the 43 SSRs employed are listed in Supplementary Table 2.1. The allele calls for the 90 diploids are displayed in Supplementary Table 2.2. Thirty-seven diploid accessions were identified as having multi-locus genotypes that were identical to at least one other and up to 17 other accessions (Table 2.3). Removal of the duplicates reduced the original panel from 90 accessions to 60 unique multi-locus genotypes excluding the six non-redundant putative polyploids.

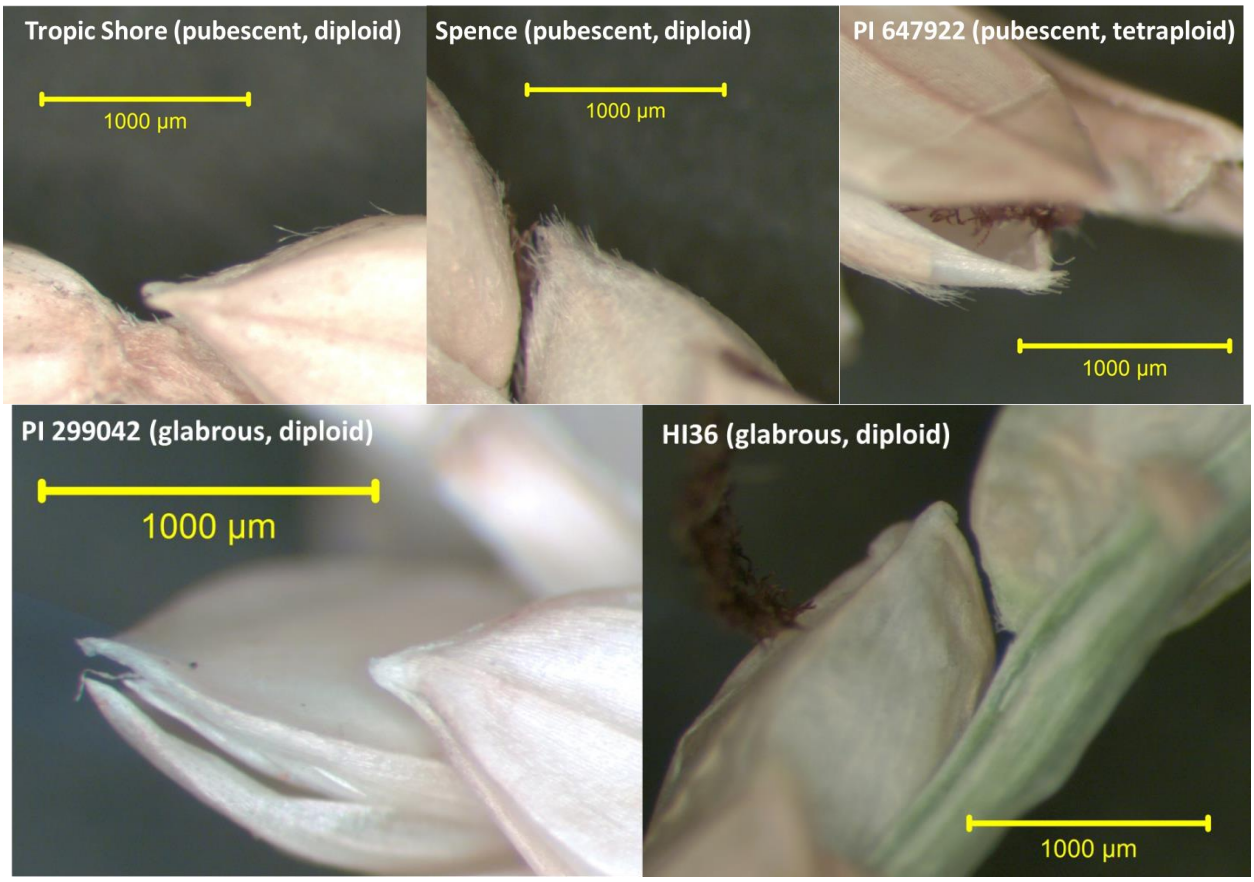

Figure 2.4: Glumes (pubescent or glabrous) in five seashore paspalum accessions

Images document the presence or absence of pubescence on glumes. This feature has traditionally been considered diagnostic in distinguishing *P. vaginatum* and *P. distichum*.

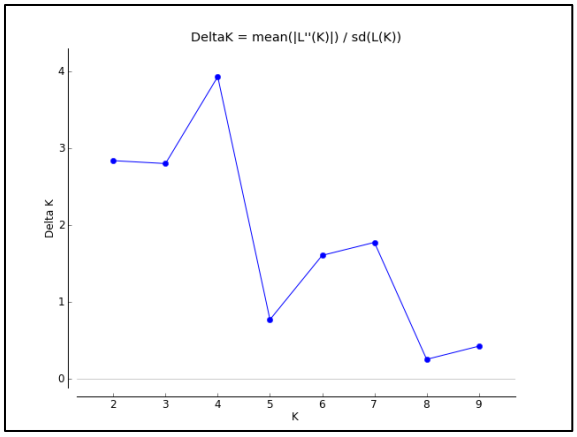

Figure 2.5: Delta K plot of STRUCTURE results following Evanno et al., (2005)

This plot identifies k=4 as the most likely number of subpopulations to recognize in the results from STRUCTURE.

Population Structure Analysis

The Evanno et al., (2005) method for selecting the best value for K identified four as the most probable number of subpopulations (Figure 2.5). The results of the structure analysis are displayed as a bar plot (Figure 2.6). Lines with a probability of assignment greater than 80% were considered pure. Under this criterion, members of subpopulations 1 (red) and 2 (green) do not exhibit patterns indicative of admixture while thirteen individuals assigned to subpopulations 3 and 4 do.

Genetic Distance and Principal Coordinates Analysis (PCoA)

A genetic distance matrix was calculated in GenAlEx. This matrix was used to complete a principal coordinates analysis, also in GenAlEx. The first three principal coordinates (PCos) explained 35.2 % of the total variation. The first and second PCos and the second and third PCos are plotted with individual genotypes color-coded with population membership as assigned in STRUCTURE (Figure 2.7).

Table 2.3: Redundant diploid genotypes

Geno IDs reference the identity of genotypes displayed in the STRUCTURE bar plot (Figure 2.6)

Summary Table Identifying Redundant Diploid Genotypes							
Geno ID	**Accession**	**Collection**	**Source**	**Geno ID**	**Accession**	**Collection**	**Source**
1	PI 276245	USDA	Uruguay	22	PI 647908	USDA	Georgia (USA)
2	PI 299042	USDA	Zimbabwe	23	PI 647913	USDA	Argentina
3	PI 364368	USDA	Mozambique	24	PI 647915	USDA	Texas (USA)
*	509021	UGA	Argentina	25	PI 647920	USDA	Louisiana (USA)
*	PI 647893 (HI10)	USDA	Hawaii (USA)	26	PI 647923	USDA	Australia
*	PI 647896 (HI26)	USDA	Hawaii (USA)	27	509018-2	UGA	
*	PI 647900	USDA	Thailand	28	509018-3	UGA	
*	PI 647901	USDA	Guam	*	Prince	UGA	
*	PI 647914	USDA	Argentina	*	TF P7-4	UGA	
*	PI 647919	USDA	Hawaii (USA)	29	HI26 (PI 647895)	UGA	Hawaii (USA)
*	PI 647921	USDA	Louisiana (USA)	30	Taylor 1 (PI 647903)	UGA	North Carolina (USA)
*	HI14 (PI 647894)	UGA	Hawaii (USA)	31	Taylor 2 (PI 647904)	UGA	North Carolina (USA)
*	HI33 (PI 647897)	UGA	Hawaii (USA)	32	310-79 (PI 647906)	UGA	Argentina
*	HI36 (PI 647898)	UGA	Hawaii (USA)	*	Excal	UGA	
*	Adalayd (PI 647902)	UGA	Australia	33	561-79 (PI 647907)	UGA	UGA
*	Aloha (PI 652948)	UGA	Hawaii	*	Temple 2 (PI 647910)	UGA	Texas (USA)
*	Cloister	UGA		*	TG Kona	UGA	
*	KC9	UGA		34	Temple 1 (PI 647909)	UGA	Texas (USA)
*	Salam	UGA		*	HI 101	UGA	
*	TRB2	UGA		35	SIPV28-1 (PI 647912)	UGA	Georgia (USA)
4	PI 377709	USDA	South Africa	36	Bahama	UGA	
5	PI 403999	USDA	Australia	37	Belize	UGA	
6	PI 508729	USDA	Argentina	38	Collier	UGA	
7	PI 508735	USDA	Argentina	39	Cuba 223	UGA	
*	HI39 (PI 647899)	UGA	Hawaii (USA)	40	Darien	Eudy	Georgia (USA)
*	Cal	UGA		41	FR-4	UGA	
*	K8	UGA		42	FSP1	UGA	
*	Utah1	UGA		43	Spence	UGA	
8	PI 508737	USDA	Argentina	44	Hignight S	UGA	
9	509020	UGA	Argentina	45	HYB5	UGA	
10	509022	UGA	Argentina	46	HYB7	UGA	
*	HH	UGA		47	Kai Luna	UGA	
*	Sea Isle 1	UGA		48	Kim1	UGA	
*	Taliaferro	UGA		49	Polo	UGA	
11	509023	UGA	Argentina	50	Q36313	UGA	
12	Tropic Shore (PI 543854)	UGA	Hawaii (USA)	51	Q36315	UGA	
13	PI 576134	USDA	Brazil	52	Q37956	UGA	
14	PI 576138	USDA	Brazil	53	SeaDwarf	UGA	
15	PI 612771	USDA	Argentina/Peru	54	Sea Isle 2000	UGA	
16	Durban (PI 614678)	USDA	South Africa	55	Sea Isle Supreme	UGA	
17	PI 614679	USDA	Bahamas	56	TCR3	UGA	
18	PI 647885	USDA		57	TCR6	UGA	
19	PI 647886	USDA		58	TOCGC	UGA	
20	PI 647888	USDA		59	Vero Beach	UGA	
21	PI 647891	USDA	Isreal	60	Wai Lua Kauai	UGA	
Any entry with a Geno ID indicated as * is indistiguishable from the other indiviudals in that set							

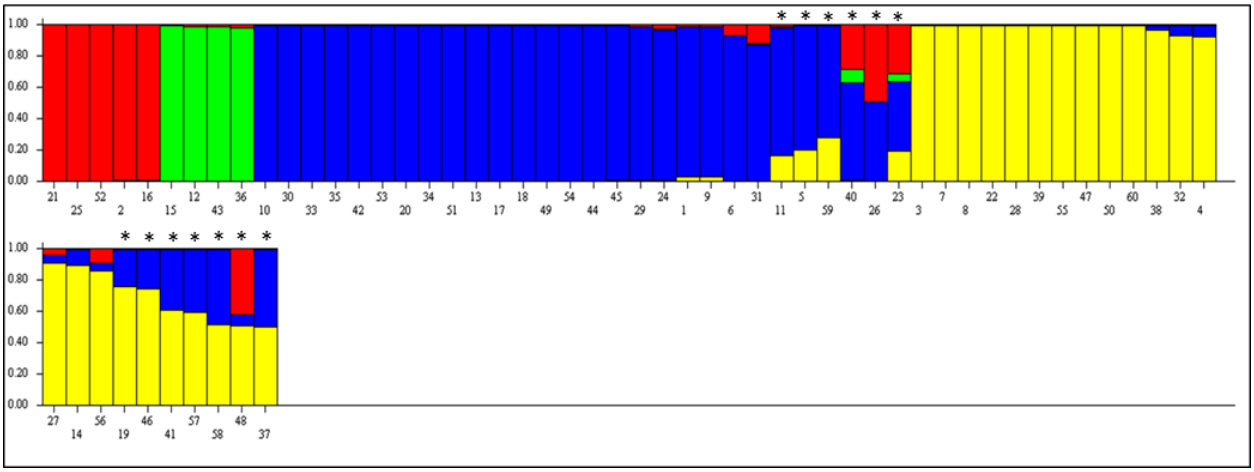

Figure 2.6: Bar plot generated in STRUCTURE

The four colors (red, green, blue, and yellow) represent an individual genotype's membership to the 4 subpopulations. Admixed individuals (<80% membership to any single subpopulation) are indicated with an asterisk (*). Genotype IDs are indicated by the numbers below each bar and refer to non-redundant individuals identified in Table 2.3.

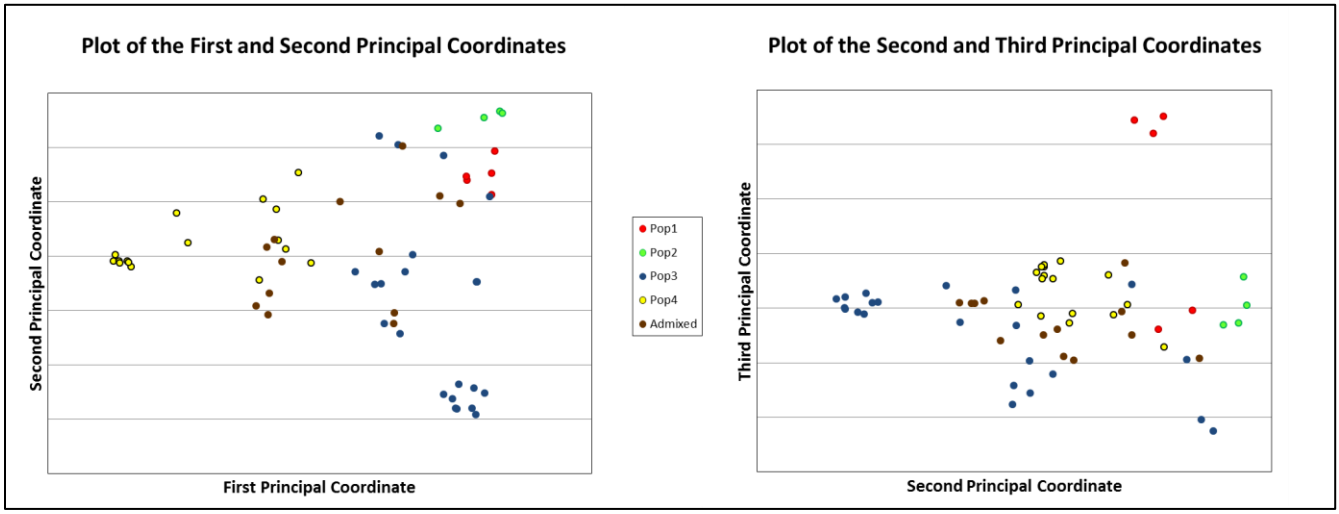

Figure 2.7: Plots of the first and second and second and third principal coordinates

Individual accessions are represented by circles and are color-coded with the population identity assigned in STRUCTURE, with brown indicating admixed individuals; the first PCo explained 14.69 % of the variation, the second 13.88 % and the third 6.63%.

Discussion

DNA Content, Ploidy and Species Identity

Ninety percent of the *P. vaginatum* and *P. distichum* germplasm surveyed were determined to have a DNA content consistent with being diploid. Chromosome counts on three lines across the diploid range confirmed twenty chromosomes in each. Chromosome counts were more difficult on spreads which contained greater numbers of chromosomes, but generally confirm that ploidy levels extend above the diploid state in at least 9 accessions. Within the diploids, the estimated range of genome size extends from 0.48 pg/1C to 0.81 pg/1C. With such a broad range within the diploid context, relying exclusively on flow cytometric data to accurately estimate higher ploidy levels would be tenuous. Chromosome counts suggest that PI 645598 is triploid and its DNA content relative to PIs 647917 and 647918 suggest that they may be triploid as well. A count for PI 364891 suggests it has fifty chromosomes, making that line putatively pentaploid; this also suggests that PIs 284500, 576140, and 647922 are tetraploid. The greatest amount of DNA content was detected in PIs 364977 and 222796, which may be hexaploid. Using the formula for the regression line and the DNA content measured with flow cytometry estimates PI 364977 to contain 3.54 Gb of total DNA, which is ~6 times as large as the haploid genome size estimate.

Based on the presence of glumes, it would appear that two of the potentially *P. distichum* (Spence and Tropic Shore) accessions screened were diploid. Both were previously identified as such. Both of these lines were members of subpopulation 2 (green) which was morphotypically as well as genetically very distinct from the rest of the germplasm. This subpopulation has four members including the two named above. PI 612771, another member of subpopulation 2, had glabrous glumes suggesting a *P. vaginatum* identity, while Bahama, the fourth member, never

flowered and could not be evaluated. The fact that (1) microsatellite markers developed from *P. vaginatum* amplify well in *P. distichum* and (2) both species group in the same subpopulation suggests either that both species are very closely related or that glume pubescence should not be used as the primary feature to distinguish these species. If the first hypothesis is correct, one would expect both species to carry the same genome and to be able to intercross freely. The genome of *P. vaginatum* has been designated as DD, but that of *P. distichum* is currently unknown (Jarret et al., 1998). No crosses between *P. vaginatum* and *P. distichum* have been reported. However, it is possible that the lack of success in crossing the two species is due to the fact that both species typically vary in their ploidy level. More detailed morphological analyses are needed to determine to which species the members of subpopulation 2 belong.

Members of subpopulation 2 had the coarse leaf texture heretofore associated with polyploid paspalum germplasm. Because the members of subpopulation 4 are diploid, our data suggest that polyploidy itself is not required for conditioning the broad-textured morphotype. If leaf texture is not determined by ploidy level, then it should be possible to develop polyploid paspalums with a leaf texture suitable for turf.

Marker and Germplasm Resources

The 43 microsatellite markers used to screen this collection comprise a novel set of SSR markers for *P. vaginatum* germplasm. They amplified equally well in *P. distichum* as in *P. vaginatum*, supporting the close genetic relationship between these species. Amplification from *P. notatum* and *P. lividum* was typically weak or failed altogether, suggesting that the utility of the SSRs presented here will be limited to the Disticha subgroup. Allele numbers identified by these markers in the *P. vaginatum* and *P. distichum* germplasm evaluated ranged from one allele to

twenty-three. Among markers with di-nucleotide repeats, increases in motif repeat number correlated with increases in the number of alleles detected. (Figure 2.8).

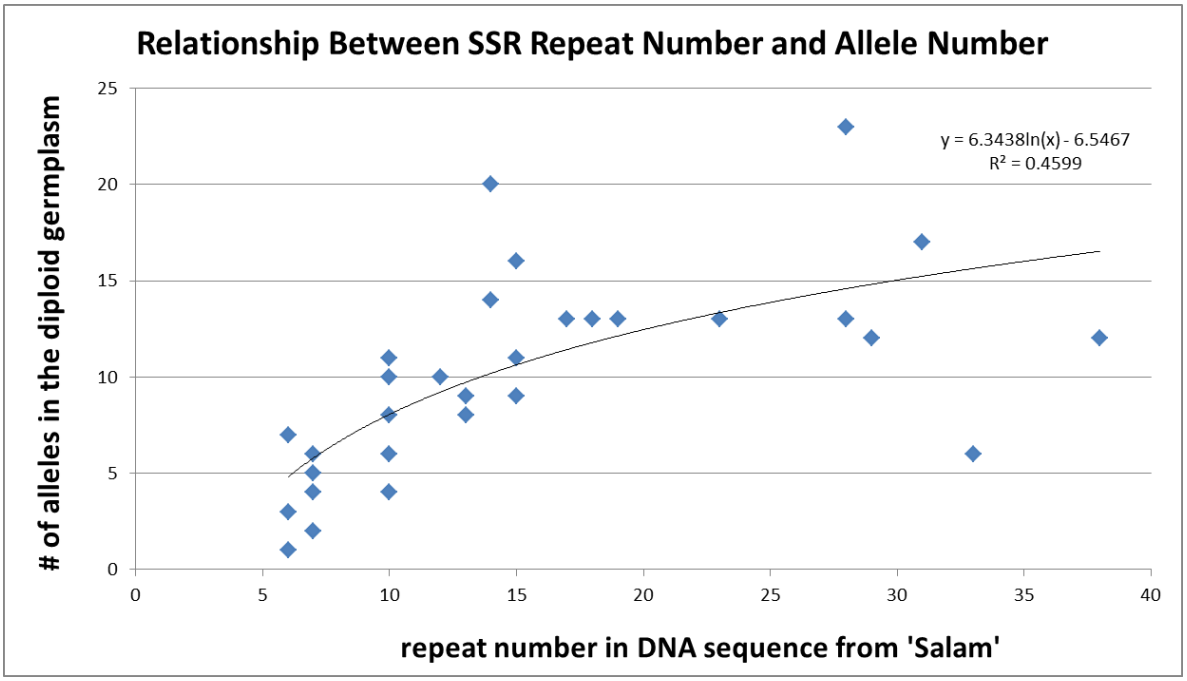

Figure 2.8: Relationship between motif repeat number in dinucleotide repeats and allele number in the diploid paspalum germplasm panel

To reduce phenotyping costs when maintaining germplasm collections, characterizing newly collected accessions, or surveying the collections conserved in herbaria, we identified a core set of 15 highly informative markers. The fourteen markers with the highest PIC score in the panel of seashore paspalum germplasm genotyped, as well as the fourteen with the highest Shannon Information Index (15 markers total) are listed in Table 2.4.

Table 2.4: Fifteen most informative markers from among the 43 developed

This list was screened based on PIC values (Phylogenetic Information Content) and Shannon indices.

15 Most Informative SSR Markers			
Locus	Primer set	PIC	Shannon Index
Locus 2	SSR038	0.803	1.867
Locus 8	SSR023	0.718	1.665
Locus 12	SSR037	0.751	1.772
Locus 15	SSR057	0.773	1.593
Locus 19	SSR086	0.843	2.128
Locus 23	SSR026	0.727	1.764
Locus 25	SSR073	0.801	1.929
Locus 26	SSR034	0.844	2.095
Locus 31	SSR077	0.803	2.174
Locus 33	SSR059	0.829	2.132
Locus 35	SSR065	0.758	1.576
Locus 36	SSR024	0.839	2.298
Locus 38	SSR002	0.834	2.151
Locus 39	SSR072	0.706	1.597
Locus 43	SSR041	0.745	1.743

Clonality

Thirty-seven diploid accessions from the panel had the same microsatellite profile as at least one other accession and were identified as putative clonal duplicates (Table 2.3). It is unclear whether these lines are truly identical, or merely too closely related to be differentiated with our set of 43 SSR markers. If the duplicates are clones, the question remains whether this genetic redundancy characterizes the original nature of the germplasm or whether it is indicative of errors in germplasm maintenance. It seems likely that maintenance errors are responsible to some degree, since several genotypes expected to be clonally identical between the USDA and UGA collections were not (e.g. PI 647885 and HYB5; PI 647886 and HYB7; PI 647913 and PI 647914, 509018-2 and 509018-3). Maintaining clonal identity in seashore paspalum is complicated by the fact that accessions are maintained vegetatively. Seashore paspalum exhibits

aggressive growth rates and can grow into closely spaced pots in a few days' time. Additionally, the materials are maintained as living clones, not as seeds and as such, these materials require periodic regeneration from stolon cuttings which is another occasion for maintenance errors to be introduced. Genotyping materials immediately upon the accession's deposition into the collection, storing this DNA fingerprint, and periodically reconfirming identity may be an appropriate approach to helping to confirm line identity in the future.

Diversity and Population Structure

Because of limitations in the number of alleles that could be entered for a single genotype in STRUCTURE, polyploids were omitted from the diversity analyses. The non-redundant diploid germplasm formed four subpopulations: five genotypes or ~8% of the assayed diploids were partitioned into subpopulation 1, while four genotypes or ~7% of the total were partitioned into subpopulation 2. Subpopulations 3 and 4 comprised ~37% and ~25% of the accessions respectively. Some 22% of the lines had <80% membership to any single subpopulation and were considered admixed. Though subpopulation 1, subpopulation 3 and subpopulation 4 can be differentiated genetically, their members are morphologically highly similar. Subpopulation 2, on the other hand, was morphotypically distinct from the other three subpopulations. All of the members of subpopulations 1, 3, and 4 display an intermediate leaf texture, except for 'SeaDwarf' which is a fine-textured dwarf cultivar, while each member of subpopulation 2 exhibits a course texture.

The populations derived from the genetic data do not indicate geographic origins as being implicated in the structuring. Genotypes recorded as having South American origins as well as lines collected in North America are present in both of the two larger populations, although, in light of the issues with clonal identity, the origin as listed in GRIN may not be correct if the

original line has lost its identity. The results of the PCoA are largely in agreement with the results from STRUCTURE and support the overall patterning apparent in the germplasm. Only two inconsistencies between the analyses are notable: two members identified as belonging to subpopulation 1 (PI 614678 and Q37956) appear differentiated from the other members of that population to a greater extent than expected in the results of the PCoA, specifically, in the plot of the second and third principal coordinates.

Conclusions

Paspalum vaginatum is a grass species of increasing importance worldwide due to its salt tolerance and its ability to serve as forage, erosion control, and as turf for sports surfaces in vulnerable areas in the tropics and sub-tropics. Though it is a member of one of the largest grass genera, and has achieved a pan-tropical distribution along coastlines and in marshes, the details of its genetic diversity, population structure, and distribution history have not been adequately resolved. We have developed forty-three microsatellite markers which were used to survey a collection of paspalum germplasm for genetic diversity. A large amount of clonal redundancy, as well as population structure within the panel members was detected. Future work to characterize the genetic components contributing to phenotypic diversity in this species would benefit from utilizing the knowledge of genetic relatedness presented here to inform choices of distinct or diverse individuals. Additionally, as germplasm is maintained, periodically assaying the identity of materials in the collection, as well new plant introductions received will be possible with these molecular marker resources.

References

Brown, S. M., S. E. Mitchell, C. A. Jester, Z. W. Liu, S. Kresovich and R. R. Duncan (1996). DNA typing (profiling) of seashore paspalum (*Paspalum vaginatum* Swartz) ecotypes and cultivars. Recent Cellular and Molecular Genetic Approaches to Turfgrass Improvement. Chelsea, MI, Ann Arbor Press: 39-51.

Brugnoli, E. A., M. H. Urbani, C. L. Quarin, E. J. Martínez and C. A. Acuña (2013). "Diversity in diploid, tetraploid, and mixed diploid–tetraploid populations of *Paspalum simplex*." Crop Science 53(4).

Chase, A. (1929). The North American species of *Paspalum*. Washington, G.P.O.

Chen, Z., M. Newman, W. Kim, M. Wang and P. Raymer (2005). "Molecular characterization of genetic diversity in the USDA seashore paspalum germplasm collection." ITS Research Journal 10: 543-549.

Doyle, J. and J. Doyle (1987). "A rapid DNA isolation procedure for small quantities of fresh leaf tissue." Phytochemistry Bulletin 19: 11-15.

Duncan, R. and R. Carrow (2000). Seashore Paspalum: the Environmental Turfgrass, Sleeping Bear Press.

Echarte, A. and A. Clausen (1993). "Morphological affinities between *Paspalum distichum* senso lato and *Paspalum vaginatum*." Boletin de la Sociedad Argentina de Botanica 29(3-4): 143-152.

Echarte, A. M., A. M. Clausen and C. A. Sala (1992). "Numeros cromosomicos y variabilidad morfologica de *Paspalum distichum* (poaceae) en la provincia de Buenos Aires (Argentina)." Darwiniana 31(1/4): 185-197.

Gates, R. N., C. L. Quarin and C. G. S. Pedreira (2004). Bahiagrass. Warm-Season (C4) Grasses. L. E. Moser, B. L. Burson and L. E. Sollenberger. Madison, WI, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America: 651-681.

Harris-Shultz, K., P. Raymer, B. E. Scheffler and R. S. Arias (2013). "Development and characterization of seashore paspalum SSR markers." Crop Science 53(6): 2679-2685.

Hojsgaard, D., A. I. Honfi, G. Rua and J. Davina (2009). "Chromosome numbers and ploidy levels of *Paspalum* species from subtropical South America (Poaceae)." Genetic Resources and Crop Evolution 56(4): 533-545.

Jarret, R. L., Z. W. W. Liu and R. W. Webster (1998). "Genetic diversity among *Paspalum* spp. as determined by RFLPs." Euphytica 104(2): 119-125.

Jarret, R. L., P. Ozias-Akins, S. Phatak, R. Nadimpalli, R. Duncan and S. Hiliard (1995). "DNA contents in *Paspalum* spp. determined by flow-cytometry." Genetic Resources and Crop Evolution 42(3): 237-242.

Kato, A. (1999). "Air drying method using nitrous oxide for chromosome counting in maize." Biotech Histochem 74(3): 160-166.

Liu, Z. W., R. L. Jarret, R. R. Duncan and S. Kresovich (1994). "Genetic-relationships and variation among ecotypes of seashore paspalum (*Paspalum vaginatum*) determined by random amplified polymorphic DNA markers." Genome 37(6): 1011-1017.

Morrone, O., L. Aagesen, M. A. Scataglini, D. L. Salariato, S. S. Denham, M. A. Chemisquy, S. M. Sede, L. M. Giussani, E. A. Kellogg and F. O. Zuloaga (2012). "Phylogeny of the Paniceae (Poaceae: Panicoideae): integrating plastid DNA sequences and morphology into a new classification." Cladistics 28(4): 333-356.

Quarin, C. L. and B. L. Burson (1991). "Cytology of sexual and apomictic paspalum species." Cytologia 56: 223-228.

Quarin, C. L., F. Espinoza, E. J. Martinez, S. C. Pessino and O. A. Bovo (2001). "A rise in ploidy level induces the expression of apomixis in *Paspalum notatum*." Sex Plant Reproduction 13: 243-249.

Rozen, S. and H. Skaletsky (2000). "Primer3 on the WWW for general users and for biologist programmers." Methods Mol Biol 132: 365-386.

Rua, G. H., P. R. Speranza, M. Vaio and M. Arakaki (2010). "A phylogenetic analysis of the genus *Paspalum* (Poaceae) based on cpDNA and morphology." Plant Systematics and Evolution 288(3-4): 227-243.

Skerman, P. and F. Riveros (1990). Tropical Grasses. Rome, Italy, Food and Agriculture Organization of the United Nations.

Souza-Chies, T. T., L. Essi, G. H. Rua, J. F. M. Valls and R. B. Miz (2006). "A preliminary approach to the phylogeny of the genus *Paspalum* (Poaceae)." Genetica 126(1-2): 15-32.

Urbani, M. H., C. L. Quarin, F. Espinoza, M. I. O. Penteado and I. F. Rodrigues (2002). "Cytogeography and reproduction of the *Paspalum simplex* polyploid complex." Plant Systematics and Evolution 236(1-2): 99-105.

Wang, M. L., Z. B. Chen, N. A. Barkley, M. L. Newman, W. Kim, P. Raymer and G. A. Pederson (2006). "Characterization of seashore paspalum (*Paspalum vaginatum* Swartz) germplasm by transferred SSRs from wheat, maize and sorghum." Genetic Resources and Crop Evolution 53(4): 779-791.

Zuloaga, F. O. and O. Morrone (2005). Revisión de las especies de Paspalum para América del Sur austral: (Argentina, Bolivia, sur del Brasil, Chile, Paraguay y Uruguay), Missouri Botanical Garden Press

Supplementary Table 2.1: Forty-three microsatellite loci developed

The table includes tailed primer sets, estimated fragment size, motif and repeat number as well as marker diversity information and % missing data from the screen. PIC or Shannon Index values that were among the fifteen best are indicated in bold.

Locus #	Primer set	Primer Sequence	estimated fragment size in 'Salam' (bp)	motif(repeat)	PIC	Shannon Index	# alleles	expect # alleles	Ho	He	%missing data
Locus 1	**SSR080_D_Ft**	**CGTTGTAAAACGACGGCCAGT**AGGGAGTGCACCAGTACGAC	162	AG(15)	0.549	1.264	9	2.216	0.212	0.549	13.33%
	SSR080_D_R	GACCTGCAACAAAACGAACC									
Locus 2	**SSR038_Ft**	**CGTTGTAAAACGACGGCCAGT**AGTGCTCGAAAGCAAAGCTC	332	AG(38)	**0.803**	**1.867**	12	5.065	0.878	0.803	18.33%
	SSR038_R	AACACCAACAGAGGCCTGAC									
Locus 3	**SSR055_Ft**	**CGTTGTAAAACGACGGCCAGT**AAGAACGCCGATGTGGTAAG	270	TG(10)	0.621	1.220	8	2.637	0.385	0.621	13.33%
	SSR055_R	ACTTGCAGATGCTCGTCAAC									
Locus 4	**SSR007_Ft**	**CGTTGTAAAACGACGGCCAGT**AATCTACACCCACACGCCTC	163	GCC(6)	0.018	0.051	2	1.018	0.018	0.018	6.67%
	SSR007_R	CTGCCTAGGAAACAAGCAGG									
Locus 5	**SSR046_Ft**	**CGTTGTAAAACGACGGCCAGT**AAGAACGCCGATGTGGTAAG	332	TG(13)	0.664	1.363	9	2.975	0.433	0.664	0.00%
	SSR046_R	CCAGTGAAACCCAGGTAAGC									
Locus 6	**SSR040_Ft**	**CGTTGTAAAACGACGGCCAGT**GGGTGGGATTCTGTTTTCAG	271	TC(7)	0.502	0.877	4	2.008	0.431	0.502	3.33%
	SSR040_R	CGTTTGGTGACATGTCCTTG									
Locus 7	**SSR003_Ft**	**CGTTGTAAAACGACGGCCAGT**AGCCGGTATGGTGTTCTGTC	167	TTA(6)	0.255	0.526	5	1.342	0.169	0.255	1.67%
	SSR003_R	GCGTACATGCAGCAGAATTG									
Locus 8	**SSR023_Ft**	**CGTTGTAAAACGACGGCCAGT**GAGACCAGATGCATCACAGG	340	AC(18)	**0.718**	**1.665**	13	3.552	0.683	0.718	0.00%
	SSR023_R	GGACACAATCGACATGCTTC									
Locus 9	**SSR001_Ft**	**CGTTGTAAAACGACGGCCAGT**GCGAACCAGAGAGGAAGATG	279	CT(10)	0.333	0.852	10	1.498	0.136	0.333	1.67%
	SSR001_R	AAACAAATTAAAGCACGGCG									
Locus 10	**SSR010_Ft**	**CGTTGTAAAACGACGGCCAGT**CTCTTAATTTGACCGCTGCC	184	CG(10)	0.098	0.257	4	1.109	0.000	0.098	1.67%
	SSR010_R	ACGTGCACATGAGAACGAAC									
Locus 11	**SSR063_Ft**	**CGTTGTAAAACGACGGCCAGT**GCATATATCGCCGGATGACT	357	GGA(6)	0.283	0.519	4	1.395	0.211	0.283	5.00%
	SSR063_R	TGATTCAGAATACGCGCAAG									
Locus 12	**SSR037_Ft**	**CGTTGTAAAACGACGGCCAGT**TGCAGTTTGGTTCTGGGTAAG	281	TC(23)	**0.751**	**1.772**	13	4.010	0.607	0.751	6.67%
	SSR037_R	TTGTCAATGTTGAGGTTTTGAAC									
Locus 13	**SSR074_D_Ft**	**CGTTGTAAAACGACGGCCAGT**AAACAAACATGGAGAAGGCG	184	AAG(6)	0.132	0.296	3	1.152	0.020	0.132	16.67%
	SSR074_D_R	GAACCCATTTCGTAGCCTTG									
Locus 14	**SSR081_D_Ft**	**CGTTGTAAAACGACGGCCAGT**CAAGAGCCTTCAAGAGGGTG	365	GT(7)	0.132	0.335	5	1.151	0.086	0.132	3.33%
	SSR081_D_R	AGGCATACAGGTTCCAGCAG									
Locus 15	**SSR057_Ft**	**CGTTGTAAAACGACGGCCAGT**CTCCTGTTGATGGCTGCATA	290	CT(7)	**0.773**	**1.593**	6	4.401	0.729	0.773	1.67%
	SSR057_R	ATTTATGAGAGCGCGTCGAT									
Locus 16	**SSR004_Ft**	**CGTTGTAAAACGACGGCCAGT**ACCAGTCCAGTACCACCGAG	236	CGTG(4)	0.196	0.401	4	1.244	0.217	0.196	0.00%
	SSR004_R	CGAAGCAAGCCATCCTTATC									
Locus 17	**SSR012_Ft**	**CGTTGTAAAACGACGGCCAGT**CCTCTTGATTGGAAACAGGG	238	TG(6)	0.033	0.096	3	1.034	0.033	0.033	0.00%
	SSR012_R	GCCAGCATGGACATGTATTG									
Locus 18	**SSR014_Ft**	**CGTTGTAAAACGACGGCCAGT**TAGTGGTAGGGGCAGGTGAC	298	TCT(14)	0.033	0.086	2	1.034	0.034	0.033	1.67%
	SSR014_R	AGCAAGCCATCATTGGCTAC									
Locus 19	**SSR086_D_Ft**	**CGTTGTAAAACGACGGCCAGT**AGTCACAGCACGACGACAAG	239	CT(14)	**0.843**	**2.128**	14	6.367	0.444	0.843	10.00%
	SSR086_D_R	ACGACATCGAGGACGAGG									
Locus 20	**SSR054_Ft**	**CGTTGTAAAACGACGGCCAGT**ATCGATCGAGGCATCAAATG	381	TG(22)CT(7)	0.705	1.583	12	3.394	0.780	0.705	1.67%
	SSR054_R	CTTTTCGCCTCTGATCTTGC									
Locus 21	**SSR008_Ft**	**CGTTGTAAAACGACGGCCAGT**AATCAGCACAGGGAACTTGG	300	CACTC(4)	0.287	0.555	4	1.403	0.178	0.287	25.00%
	SSR008_R	CAGAGTGCTGTGTACCGTGC									
Locus 22	**SSR078_D_Ft**	**CGTTGTAAAACGACGGCCAGT**ATCGGGAGCACTTCACTGTC	406	CT(13)/AAG(7)	0.516	0.951	6	2.065	0.143	0.516	18.33%
	SSR078_D_R	AACCACTGCAATCGAAGACC									

Supplementary Table 2.1 (continued): Forty-three microsatellite loci developed

The table includes tailed primer sets, estimated fragment size, motif and repeat number as well as marker diversity information and % missing data from the screen. PIC or Shannon Index values that were among the fifteen best are indicated in bold.

Locus #	Primer set	Primer Sequence	estimated fragment size in 'Salam' (bp)	motif(repeat)	PIC	Shannon Index	# alleles	expect # alleles	Ho	He	%missing data
Locus 23	**SSR026_Ft_N**	**CGTTGTAAAACGACGGCCAGT**GTTTCGTTCCGTCTCGTCTC	308	CT(17)	**0.727**	**1.764**	13	3.657	0.733	0.727	0.00%
	SSR026_R_N	TTACGTAGTGCGTGGTGCAG									
Locus 24	**SSR060_Ft**	**CGTTGTAAAACGACGGCCAGT**GTCGATTAAATCACCGACAGTAAG	250	CT(6)	0.674	1.265	7	3.066	0.424	0.674	1.67%
	SSR060_R	CTTAGCGTACGTAACCGTCAGAG									
Locus 25	**SSR073_D_Ft**	**CGTTGTAAAACGACGGCCAGT**ATCTGGGCATCTTCCATCTG	411	CT(19)	**0.801**	**1.929**	13	5.017	0.782	0.801	8.33%
	SSR073_D_R	TCATGAGTGCACCGAGAGAC									
Locus 26	**SSR034_Ft**	**CGTTGTAAAACGACGGCCAGT**TCTTAGTTCGGAGGTTTTCCAG	308	CA(28)	**0.844**	**2.095**	13	6.429	0.500	0.844	0.00%
	SSR034_R	GGCGGTTTTGGATTCCTTAC									
Locus 27	**SSR062_Ft**	**CGTTGTAAAACGACGGCCAGT**AAAGGAGTGGTTGGAGTTGC	254	AAT(7)	0.512	1.029	6	2.049	0.490	0.512	15.00%
	SSR062_R	GGGTGCACTGCATAGCATAG									
Locus 28	**SSR083_D_Ft**	**CGTTGTAAAACGACGGCCAGT**AAAGGAGGAGGTTGTGAGGG	412	CT(13)	0.652	1.317	8	2.877	0.717	0.652	0.00%
	SSR083_D_R	GGTGCAATGATTATGGAATGG									
Locus 29	**SSR056_Ft**	**CGTTGTAAAACGACGGCCAGT**GATCGATGGTGATGGGAATC	309	TG(7)	0.143	0.349	4	1.166	0.067	0.143	0.00%
	SSR056_R	GCCCGGTAGCTGGTAGTACG									
Locus 30	**SSR013_Ft**	**CGTTGTAAAACGACGGCCAGT**GAGGCAATTGATCTTGGAGG	256	TG(7)	0.067	0.150	2	1.071	0.000	0.067	3.33%
	SSR013_R	CCATGTCTCTACCAATGGGC									
Locus 31	**SSR077_D_Ft**	**CGTTGTAAAACGACGGCCAGT**GTCGTCGTCGAGGAGAGG	422	GA(14)	**0.803**	**2.174**	20	5.069	0.474	0.803	5.00%
	SSR077_D_R	ATACAACGTGCCTCCATTCC									
Locus 32	**SSR021_Ft**	**CGTTGTAAAACGACGGCCAGT**AGGCCATCAAGGTGAGTACG	317	AC(15)AG(18)	0.654	1.251	6	2.891	0.404	0.654	5.00%
	SSR021_R	GCCGTCAATTGGTTGCTATC									
Locus 33	**SSR059_Ft**	**CGTTGTAAAACGACGGCCAGT**CCGTCTGGGTAGAAGCTGAA	257	GC(10)AC(21)	**0.829**	**2.132**	17	5.835	0.600	0.829	0.00%
	SSR059_R	GACAACAAGATCCCGTGCTT									
Locus 34	**SSR087_D_Ft**	**CGTTGTAAAACGACGGCCAGT**TTCTGTTGCTGGGACTTCAG	422	CA(10)	0.412	0.821	6	1.701	0.288	0.412	1.67%
	SSR087_D_R	GCCAACAACACACGCATATC									
Locus 35	**SSR065_Ft**	**CGTTGTAAAACGACGGCCAGT**CCCTATCATCAGCAGGGCTA	324	TACG(6)	**0.758**	1.576	9	4.128	0.617	0.758	0.00%
	SSR065_R	ATGGTTTGGAGGAGACGTTG									
Locus 36	**SSR024_Ft_N**	**CGTTGTAAAACGACGGCCAGT**CATACCGGGGATACCAGTTC	267	AG(28)	**0.839**	**2.298**	23	6.206	0.702	0.839	5.00%
	SSR024_R_N	CTTTCGCAGGGATTTCAGAG									
Locus 37	**SSR084_D_Ft**	**CGTTGTAAAACGACGGCCAGT**ACCCAGTCGATCTCATCCTG	449	CAT(5)/GGA(5)	0.521	0.819	5	2.087	0.431	0.521	3.33%
	SSR084_D_R	GTCATAGAAACCGTCAGCCC									
Locus 38	**SSR002_Ft**	**CGTTGTAAAACGACGGCCAGT**CTGGAGAGGAGAGCTAACCG	270	CT(15)	**0.834**	**2.151**	16	6.019	0.893	0.834	6.67%
	SSR002_R	GCAAACATGCAACACAAAGG									
Locus 39	**SSR072_D_Ft**	**CGTTGTAAAACGACGGCCAGT**AACACATGAGCTCACCATGC	453	CT(10)	0.706	**1.597**	11	3.399	0.439	0.706	5.00%
	SSR072_D_R	AGTCGTTGGCCCTAGTTGTG									
Locus 40	**SSR029_Ft_N**	**CGTTGTAAAACGACGGCCAGT**CCAGTGAAACCCAGGTAAGC	330	AC(12)	0.674	1.418	10	3.064	0.441	0.674	1.67%
	SSR029_R_N	AAGAACGCCGATGTGGTAAG									
Locus 41	**SSR011_Ft**	**CGTTGTAAAACGACGGCCAGT**AATCAGGCCAGAATGTGAGG	270	CA(6)	0.000	0.000	1	1.000	0.000	0.000	0.00%
	SSR011_R	GAGGAAGAAGGCTTCCAACC									
Locus 42	**SSR066_Ft**	**CGTTGTAAAACGACGGCCAGT**ATGTCTTTGCCTGCTGCTCT	314	ATT(6)	0.567	1.245	9	2.311	0.579	0.567	5.00%
	SSR066_R	AGGGACCCTTGGCTTGTACT									
Locus 43	**SSR041_Ft**	**CGTTGTAAAACGACGGCCAGT**ACGGTGAATAAAGCGGAGTG	331	CT(15)	**0.745**	**1.743**	11	3.917	0.370	0.745	10.00%
	SSR041_R	GCAAACATGCAACACAAAGG									

Supplementary Table 2.2: Genotyping calls for forty-three microsatellite markers

Genotyping calls for 90 diploid lines are listed as the fragment size; -9 indicates a missing data point; VIC, NED, or FAM I identify the fluorochrome used with a particular marker

Accession ID:	Locus 1		Locus 2		Locus 3		Locus 4		Locus 5		Locus 6		Locus 7		Locus 8		Locus 9		Locus 10		Locus 11	
	80 VIC		38 NED		55 FAM		07 VIC		46 NED		40 FAM		03 VIC		23 NED		01 FAM		10 VIC		63 NED	
PI 276245	184	184	-9	-9	295	299	-9	-9	352	356	-9	-9	189	189	360	363	-9	-9	200	200	369	375
PI 299042	188	188	357	363	287	287	183	183	344	344	294	294	189	189	346	363	296	296	200	200	375	375
PI 364368	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
PI 377709	185	185	-9	-9	295	299	183	183	352	356	294	294	189	189	363	363	296	296	200	200	375	375
PI 403999	-9	-9	-9	-9	-9	-9	183	183	352	356	294	294	189	189	348	354	296	296	200	200	375	375
PI 508729	184	184	357	357	295	299	183	183	352	356	294	294	189	189	363	363	304	304	200	200	369	375
PI 508735	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	300	300	200	200	375	375
PI 508737	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
509020	185	185	355	355	295	299	183	183	352	356	294	294	189	189	352	363	296	296	200	200	369	375
509021	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
509022	185	195	352	355	299	299	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
509023	204	204	355	358	295	295	183	183	352	352	294	294	189	189	348	350	296	296	200	200	375	375
PI 543854 (Tropic Shore)	-9	-9	-9	-9	-9	-9	183	183	344	346	292	292	189	198	344	348	292	292	200	200	369	375
PI 576134	184	195	-9	-9	295	299	183	183	342	356	294	294	189	189	348	363	296	304	200	200	369	375
PI 576138	185	185	357	363	295	295	183	183	352	352	294	294	189	189	360	363	296	296	200	200	369	375
PI 612771	164	164	341	353	260	316	183	183	372	372	291	291	183	189	342	350	296	296	133	133	366	372
Durban (PI 614678)	185	185	355	361	297	297	-9	-9	354	354	294	294	189	189	339	352	296	304	200	200	369	369
PI 614679	185	185	352	363	295	295	183	183	352	356	294	296	189	189	348	363	296	296	200	200	-9	-9
PI 647885	185	185	342	352	295	299	183	183	352	356	294	296	183	189	348	348	296	296	200	200	375	375
PI 647886	185	185	363	363	295	295	183	183	352	352	294	296	183	189	348	358	296	296	200	200	375	375
PI 647888	185	195	352	355	299	299	183	183	356	356	294	294	189	189	348	363	296	296	197	197	375	375
PI 647891	188	188	357	363	287	287	183	183	344	344	294	294	189	189	346	363	296	296	-9	-9	375	375
PI 647893 (HI10)	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
PI 647896 (HI32)	-9	-9	-9	-9	-9	-9	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
PI 647900	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
PI 647901	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	-9	-9
PI 647908	185	185	352	363	-9	-9	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
PI 647913	164	230	355	355	295	295	183	183	352	352	296	296	189	189	338	354	296	306	200	200	369	369
PI 647914	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
PI 647915	164	164	342	363	295	295	183	183	352	352	296	296	183	189	348	348	312	316	200	200	375	375
PI 647919	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
PI 647920	188	188	357	363	287	287	183	183	344	344	294	294	189	189	346	363	296	296	200	200	375	375
PI 647921	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
PI 647923	-9	-9	-9	-9	-9	-9	183	183	340	344	294	296	183	189	348	348	296	316	200	200	369	375
509018-2	185	185	352	355	295	299	183	183	352	356	296	296	189	189	354	363	296	296	200	200	369	375
509018-3	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
HI14 (PI 647894)	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
HI26 (PI 647895)	-9	-9	-9	-9	295	295	183	183	352	352	296	296	183	189	348	348	296	296	200	200	375	375
HI33 (PI 647897)	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
HI36 (PI 647898)	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
HI39 (PI 647899)	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	300	300	200	200	375	375
Adalayd (PI 647902)	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
Taylor 1 (PI 647903)	185	195	352	355	299	299	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
Taylor 2 (PI 647904)	195	195	342	355	299	299	183	183	342	356	292	294	-9	-9	348	354	296	296	200	200	369	375

Supplementary Table 2.2 (continued): Genotyping calls for forty-three microsatellite markers

Genotyping calls for 90 diploid lines are listed as the fragment size; -9 indicates a missing data point; VIC, NED, or FAM identify the fluorochrome used with a particular marker

Accession ID:	Locus 12		Locus 13		Locus 14		Locus 15		Locus 16		Locus 17		Locus 18		Locus 19		Locus 20		Locus 21		Locus 22	
	37 FAM		74 VIC		81 NED		57 FAM		04 VIC		12 VIC		14 FAM		86 VIC		54 NED		08 FAM		78 NED	
PI 276245	284	284	204	204	-9	-9	308	309	257	257	262	262	317	317	249	249	379	385	-9	-9	428	432
PI 299042	-9	-9	204	204	383	383	-9	-9	253	257	262	262	317	317	248	257	385	407	-9	-9	-9	-9
PI 364368	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
PI 377709	284	294	204	204	383	383	309	314	257	257	262	262	317	317	249	261	379	385	318	318	428	428
PI 403999	294	302	204	204	383	383	308	308	257	257	262	262	317	317	256	256	373	403	318	318	430	430
PI 508729	284	284	-9	-9	383	383	309	309	257	257	262	262	317	317	251	251	379	385	-9	-9	428	432
PI 508735	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
PI 508737	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
509020	286	302	204	204	383	383	308	308	257	257	260	262	317	317	255	255	403	403	318	318	-9	-9
509021	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
509022	302	302	-9	-9	383	383	308	308	257	257	262	262	317	317	255	255	385	403	318	318	430	430
509023	294	302	207	207	383	383	308	309	257	257	262	262	317	317	249	256	379	385	318	318	432	432
PI 543854 (Tropic Shore)	279	284	207	207	381	383	312	323	249	257	262	262	317	321	248	250	363	385	291	295	-9	-9
PI 576134	284	302	204	207	383	383	308	312	257	257	262	262	317	317	249	257	385	385	318	321	428	430
PI 576138	284	294	204	204	383	383	309	311	257	257	262	262	317	317	249	261	385	385	318	318	428	428
PI 612771	280	292	204	204	385	385	309	312	257	257	262	262	317	317	252	252	361	361	-9	9	428	428
Durban (PI 614678)	304	304	204	204	383	385	312	312	253	257	262	262	317	317	263	263	379	385	321	321	428	430
PI 614679	386	302	-9	-9	383	383	308	309	257	257	262	262	317	317	249	255	377	403	318	321	428	428
PI 647885	286	302	-9	-9	383	383	308	312	257	257	262	262	317	317	-9	-9	385	403	-9	-9	412	414
PI 647886	302	302	204	204	383	383	308	314	257	257	262	262	317	317	-9	-9	403	403	318	318	428	428
PI 647888	284	302	-9	-9	383	383	308	308	257	257	262	262	-9	-9	255	255	385	403	318	318	430	430
PI 647891	294	294	-9	-9	383	383	309	314	253	257	262	262	317	317	248	257	385	407	-9	-9	-9	-9
PI 647893 (HI10)	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
PI 647896 (HI32)	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
PI 647900	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
PI 647901	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
PI 647908	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
PI 647913	286	286	204	204	383	383	308	312	253	257	262	262	317	317	250	250	389	403	-9	-9	-9	-9
PI 647914	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
PI 647915	286	286	204	204	383	383	309	312	253	257	262	262	317	317	249	249	377	403	318	321	428	428
PI 647919	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	-9	-9
PI 647920	294	294	-9	-9	383	383	309	314	253	257	262	262	317	317	248	257	385	407	-9	-9	430	430
PI 647921	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
PI 647923	280	294	204	204	383	383	309	314	257	257	262	262	317	317	254	254	379	385	321	321	428	428
509018-2	-9	-9	204	204	383	383	311	312	253	257	262	262	317	317	250	256	385	389	-9	-9	428	428
509018-3	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
HI14 (PI 647894)	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
HI26 (PI 647895)	286	286	204	204	383	383	309	312	253	257	262	262	317	317	249	249	377	403	318	321	-9	-9
HI33 (PI 647897)	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
HI36 (PI 647898)	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
HI39 (PI 647899)	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
Adalayd (PI 647902)	-9	-9	204	204	-9	-9	311	314	-9	-9	262	262	317	317	256	256	385	403	318	318	428	428
Taylor 1 (PI 647903)	302	302	204	204	383	383	308	308	257	257	262	262	317	317	255	255	385	403	318	318	430	430
Taylor 2 (PI 647904)	280	302	204	204	383	383	308	309	253	257	262	262	317	317	251	255	-9	-9	318	318	430	430

Supplementary Table 2.2 (continued): Genotyping calls for forty-three microsatellite markers
Genotyping calls for 90 diploid lines are listed as the fragment size; -9 indicates a missing data point; VIC, NED, or FAM identify the fluorochrome used with a particular marker

Accession ID:	Locus 23		Locus 24		Locus 25		Locus 26		Locus 27		Locus 28		Locus 29		Locus 30		Locus 31		Locus 32		Locus 33	
	26 FAM		60 VIC		73 NED		34 FAM		62 VIC		83 NED		56 FAM		13 VIC		77 NED		21 FAM		59 VIC	
PI 276245	328	347	269	269	428	434	304	312	-9	-9	424	424	331	331	281	281	337	387	434	434	196	222
PI 299042	320	332	265	265	428	432	310	310	273	275	424	424	331	331	281	281	417	417	432	432	240	258
PI 364368	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
PI 377709	324	328	271	271	432	434	304	304	273	275	424	424	331	331	281	281	337	337	434	434	222	222
PI 403999	324	328	265	269	428	432	312	318	275	275	430	430	331	331	281	281	385	392	436	436	240	271
PI 508729	328	347	269	269	428	428	312	312	273	301	424	424	331	331	281	281	337	387	432	432	196	222
PI 508735	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
PI 508737	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
509020	324	334	265	269	422	426	312	334	275	275	424	426	331	331	281	281	385	392	436	436	264	271
509021	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
509022	324	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	385	385	436	436	240	240
509023	324	330	269	269	418	428	312	326	273	275	424	430	336	336	281	281	337	364	436	436	271	279
PI 543854 (Tropic Shore)	303	312	260	269	414	416	298	298	286	286	424	444	329	331	282	282	329	329	-9	-9	192	210
PI 576134	324	330	265	269	422	428	312	312	275	301	424	424	331	336	281	281	364	387	436	436	196	240
PI 576138	328	330	269	269	432	434	312	326	273	275	424	424	331	331	281	281	337	387	434	434	222	222
PI 612771	324	328	267	267	426	434	302	302	278	278	424	426	326	329	281	281	310	310	-9	-9	194	194
Durban (PI 614678)	322	324	269	269	422	430	326	353	273	275	424	434	331	331	281	281	347	347	432	436	240	240
PI 614679	324	324	269	271	418	428	304	304	275	301	424	430	331	331	281	281	356	385	430	432	198	198
PI 647885	322	324	268	271	424	428	304	334	-9	-9	424	430	331	331	281	281	385	399	434	436	271	271
PI 647886	324	324	271	271	-9	-9	310	326	-9	-9	424	432	331	331	281	281	392	392	434	436	271	286
PI 647888	320	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	385	392	436	436	222	240
PI 647891	320	332	265	269	428	432	310	310	273	275	424	424	331	331	281	281	417	417	432	432	240	258
PI 647893 (HI10)	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
PI 647896 (HI32)	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
PI 647900	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
PI 647901	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
PI 647908	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
PI 647913	336	336	269	269	422	422	304	328	273	275	424	426	331	331	281	281	371	371	430	434	236	264
PI 647914	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
PI 647915	322	324	269	269	418	424	310	310	273	301	424	434	331	331	-9	-9	356	399	434	434	198	198
PI 647919	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
PI 647920	320	332	265	265	428	432	310	310	273	275	424	424	331	331	281	281	-9	-9	432	432	240	258
PI 647921	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
PI 647923	320	320	269	269	428	428	312	326	273	275	430	430	331	331	281	281	-9	-9	420	434	240	258
509018-2	330	336	269	269	422	432	304	304	275	275	426	432	331	331	281	281	337	371	434	434	264	271
509018-3	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
HI14 (PI 647894)	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
HI26 (PI 647895)	322	324	269	269	418	424	310	310	273	301	424	434	331	331	281	281	356	399	434	434	198	198
HI33 (PI 647897)	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
HI36 (PI 647898)	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
HI39 (PI 647899)	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
Adalayd (PI 647902)	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
Taylor 1 (PI 647903)	324	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	385	385	436	436	240	240
Taylor 2 (PI 647904)	320	324	265	271	428	430	312	326	273	275	424	430	331	331	281	281	337	337	432	432	240	240

Supplementary Table 2.2 (continued): Genotyping calls for forty-three microsatellite markers
Genotyping calls for 90 diploid lines are listed as the fragment size; -9 indicates a missing data point; VIC, NED, or FAM identify the fluorochrome used with a particular marker

Accession ID:	Locus 34		Locus 35		Locus 36		Locus 37		Locus 38		Locus 39		Locus 40		Locus 41		Locus 42		Locus 43	
	87 NED		65 FAM		24 VIC		84 NED		02 VIC		72 NED		29 FAM		11 VIC		66 NED		41 FAM	
PI 276245	343	343	445	445	-9	-9	471	471	-9	-9	468	482	350	354	289	289	327	335	331	346
PI 299042	343	343	447	447	256	290	471	471	269	303	468	468	342	342	289	289	321	337	365	365
PI 364368	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
PI 377709	340	343	445	445	287	290	468	471	271	301	482	482	350	354	289	289	327	337	362	362
PI 403999	336	336	445	445	287	290	468	468	285	285	468	468	350	354	289	289	337	337	-9	-9
PI 508729	343	343	445	445	285	285	471	471	285	285	468	468	350	354	289	289	327	335	346	346
PI 508735	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
PI 508737	340	343	447	455	290	295	468	468	297	303	474	474	350	350	289	289	337	337	359	365
509020	343	343	447	455	277	292	464	468	279	297	468	482	350	354	289	289	337	337	338	359
509021	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
509022	343	343	445	455	277	287	468	471	285	297	468	468	354	354	289	289	337	337	359	359
509023	343	343	445	445	259	287	468	471	281	285	468	484	350	350	289	289	327	337	340	340
PI 543854 (Tropic Shore)	330	330	434	437	251	257	471	471	201	259	478	478	342	342	289	289	-9	-9	362	362
PI 576134	343	343	445	445	277	285	471	471	285	297	468	476	340	354	289	289	335	337	359	359
PI 576138	340	343	445	445	287	290	468	471	271	297	-9	-9	350	350	289	289	327	337	359	359
PI 612771	-9	-9	443	447	246	246	-9	-9	271	281	-9	-9	369	369	289	289	283	315	-9	-9
Durban (PI 614678)	343	343	443	443	249	282	471	471	279	281	474	478	352	352	289	289	327	337	338	338
PI 614679	343	343	443	443	287	287	471	471	285	285	468	476	350	354	289	289	335	337	-9	-9
PI 647885	343	343	443	455	267	287	468	471	283	297	468	476	350	354	289	289	335	337	359	359
PI 647886	343	343	447	455	277	290	471	471	283	301	468	482	350	350	289	289	321	337	362	362
PI 647888	343	343	445	455	277	287	468	471	285	297	468	468	354	354	289	289	327	337	359	359
PI 647891	343	343	447	447	256	256	471	471	269	303	468	468	342	342	289	289	321	337	365	365
PI 647893 (HI10)	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
PI 647896 (HI32)	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
PI 647900	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
PI 647901	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
PI 647908	340	343	445	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
PI 647913	340	343	443	447	301	301	471	471	281	285	470	476	350	350	289	289	335	337	346	346
PI 647914	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
PI 647915	343	343	443	443	267	287	471	471	283	285	468	476	350	350	289	289	321	335	344	362
PI 647919	340	343	447	455	290	290	468	468	-9	-9	474	474	350	350	289	289	337	337	359	362
PI 647920	343	343	447	447	256	256	471	471	269	303	468	468	342	342	289	289	321	337	365	365
PI 647921	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
PI 647923	343	343	445	445	258	285	471	471	269	297	468	468	338	342	289	289	327	337	359	359
509018-2	340	343	447	447	290	290	468	471	281	301	470	474	350	354	289	289	337	337	362	362
509018-3	340	343	447	455	290	290	468	468	297	303	474	474	350	350	289	289	337	337	359	365
HI14 (PI 647894)	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
HI26 (PI 647895)	343	343	443	443	-9	-9	471	471	-9	-9	468	476	350	350	289	289	321	335	359	362
HI33 (PI 647897)	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
HI36 (PI 647898)	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
HI39 (PI 647899)	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
Adalayd (PI 647902)	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
Taylor 1 (PI 647903)	343	343	445	455	277	285	468	471	285	297	468	468	354	354	289	289	337	337	359	359
Taylor 2 (PI 647904)	343	343	447	455	287	294	468	471	271	285	468	494	340	354	289	289	-9	-9	331	331

Supplementary Table 2.2 (continued): Genotyping calls for forty-three microsatellite markers
Genotyping calls for 90 diploid lines are listed as the fragment size; -9 indicates a missing data point; VIC, NED, or FAM identify the fluorochrome used with a particular marker

Accession ID:	Locus 1		Locus 2		Locus 3		Locus 4		Locus 5		Locus 6		Locus 7		Locus 8		Locus 9		Locus 10		Locus 11	
	80 VIC		38 NED		55 FAM		07 VIC		46 NED		40 FAM		03 VIC		23 NED		01 FAM		10 VIC		63 NED	
Temple 1 (PI 647909)	-9	-9	-9	-9	-9	-9	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
Temple 2 (PI 647910)	185	195	352	355	299	299	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
SIPV28-1 (PI647912)	185	195	352	355	299	299	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
Aloha (PI 652948)	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
Bahama	164	192	341	371	291	291	183	183	344	348	292	294	183	192	352	354	296	296	202	202	369	369
Belize	185	185	352	352	295	299	183	183	352	356	296	296	189	189	348	363	300	300	200	200	375	375
Cal	185	185	352	363	295	295	-9	-9	352	352	294	296	189	189	363	363	300	300	200	200	375	375
Cloister	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
Collier	185	185	342	352	295	299	183	183	352	356	294	294	189	189	354	363	296	296	200	200	375	375
Cuba 223	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
Darien	185	185	355	363	295	299	183	183	352	356	292	294	189	192	348	380	312	341	200	200	369	375
Excal	185	185	342	347	295	299	183	183	352	356	294	296	189	189	354	363	296	296	200	200	375	375
FR-4	185	185	355	363	295	299	183	183	352	356	294	296	189	189	354	363	296	296	200	200	375	375
FSP1	185	195	352	355	299	299	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
Grif Spence	158	158	338	361	291	297	183	189	348	354	292	292	189	195	346	348	292	294	200	200	369	375
HH	-9	-9	-9	-9	-9	-9	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
HI 101	185	195	352	355	299	299	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
Hignight S	185	185	352	363	299	299	183	183	356	356	294	294	189	189	358	363	296	296	200	200	375	375
HYB5	185	185	-9	-9	295	299	183	183	352	356	294	296	183	183	348	348	296	296	200	200	375	375
HYB7	185	185	363	363	295	295	183	183	352	352	294	296	183	183	348	358	296	296	200	200	375	375
K8	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	300	300	200	200	375	375
Kai Luna	185	185	342	352	295	295	183	183	352	352	294	294	189	189	358	363	296	296	200	200	375	375
KC9	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
Kim1	188	188	357	363	295	299	183	183	344	352	294	296	189	189	346	358	308	316	200	200	375	375
Polo	185	185	355	363	295	295	183	183	352	356	294	296	189	189	348	348	296	296	200	200	375	375
Prince	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
Q36313	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
Q36315	185	195	352	355	299	299	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
Q37956	-9	-9	355	361	-9	-9	183	183	354	354	294	294	189	189	338	352	304	304	200	200	-9	-9
Salam	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
Seadwarf	185	195	352	355	299	299	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
Sea Isle 1	185	195	352	355	299	299	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
Sea Isle 2000	185	185	342	355	295	299	183	183	352	356	294	296	183	183	348	348	296	296	200	200	375	375
Sea Isle Supreme	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
Talia Fero	185	195	352	355	299	299	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
TCR3	-9	-9	-9	-9	-9	-9	183	183	344	352	294	296	189	189	363	363	296	296	200	200	375	375
TCR6	185	185	355	363	295	299	183	183	352	356	294	296	189	189	348	363	296	296	200	200	375	375
TF P7-4	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
TG Kona	185	195	352	355	299	299	183	183	356	356	294	294	189	189	348	363	296	296	200	200	375	375
TOCGC	185	185	352	355	295	299	-9	-9	352	356	294	296	189	189	348	363	296	296	200	200	375	375
TRB2	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	296	296	200	200	375	375
Utah1	185	185	352	363	295	295	183	183	352	352	294	296	189	189	363	363	300	300	200	200	375	375
Vero Beach	185	185	347	355	299	305	183	183	356	356	294	294	189	189	363	363	296	296	200	200	375	375
Wai Lua Kauai	-9	-9	-9	-9	-9	-9	-9	-9	352	352	-9	-9	189	189	358	363	296	296	200	200	-9	-9

Supplementary Table 2.2 (continued): Genotyping calls for forty-three microsatellite markers
Genotyping calls for 90 diploid lines are listed as the fragment size; -9 indicates a missing data point; VIC, NED, or FAM identify the fluorochrome used with a particular marker

Accession ID:	Locus 12		Locus 13		Locus 14		Locus 15		Locus 16		Locus 17		Locus 18		Locus 19		Locus 20		Locus 21		Locus 22	
	37 FAM		74 VIC		81 NED		57 FAM		04 VIC		12 VIC		14 FAM		86 VIC		54 NED		08 FAM		78 NED	
Temple 1 (PI 647909)	302	302	-9	-9	383	383	308	308	257	257	262	262	317	317	255	255	385	403	318	318	430	430
Temple 2 (PI 647910)	302	302	-9	-9	383	383	308	308	257	257	262	262	317	317	255	255	385	403	318	318	430	430
SIPV28-1 (PI647912)	302	302	-9	-9	383	383	308	308	257	257	262	262	317	317	255	255	385	403	318	318	430	430
Aloha (PI 652948)	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
Bahama	341	417	-9	-9	385	389	314	314	245	257	262	264	317	317	258	258	377	397	-9	-9	-9	-9
Belize	286	302	204	204	383	383	308	311	257	257	262	262	317	317	249	256	385	403	321	321	428	428
Cal	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
Cloister	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
Collier	-9	-9	204	204	383	383	309	311	257	257	262	262	317	317	255	261	403	403	318	318	428	428
Cuba 223	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
Darien	280	280	204	204	383	383	309	314	253	257	262	262	317	317	249	257	377	381	-9	-9	407	407
Excal	296	302	204	204	383	383	309	314	257	257	262	262	317	317	255	261	375	403	318	318	428	428
FR-4	294	302	204	204	383	383	308	311	257	257	262	262	317	317	256	256	373	403	318	318	428	428
FSP1	302	302	204	204	383	383	308	308	257	257	262	262	317	317	255	255	385	403	318	318	430	430
Grif Spence	282	292	204	204	381	383	312	323	257	257	262	262	317	321	247	253	361	361	291	318	-9	-9
HH	302	302	-9	-9	383	383	308	308	257	257	262	262	317	317	255	255	385	403	318	318	430	430
HI 101	302	302	204	204	383	383	308	308	257	257	262	262	317	317	255	255	385	403	318	318	430	430
Hignight S	286	302	204	204	383	383	308	308	257	257	262	262	317	317	257	257	403	403	318	318	-9	-9
HYB5	286	302	204	204	383	383	308	312	257	257	262	262	317	317	249	255	385	403	318	318	428	428
HYB7	302	302	204	204	383	383	308	314	257	257	262	262	317	317	255	261	403	403	318	318	428	428
K8	294	302	204	204	383	383	311	314	257	257	262	262	317	317	-9	-9	385	403	318	318	428	428
Kai Luna	286	286	204	204	383	383	309	311	257	257	262	262	317	317	257	257	403	403	318	318	428	428
KC9	294	302	204	204	383	383	311	314	257	257	262	262	317	317	-9	-9	385	403	318	318	428	428
Kim1	-9	-9	204	204	383	383	308	314	257	257	262	262	317	317	248	255	403	407	318	318	428	428
Polo	286	302	204	204	383	383	308	309	257	257	262	262	317	317	249	255	377	385	318	321	428	428
Prince	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
Q36313	294	302	204	204	383	383	311	314	257	257	262	262	317	317	-9	-9	385	403	-9	-9	428	428
Q36315	302	302	206	206	383	383	308	308	257	257	262	262	317	317	-9	-9	385	403	-9	-9	430	430
Q37956	304	304	204	204	383	384	312	312	253	257	262	262	317	317	-9	-9	379	385	-9	-9	428	430
Salam	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
Seadwarf	302	302	204	204	383	383	308	308	257	257	262	262	317	317	255	255	385	403	318	318	430	430
Sea Isle 1	302	302	204	204	383	383	308	308	257	257	262	262	317	317	255	255	385	403	318	318	430	430
Sea Isle 2000	286	302	204	204	383	383	308	312	257	257	262	262	317	317	249	255	385	403	318	321	428	428
Sea Isle Supreme	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
Talia Fero	302	302	204	204	383	383	308	308	257	257	262	262	317	317	255	255	385	403	318	318	430	430
TCR3	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	385	318	318	428	430
TCR6	302	302	204	204	383	383	308	311	257	257	262	262	317	317	-9	-9	385	403	318	318	428	428
TF P7-4	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
TG Kona	302	302	204	204	383	383	308	308	257	257	262	262	317	317	-9	-9	385	403	318	318	430	430
TOCGC	286	294	204	204	383	383	308	314	257	257	262	262	317	317	249	256	385	403	318	318	428	428
TRB2	294	302	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
Utah1	-9	-9	204	204	383	383	311	314	257	257	262	262	317	317	256	256	385	403	318	318	428	428
Vero Beach	296	302	204	204	383	383	308	309	257	257	262	262	317	317	257	261	403	403	318	318	430	430
Wai Lua Kauai	286	302	204	204	-9	-9	309	311	257	257	262	262	317	317	257	261	403	403	-9	-9	428	428

Supplementary Table 2.2 (continued): Genotyping calls for forty-three microsatellite markers
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Accession ID:	Locus 23		Locus 24		Locus 25		Locus 26		Locus 27		Locus 28		Locus 29		Locus 30		Locus 31		Locus 32		Locus 33	
	26 FAM		60 VIC		73 NED		34 FAM		62 VIC		83 NED		56 FAM		13 VIC		77 NED		21 FAM		59 VIC	
Temple 1 (PI 647909)	324	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	385	385	436	436	240	271
Temple 2 (PI 647910)	324	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	385	392	436	436	240	271
SIPV28-1 (PI647912)	324	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	381	392	436	436	240	240
Aloha (PI 652948)	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
Bahama	318	322	224	269	420	438	302	306	-9	-9	424	428	326	326	-9	-9	327	367	430	430	198	249
Belize	324	330	269	271	428	432	326	334	275	301	424	424	331	331	281	281	337	337	432	434	271	286
Cal	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
Cloister	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
Collier	330	330	269	269	432	440	306	306	275	275	430	432	331	331	281	281	337	337	436	436	271	286
Cuba 223	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	335	335	434	436	271	286
Darien	322	324	265	269	422	428	312	324	-9	-9	424	424	331	331	281	281	382	392	436	436	237	283
Excal	330	338	269	271	432	440	306	306	275	281	430	430	331	331	281	281	337	337	428	436	222	271
FR-4	328	330	265	269	428	432	312	318	275	275	430	430	331	331	281	281	337	337	434	436	240	271
FSP1	324	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	385	385	436	436	240	271
Grif Spence	303	303	267	269	414	416	300	300	-9	-9	424	452	329	331	282	282	355	362	-9	-9	210	210
HH	324	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	385	385	436	436	240	240
HI 101	324	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	385	385	436	436	240	271
Hignight S	322	324	271	271	-9	-9	304	304	275	275	424	424	331	331	281	281	364	385	432	432	240	240
HYB5	322	324	271	271	-9	-9	304	310	275	301	424	430	331	331	281	281	-9	-9	434	436	198	198
HYB7	324	324	271	271	-9	-9	310	310	273	275	424	432	331	331	281	281	337	392	434	436	271	286
K8	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
Kai Luna	324	330	271	271	432	432	304	304	273	275	430	432	331	331	281	281	337	337	436	436	286	286
KC9	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
Kim1	320	324	271	271	422	432	312	326	273	273	424	424	331	331	281	281	337	337	434	434	258	258
Polo	324	324	265	269	418	428	312	312	273	275	424	430	331	331	281	281	385	399	436	436	271	271
Prince	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
Q36313	324	330	269	269	432	432	324	324	-9	-9	424	432	331	331	281	281	337	337	434	436	271	286
Q36315	324	324	271	271	422	428	304	312	275	275	424	430	331	331	281	281	385	392	436	436	240	271
Q37956	322	324	269	269	422	430	326	353	-9	-9	424	434	331	331	281	281	395	395	432	432	240	240
Salam	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
Seadwarf	324	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	385	392	436	436	240	240
Sea Isle 1	324	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	385	385	436	436	240	240
Sea Isle 2000	322	324	271	271	418	422	312	312	275	301	424	430	331	331	281	281	385	399	434	436	271	271
Sea Isle Supreme	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	271
Talia Fero	324	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	385	385	436	436	240	240
TCR3	320	324	265	271	-9	-9	324	324	273	275	424	432	331	331	281	281	337	337	434	436	240	271
TCR6	324	330	265	265	422	432	312	324	275	275	430	432	331	331	281	281	337	385	434	436	240	286
TF P7-4	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
TG Kona	324	324	265	271	422	428	304	312	275	275	424	430	331	331	281	281	385	392	436	436	240	271
TOCGC	322	324	271	271	422	432	326	334	275	301	430	432	331	331	281	281	337	385	434	436	271	271
TRB2	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
Utah1	324	330	271	271	432	432	324	324	275	275	424	432	331	331	281	281	337	337	434	436	271	286
Vero Beach	324	338	269	271	428	440	306	312	275	281	430	430	331	331	281	281	337	385	428	428	240	271
Wai Lua Kauai	324	330	-9	-9	426	432	304	304	-9	-9	430	432	331	331	281	281	337	337	436	436	284	284

Supplementary Table 2.2 (continued): Genotyping calls for forty-three microsatellite markers

Genotyping calls for 90 diploid lines are listed as the fragment size; -9 indicates a missing data point; VIC, NED, or FAM identify the fluorochrome used with a particular marker

Accession ID:	Locus 34		Locus 35		Locus 36		Locus 37		Locus 38		Locus 39		Locus 40		Locus 41		Locus 42		Locus 43	
	87 NED		65 FAM		24 VIC		84 NED		02 VIC		72 NED		29 FAM		11 VIC		66 NED		41 FAM	
Temple 1 (PI 647909)	343	343	445	455	277	287	468	471	285	297	468	468	354	354	289	289	337	337	359	359
Temple 2 (PI 647910)	343	343	445	455	277	287	468	471	285	297	468	468	354	354	289	289	337	337	359	359
SIPV28-1 (PI647912)	343	343	445	455	277	287	468	471	285	297	468	468	354	354	289	289	337	337	359	359
Aloha (PI 652948)	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
Bahama	322	343	447	450	357	357	-9	-9	280	280	486	490	342	346	289	289	272	289	-9	-9
Belize	340	343	445	455	287	290	468	468	285	297	468	468	350	354	289	289	335	337	357	359
Cal	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
Cloister	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
Collier	343	343	445	445	262	290	468	471	279	297	474	474	350	354	289	289	337	337	339	359
Cuba 223	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
Darien	336	343	444	444	277	285	463	471	281	285	480	494	350	354	289	289	321	324	-9	-9
Excal	343	343	445	455	262	290	448	471	279	297	474	482	350	354	289	289	324	337	339	359
FR-4	336	340	445	455	287	290	468	468	285	297	468	474	350	354	289	289	337	337	359	359
FSP1	343	343	445	455	277	287	468	471	285	295	468	468	354	354	289	289	337	337	357	357
Grif Spence	336	336	437	440	256	265	471	471	258	286	478	478	346	352	289	289	-9	-9	348	348
HH	343	343	445	455	277	287	468	471	285	297	468	468	354	354	289	289	337	337	359	359
HI 101	343	343	445	455	277	287	468	471	285	297	468	468	354	354	289	289	337	337	359	359
Hignight S	343	343	445	455	267	277	468	468	285	297	468	468	-9	-9	289	289	337	337	-9	-9
HYB5	343	343	443	455	267	277	468	471	283	297	468	476	350	354	289	289	321	337	359	362
HYB7	343	343	447	455	277	290	471	471	283	301	468	482	350	350	289	289	321	337	362	362
K8	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
Kai Luna	340	340	445	455	290	290	468	471	301	301	482	482	350	350	289	289	321	337	362	362
KC9	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
Kim1	340	340	445	445	277	290	471	471	283	303	468	468	342	350	289	289	321	321	365	365
Polo	343	343	443	443	287	287	468	471	285	285	468	476	350	354	289	289	321	337	359	362
Prince	340	343	447	455	290	290	468	468	297	303	474	474	350	350	289	289	337	337	359	365
Q36313	339	343	447	455	290	290	468	468	297	303	474	474	350	350	289	289	337	337	359	365
Q36315	343	343	445	455	277	287	468	471	285	297	468	468	354	354	289	289	337	337	359	359
Q37956	343	343	443	443	249	282	471	471	278	280	474	478	352	352	289	289	327	337	338	338
Salam	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
Seadwarf	343	343	445	455	277	287	468	471	285	297	468	468	354	354	289	289	337	337	359	359
Sea Isle 1	343	343	445	455	277	287	468	471	285	297	468	468	354	354	289	289	337	337	359	359
Sea Isle 2000	343	343	443	443	277	290	471	471	285	297	468	476	350	354	289	289	335	337	359	359
Sea Isle Supreme	340	343	447	455	290	290	468	468	297	303	474	474	350	350	289	289	337	337	359	365
Talia Fero	343	343	445	455	277	287	468	471	285	297	468	468	354	354	289	289	337	337	359	359
TCR3	343	343	445	455	256	290	468	471	285	297	474	480	342	350	289	289	321	337	359	362
TCR6	343	343	447	455	276	290	468	468	297	301	468	474	350	354	289	289	337	337	359	362
TF P7-4	340	343	447	455	290	290	468	468	297	303	474	474	350	350	289	289	337	337	359	365
TG Kona	343	343	445	455	277	287	468	471	285	297	468	468	354	354	289	289	337	337	359	359
TOCGC	340	343	445	445	277	290	468	468	297	301	468	468	350	354	289	289	335	337	359	362
TRB2	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
Utah1	340	343	447	455	290	290	468	468	297	301	474	474	350	350	289	289	337	337	359	362
Vero Beach	343	343	445	455	263	286	468	471	279	285	-9	-9	356	360	289	289	324	337	339	339
Wai Lua Kauai	340	340	445	455	-9	-9	468	471	-9	-9	474	482	350	350	289	289	321	337	362	362

CHAPTER 3

CONSTRUCTION OF GENETIC MAPS IN SEASHORE PASPALUM (*PASPALUM VAGINATUM* SWARTZ) WITH A MODIFIED GENOTYPING-BY-SEQUENCING APPROACH AND COMPARATIVE GENOMIC ANALYSIS WITH *SORGHUM BICOLOR* AND *SETARIA ITALICA*[2]

[2] Douglas M Eudy, Paul Raymer, and Katrien M. Devos. To be submitted to *G3: Genes|Genomes|Genetics*

Abstract

As a step towards the identification of the genetic loci responsible for the salt tolerance of seashore paspalum, we developed an F1 mapping population from a pair of lines that varied in salt tolerance levels and exhibited among the highest genetic diversity present in a panel of available germplasm. The individual progeny, as well as clones of the parents, were genotyped using a modified genotyping-by-sequencing (GBS) protocol and an analysis pipeline combining publicly available software with in-house scripts. The genotypic data was used with a pseudo test cross approach to generate a pair of genetic maps (maternal and paternal). The resulting maps contained 1262 and 1092 SNP markers, respectively. In both maps, ten linkage groups were unambiguously resolved with an average marker resolution of ~1.9 cM. Additionally, comparisons were made between the paspalum-derived tags and the *Sorghum bicolor* and *Setaria italica* genomes. The resulting comparative information was used to identify potential paspalum-specific rearrangements. We found that the seashore paspalum genome is largely collinear to that of sorghum with the only exceptions being previously reported inversions on sorghum chromosome 4 and chromosome 7. No paspalum-specific rearrangements were identified. These high-density maps are the first available maps for seashore paspalum. The maps will provide a valuable tool for plant breeders and will also be used to anchor the genome sequence that is currently being developed.

Introduction

The salt-sensitivity of crop plants is limiting yields in many locations around the world. This will become an even more important issue in the future as crop cultivation expands into marginal areas which are already salt-affected or are vulnerable to salinization through seawater intrusion,

storm surges and/or the salinizing effects of irrigation in arid areas (Flowers 2004). With these future challenges in mind, we initiated research on the salt tolerance mechanism/s in the highly salt tolerant species seashore paspalum (*Paspalum vaginatum* Sw.) as a gateway to improving cereal crops for salt tolerance. Seashore paspalum has demonstrated an unrivaled ability to survive salt exposure and as such has become an important turf grass in coastal and salt-affected areas of the world (Duncan and Carrow 2000). To begin the process of identifying the specific genes responsible for this/these processes, we generated a genetic map of seashore paspalum and conducted a comparative genomic analysis to establish the syntenic relationships between the seashore paspalum genome and cereal crop genomes.

Despite having been utilized as a turf for almost one hundred years, few molecular marker resources are available for seashore paspalum. Previous studies have used random amplified polymorphic DNA (RAPD) markers (Liu et al. 1994) and amplified fragment length polymorphisms (AFLPs) (Chen et al. 2005). In addition, five microsatellite markers were developed in the cultivar 'Excalibur' by (Liu et al. 1995) and another seventy-nine from line "HI33"were published by Harris-Shultz et al. (2013). Forty microsatellites transferred from wheat, maize, and sorghum have also been used to analyze seashore paspalum germplasm (Chen et al. 2005). In addition to the aforementioned, we have introduced forty-three new microsatellites developed in cultivar 'Salam' (Chapter 2).

As a result of the revolution in molecular genetic technologies, analyzing the molecular biological variation present in any plant germplasm, irrespective of their genome size and complexity has become feasible (Unamba et al. 2015). In species with small and relatively non-complex genomes, whole genome (re)sequencing has become commonplace (*e.g.* rice, Setaria, tomato, soybean) (Xu et al. 2012; Bai et al. 2013; Causse et al. 2013; Zhou et al. 2015) For

species with larger and/or more complex genomes (*e.g.* wheat, barley, maize, pearl millet), reduced representation library sequencing such as restriction site associated DNA (RAD) sequencing and genotyping-by-sequencing (GBS) methods have paved the way for exploring genomic diversity and structure (Elshire et al. 2011; Poland et al. 2012) The utility of these approaches is not limited to species whose genomes are sequenced, rather it is enabling investigations into species such as seashore paspalum that have long suffered from a dearth of molecular resources. We employed GBS to develop thousands of SNP (single-nucleotide polymorphism) markers in *Paspalum vaginatum*. Those markers were used to construct the first genetic maps in the species as well as to perform comparative genomic analyses between paspalum and two closely related panicoid grasses with completely assembled genomes: *Sorghum bicolor* and *Setaria italica*.

Materials and Methods

Mapping Population

We developed an F1 mapping population comprising 226 progeny by crossing accession HI33 with accession 509022. The parental lines were chosen because they exhibited a large difference in their ranking for salt tolerance in multiple tolerance screens (Lee, Duncan et al. 2004, P. Raymer pers. comm.) Furthermore, they were among the most genetically distant in a set of more than one hundred accessions from the USDA and UGA germplasm collections analyzed (Chapter 2). Mapping was done using a pseudo testcross approach in the F1 generation because seashore paspalum is largely self-incompatible and highly heterozygous (Grattapaglia and Sederoff 1994a).

Tissue Collection/DNA Extraction

Healthy green leaves were collected from the parents and mapping progeny in 2mL microcentrifuge tubes. A single zinc-plated steel .177 caliber BB (Daisy) was added to each tube, which was sealed and flash frozen in liquid nitrogen. Samples were stored at -80°C until processed further. The samples were ground in a TissueLyzerII bead mill (Qiagen) which was run at 30RPM for 30 seconds at a time. The samples were refrozen in liquid nitrogen between 30 second grinding cycles and the total grind time per sample was 90 seconds. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions, except that the volume of elution buffer (BufferAE) was reduced to 65 µL. Extracted DNA was quantified using a Nanodrop spectrophotometer and diluted to a working concentration of 50 ng/µL.

GBS-library Construction

The libraries for the parents and each of the progeny were generated in 96-well plates. For each library, 200ng DNA from the 50 ng/µL stock was added to 26 µL of restriction digest master mix so that the final reaction mixture included 1x CutSmartBuffer (NEB) and 8 units each of *Pst*I-HF, *Nde*I, and *Msp*I restriction endonucleases (NEB). The mix was incubated for 2 hours at 37°C. In an initial pilot study, we also used the two-enzyme combination *Pst*I-HF and *Nde*I. Single-stranded oligonucleotides corresponding to the common and barcoded adapters (Supplementary Table 3.1 and Supplementary Table 3.2) were dissolved in 10mM Tris-HCl (pH 8.0) and brought to a concentration of 100µM. Complementary pairs of oligos (top and bottom strand) were combined and diluted in 1x Adapter Buffer (50mM NaCl, 10mM Tris-HCl) to a concentration of 10µM. The paired oligos were annealed by heating them to 95°C and then

cooling them at a rate of 1°C per minute until the temperature reached 30°C. The annealed barcoded adapters (*Pst*I-site) were subsequently diluted 100 fold to 0.1 µM, while the common adapter (*Nde*I-site) was kept at 10 µM.

20 µL from the restriction digest was mixed with 0.1 pmol (1 µL @ 0.1µM) of a sample-specific, double stranded (annealed) barcoded adapter. To that, 19 µL of ligation reaction mix was added. The final ligation reaction mix contained ~133 ng of digested DNA, 1x CutSmart buffer, 1 mM adenosine triphosphate (ATP) (NEB), 200U of T4 DNA ligase (NEB), 15 pmol of common adapter, and 0.1 pmol barcoded adapter. The samples were spun briefly in a centrifuge and incubated for 2 hours at 22°C.

Sera-mag SpeedBeads (Thermo Scientific) prepared according to the procedure of Faircloth and Glenn (2014) were used to remove DNA fragments shorter than 300 bp. A ratio of 0.7:1 volume of beads:reaction mixture was used, as this rate had been shown in tests with a molecular weight ladder to bind fragments of the desired size. The bead/DNA mixture was pipetted up and down 10 times and incubated at room temperature for 5 minutes. Subsequently, the bead/DNA mixture was moved to a magnetic plate stand (SPRIplate 96-Ring Agencourt) and left for 10 minutes to allow the beads to be drawn to the magnets. Leaving the plate on the magnetic stand, the supernatant containing small fragments was removed and discarded. The beads were then washed three times with 200 µL of freshly prepared 70% ethanol. The plate was incubated at 37°C until dry (~3 minutes) and then the beads were rehydrated with 40 µL of 10mM Tris (pH 8.0).

The polymerase chain reaction (PCR) was used to generate individual, barcoded, genotype-specific libraries. The reaction mix contained 3 µL of the bead-cleaned DNA as a template, as well as 1x Taq Master Mix (NEB) and 0.2 µM each of a barcode adapter-specific

forward primer and a common adapter-specific reverse primer in a total of 25 microliters (Supplementary Table 3.2). The reaction mixes were briefly vortexed and then spun down in a centrifuge. The reactions were incubated in a thermocycler with the following steps/conditions:

1	Initial denaturation	95°C for 30 sec
2	Cycle denaturation	95°C for 30 sec
3	Primer annealing	62°C for 20 sec
4	Fragment elongation	68°C for 15 sec
5	Go to step 2 for 15 more cycles	
6	Final fragment elongation	68°C for 5 min
7	Hold	8°C

Following library production, 8 µL of amplification product was run on a 1.5 % agarose gel containing ethidium bromide at a concentration of 0.2 µg/mL to confirm the presence of amplified product as well as to observe the fragment size range. Each library was individually quantified using the dsDNA HS (High Sensitivity) Assay Kit and a Qubit 2.0 Fluorometer (Invitrogen). For sample pooling, the volume required to contribute 45 ng of library was used, with the exception of the maternal parent, which contributed 95 ng of library. Libraries from 184 progeny and the two parents were combined into a single pool for sequencing.

The pool of libraries was subjected to a double-Solid Phase Reversible Immobilization (SPRI) selection at the Georgia Genomics Facility (GGF) to eliminate residual primers as well as DNA fragments outside of the target range of 350-900bp. A successful SPRI selection was confirmed by fragment analysis also performed at GGF. Material from the double-SPRI treated pool was sequenced using 150 bp paired-end sequencing on an Illumina NextSeq instrument at the GGF.

GBS-Tag Creation/Marker Development

Genotyping-by-sequencing (GBS) reference tags were developed from the raw sequencing data using an analysis pipeline incorporating components from several standalone software packages

and in-house scripts. Raw reads were first screened for quality using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Raw reads were split according to their barcode sequence using 'process_radtags' from the Stacks package (Catchen et al. 2011). Following de-multiplexing, 'fastx_trimmer' from the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) was used to trim forward reads and reverse reads, as well as to remove the enzyme recognition sequences. FLASH, (Magoč and Salzberg 2011), was used to merge paired reads that overlapped and non-overlapping paired reads were joined to form a contiguous sequence using the in-house script PERJ (script developed by X. Wang). To prepare sequence tags for processing by the Stacks program, which requires input sequences to be of equal length, all overlapping read pairs combined by FLASH were extended to a total length of 271 bases by the addition of 'A's at the 3' end (in-house script developed by D. Chakraborty). Sequences that had been joined together due to lacking an overlap, but were shorter than the expected 271bp length, were dropped from the dataset (in-house script written by D. Chakraborty). The final complement of overlapping and non-overlapping joined read pairs were combined into a single data file and used to construct sequence tags.

Ustacks, a component of the Stacks package, was used to generate the sequence tags within each library (Catchen et al. 2011). The parameters implemented in Ustacks were to require at least three raw reads to create a stack (-m 3), to set the maximum allowed distance between stacks at three (-M 3), and to set the maximum distance allowed to align secondary reads to primary stacks as one (-N 1). Following tag generation in each sample independently, a single catalog of tags from among all the libraries was assembled with Cstacks, another component of the Stacks package (Catchen et al. 2011). The distance allowed between catalog loci (-n) was set to 1 for Cstacks. The resulting catalog was filtered with a custom script (D.

Chakraborty) so that only tags present in at least 70% (132 of 189) of the libraries were retained for the tag reference set.

Bowtie2 (Langmead and Salzberg 2012) was used to align reads from each individual library to the tag reference set and the genome analysis toolkit (GATK) (DePristo et al. 2011) was used to call SNPs in each of the parental clones as well as in the 184 members of the mapping population analyzed. The resulting file which held the genotype calls for each individual at all loci was then processed using several filtering steps to create the final set of markers.

Post-GATK filtering included: selection for biallelic SNPs, removal of SNPs with allele frequencies <0.1 and >0.9, and removal of tags containing consecutive SNPs. SNP genotypes were converted to the mapping scores A, B, H, D (A or H) and C (B or H) from read counts using an in-house script (by D. Chakraborty). SNPs were considered homozygous (A or B) if the second allele was absent or present at a frequency ≤10%, heterozygous (H) if the second allele was present at a frequency between 25% and 75% (expected: 50%), and ambiguous heterozygous or homozygous (C and D) if the second alleles was present at frequencies between 10% and 25%. Calls were made only for loci with a minimum of eight total reads.

Creating Maternal and Paternal Datasets

Based on the parental genotypes, the marker set was divided into three datasets: markers segregating only in the gametes contributed by the maternal parent (genotype H in mother and A or B in father), markers segregating only in the gametes contributed by the paternal parent (genotype A or B in the mother and H in the father), and markers segregating in the gametes of both parents (both parents H). Segregation ratios were calculated for each marker in Microsoft

Excel to determine deviations from the expected 1:1 ratio. Markers for which more than 10% of progeny carried an allele that was not observed in the parents were moved into a separate file for further evaluation. For the markers segregating in the expected pattern, ambiguous bases (coded as C or D) were converted into missing data (-). B scores in markers that were initially scored as B in one parent and H in the other parent were recoded to A.

For markers with allelic scores A, B and H across the progeny, the segregation ratios suggested that the homozygous parental genotype was behaving in a manner consistent with the segregation of a null allele (*e.g.* ab x a-). As such, the unexpected allele state was converted to 'H' and the marker identified as such by the addition of the prefix "W_". The final dataset curation step was to identify tags containing multiple co-segregating SNPs, and to select the SNP with the fewest number missing allele calls among the progeny to represent that tag. Because the linkage phase of the markers was unknown, the scores for all the markers were duplicated and inverted. Inverted markers were identified with the suffix "_R".

Genetic Map Construction

Maternal and paternal genetic maps were constructed in JOINMAP 4.1 (Stam 1993). Population type was set as backcross (BC). Markers were assembled into linkage groups using the linkage LOD test statistic with the default threshold ranges: start 2.0, end 10.0 with a step of 1.0. For map construction, the maximum likelihood mapping algorithm was used with the default parameters. Framework maps were constructed initially and included only the non-distorted markers segregating in the expected 1:1 ratio. Three genotypes showed patterns consistent with sample contamination and were removed from the dataset at this time. The framework maps were recalculated and then the remaining markers added to the maps. Where possible, map

orders of markers that co-segregated were inferred from the physical positions of orthologous loci identified in the *Sorghum bicolor* (v2.0) and *Setaria italica* (v2) genomes. The final map distances were calculated in centiMorgans (cM) using the Kosambi function implemented in MapMaker (Lander et al. 1987).

Comparative Maps Construction (Seashore Paspalum- Sorghum –Setaria)

The GBS-tags were used as queries in BLASTn searches using default parameters to the *Sorghum bicolor* genome v2.0 (Paterson et al. 2009) and the *Setaria italia* genome v2 (Bennetzen et al. 2012). For tags that returned hits at an e-value ≤ 1e-6, the first and second best hits were recorded. The top hits of sorghum tags and Setaria tags mapped in seashore paspalum were each used to construct comparative genetic maps. If the top hit did not fit the general pattern of observed synteny, the second best hit was also considered in the comparative analysis. The locations of the top hits in the sorghum and Setaria genomes were plotted against the map positions of the GBS tags in seashore paspalum (Supplementary Figures 2 and 3).

Results

GBS-Tags

A pilot GBS study in which 900,000 reads were analyzed from each of four genotypes digested with two different enzyme combinations (*Pst*I and *Nde*I, and *Pst*I, *Nde*I, and *Msp*I) showed that 15,583 tags were common between the four genotypes in the *Pst*I/*Nde*I double digest, and 10,699 tags were common between the four genotypes in the *Pst*I/*Nde*I+*Msp*I triple digest. 23% of the *Pst*I/*Nde*I fragments carried at least one *Msp*I site. The number of *Msp*I sites in the *Pst*I/*Nde*I+*Msp*I triple digest was reduced to 17%.

For our mapping population, the Stacks derived reference set (obtained following digestion with the triple digest *Pst*I/*Nde*I+*Msp*I) filtered to include tags present in at least 70% of samples contained a total of 7480 sequence tags. After filtering, the number of sequence tags carrying segregating SNPs was reduced to 2748 and the total number of polymorphic SNPs was 5085 (many tags contained more than one SNP). An analysis of the distribution of polymorphic sites across the length of the GBS-tags revealed abnormally high numbers of SNPs at positions 130 and 131 in the tags (Figure 3.1).

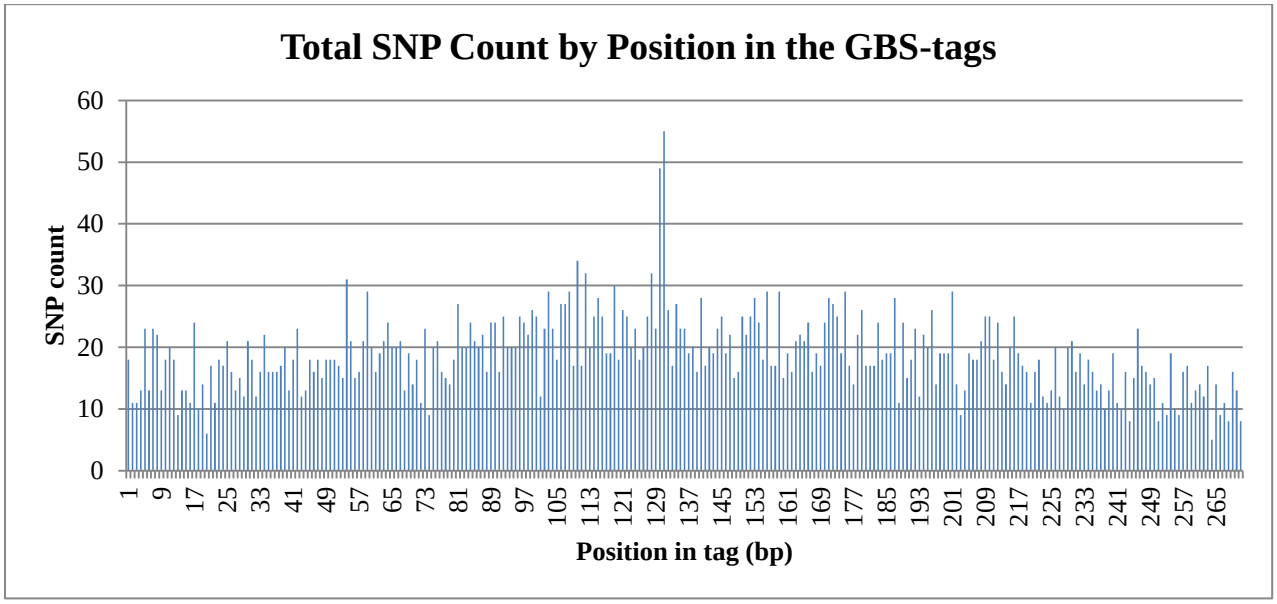

Figure 3.1: Distribution of SNPs occurring in the tags of the paspalum reference set

Paspalum Genetic Maps

The maternal genetic map contains 1262 markers with a total span of 1260.0 cM. The average genome-wide resolution in the maternal map is 1.9 cM (Table 3.1; Figure 3.2). The paternal map contains 1092 markers and spans 1334.4 cM. The average genome-wide resolution is also 1.9 cM in the paternal map (Table 3.2; Figure 3.3). There were forty-two marker intervals in the maternal map which equaled or exceeded 5 cMs in size and forty analogous intervals in the

paternal map (Table 3.3 and Table 3.4). A number of regions (identified as three or more adjacent markers) exhibited segregation distortion at the α=0.05 level or greater. There were six such regions on six total chromosomes in the mother and nine such regions on nine total chromosomes in the father (Figure 3.2 and 3.3).

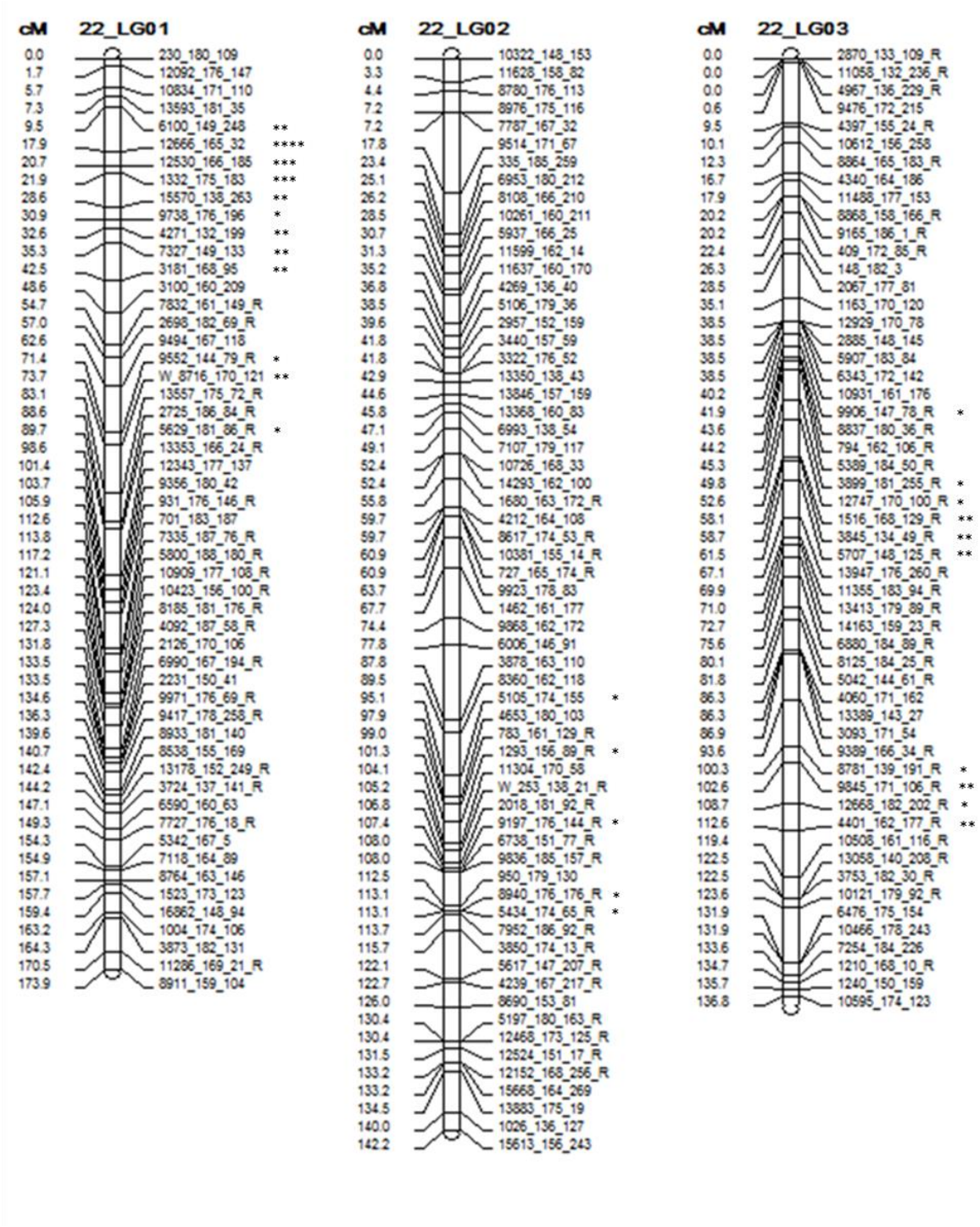

Figure 3.2: Genetic map of the maternal parent (509022)

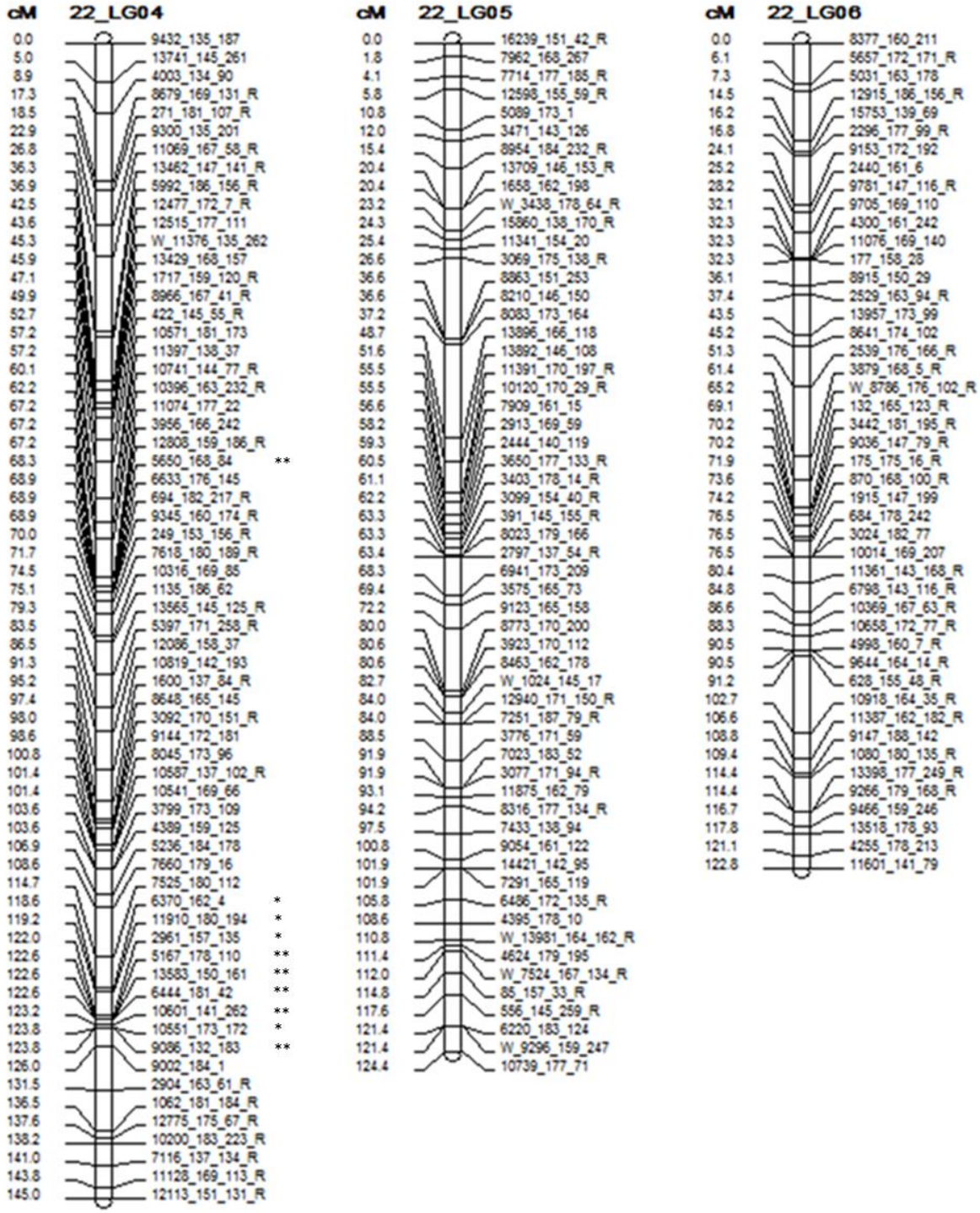

Figure 3.2: Genetic map of the maternal parent (509022)

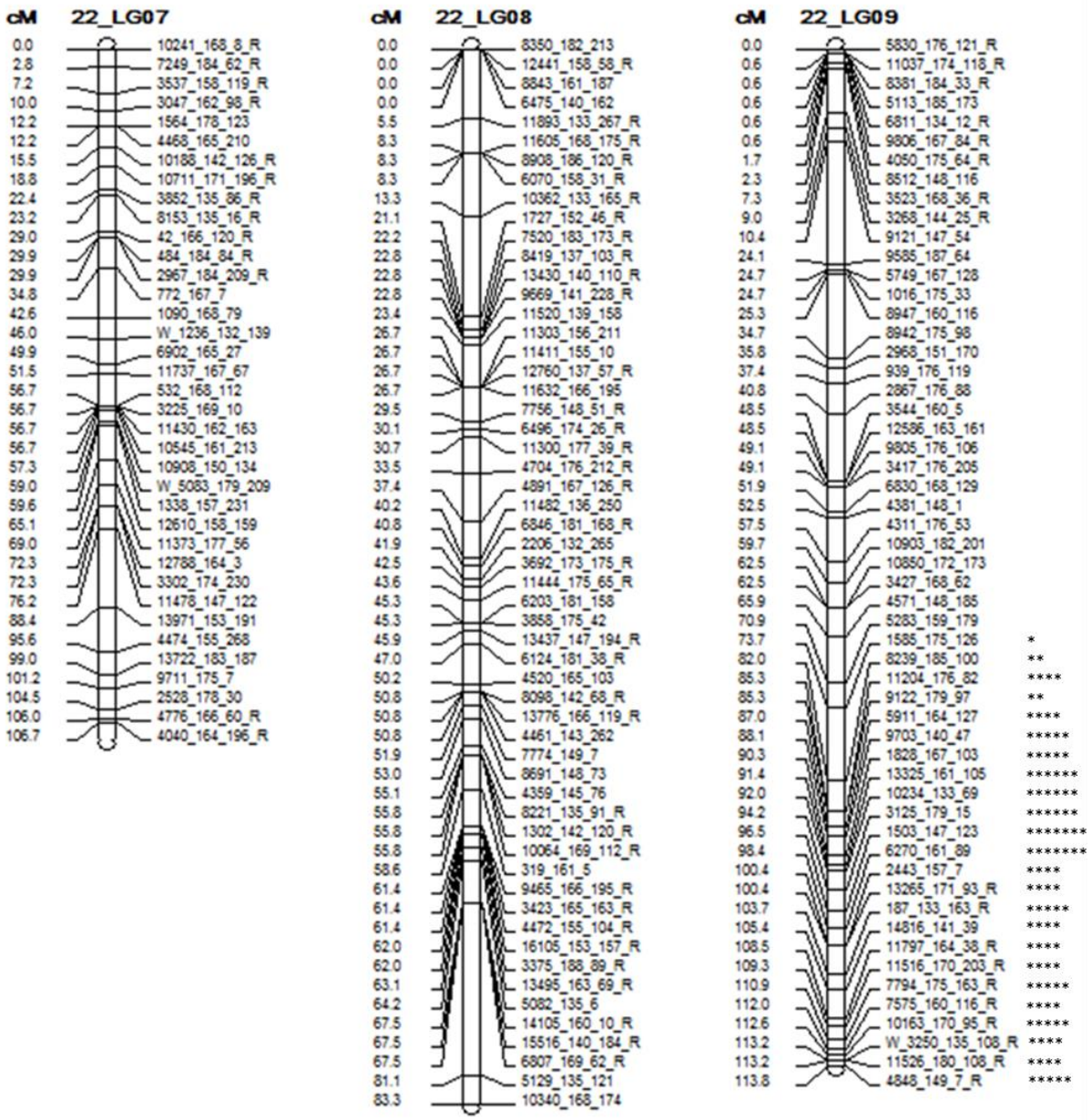

Figure 3.2: Genetic map of the maternal parent (509022)

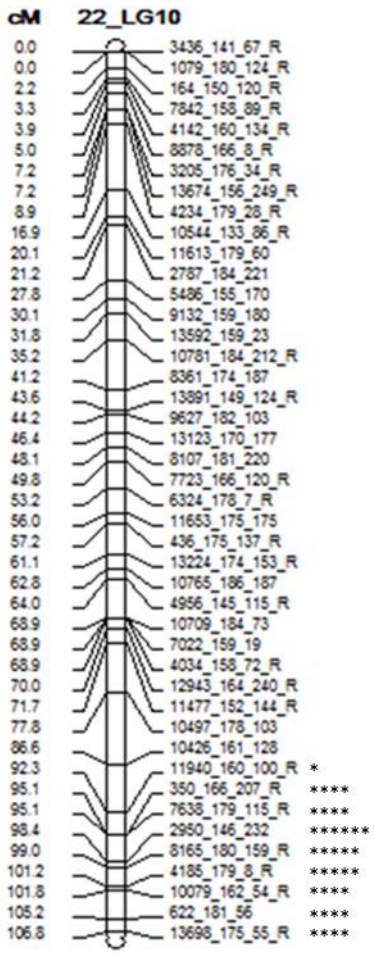

Figure 3.2: Genetic map of the maternal parent (509022)

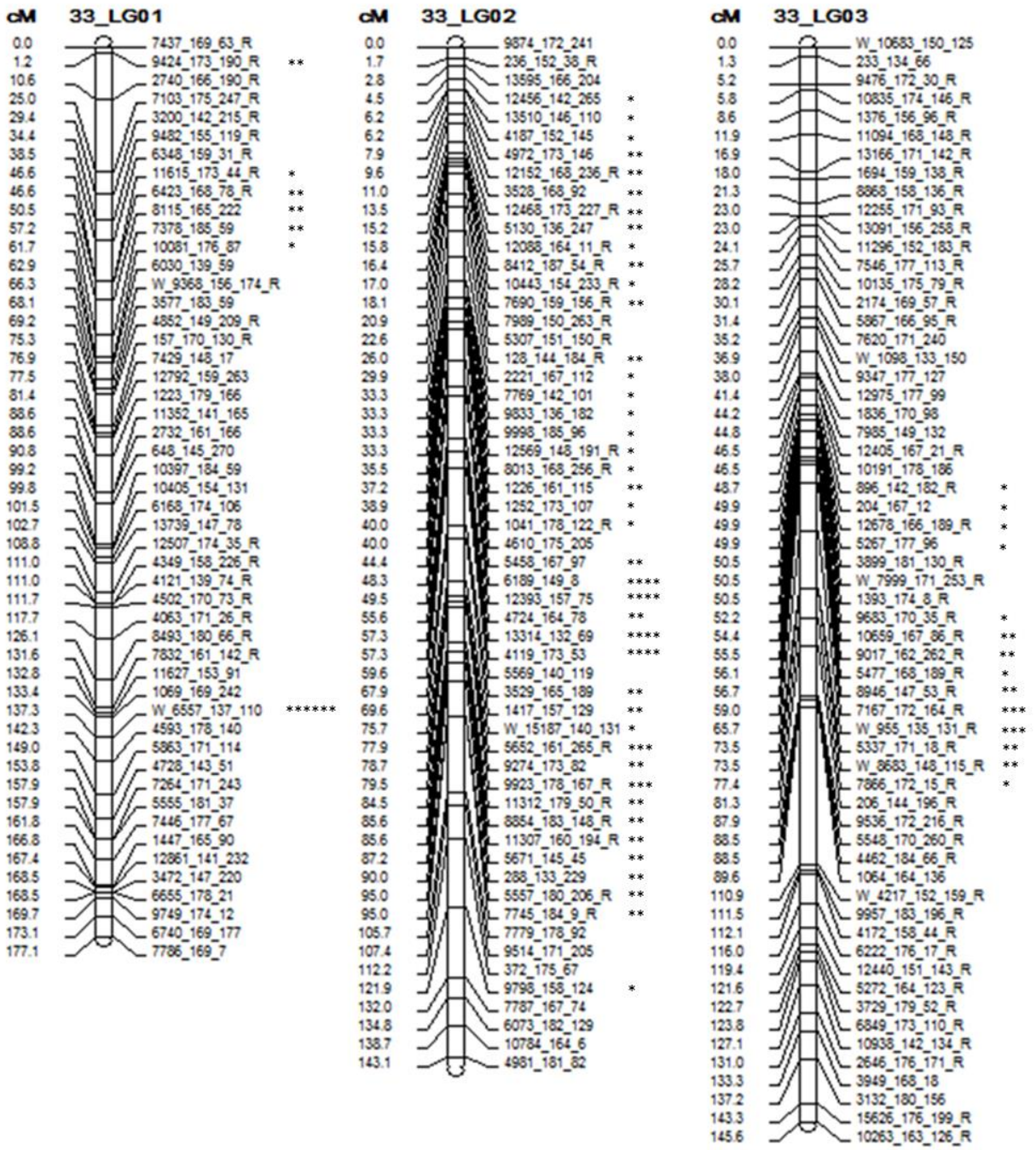

Figure 3.3: Genetic map of the paternal parent (HI33)

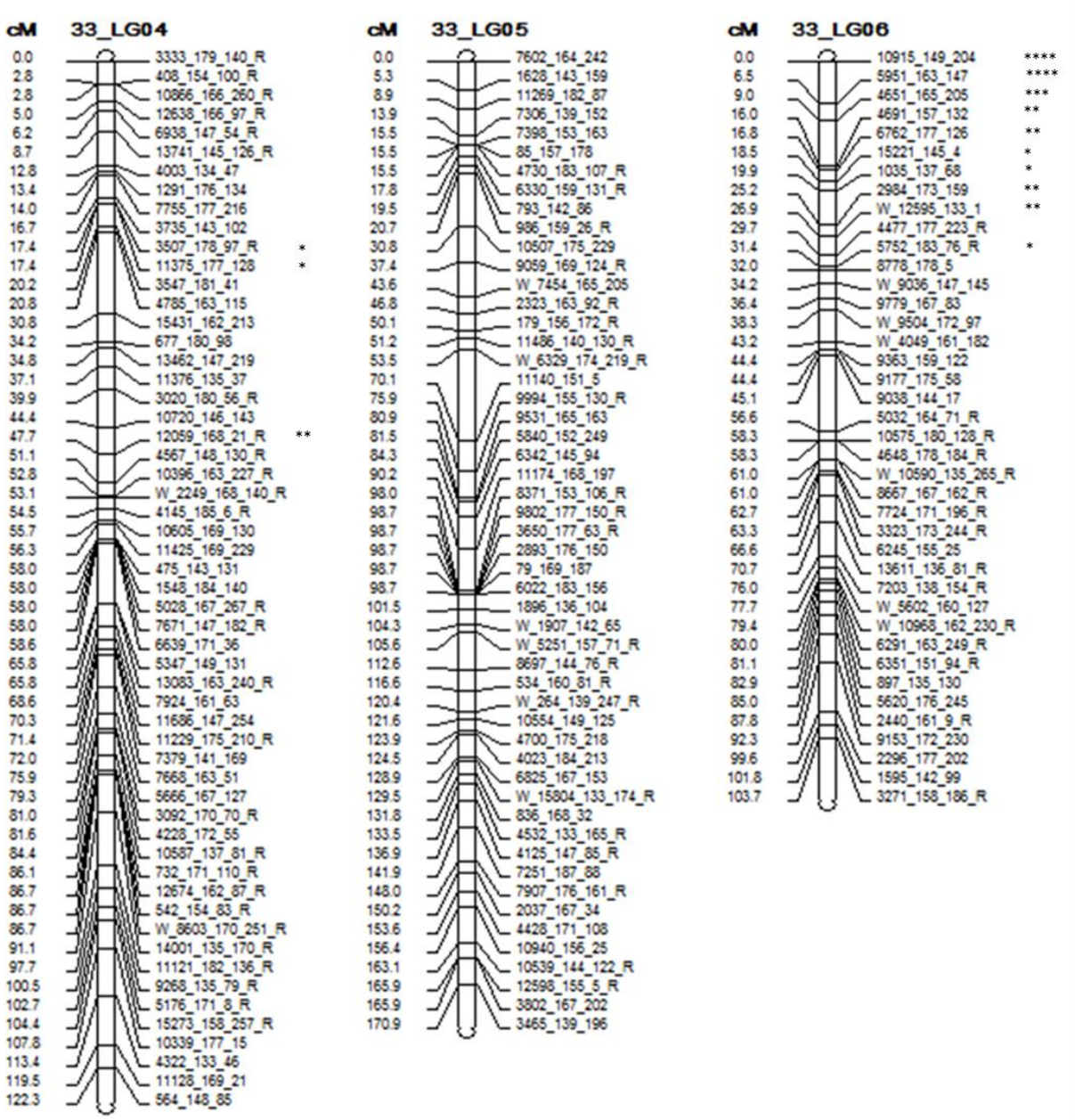

Figure 3.3: Genetic map of the paternal parent (HI33)

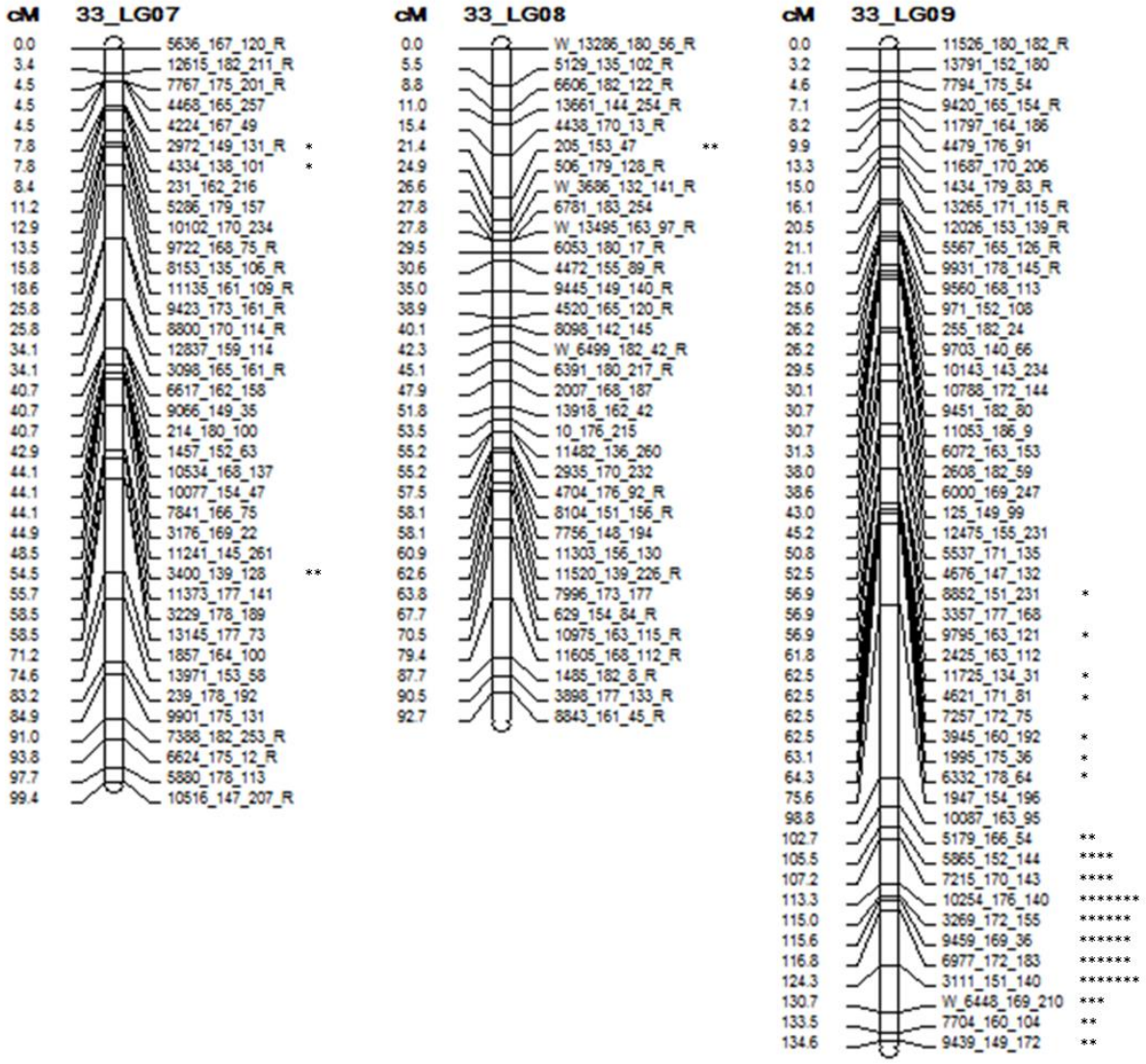

Figure 3.3: Genetic map of the paternal parent (HI33)

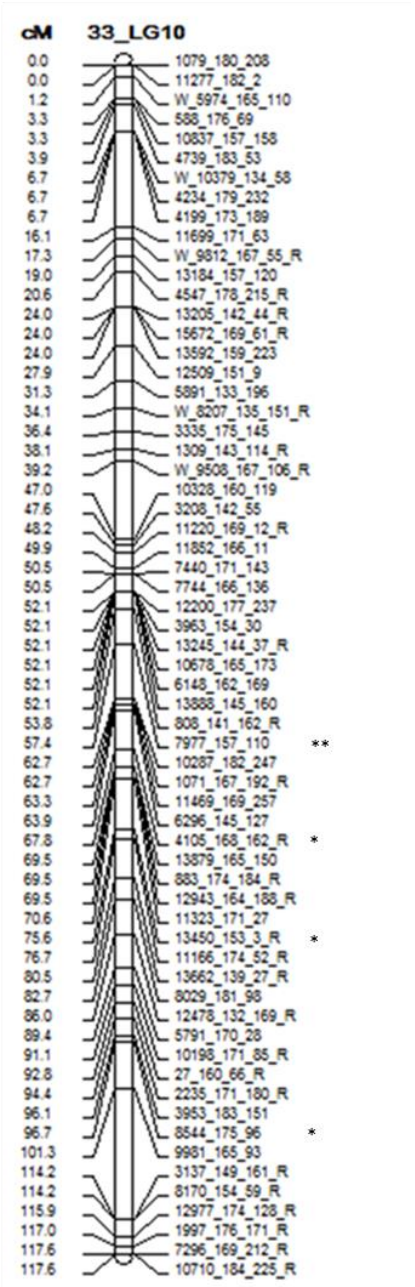

Figure 3.3: Genetic map of the paternal parent (HI33)

Map distances are indicated in centiMorgans (cM) calculated with the Kosambi map function. Markers exhibiting segregation distortion are indicated with asterisks (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, etc.)

Table 3.1: Summary of maternal (509022) linkage groups

This summary includes marker number per chromosome, average mapping resolution and map length with totals for the map.

509022 (maternal parent)			
Linkage Group (LG)	# of Markers	AVG Marker Resolution (cM)	Total Map Length (cM)
LG01	209	1.5	173.9
LG02	185	1.6	142.2
LG03	162	1.6	137.9
LG04	128	1.9	145.0
LG05	113	1.8	124.4
LG06	138	1.8	122.8
LG07	74	2.2	109.3
LG08	56	2.4	83.3
LG09	109	1.9	113.8
LG10	88	1.9	107.4
TOTAL	**1262**	**1.9**	**1260.0**

Table 3.2: Summary of paternal (HI33) linkage groups

This summary includes marker number per chromosome, average mapping resolution and map length with totals for the map.

HI33 (paternal parent)			
Linkage Group (LG)	# of Markers	AVG Marker Resolution (cM)	Total Map Length (cM)
LG01	197	1.6	177.1
LG02	112	2.0	144.4
LG03	119	1.9	145.6
LG04	112	1.8	126.5
LG05	104	2.3	178.7
LG06	80	1.9	113.2
LG07	75	2.1	99.4
LG08	68	2.0	96.7
LG09	100	2.1	135.2
LG10	125	1.7	117.6
TOTAL	**1092**	**1.9**	**1334.4**

Table 3.3: Marker intervals ≥ 5 cM in 509022 (maternal parent)

Linkage Group	Total	Marker Intervals in 509022 (maternal parent) ≥ 5 cM
LG01	4	m19-m20, m29-m30, m77-m78, m88-m89
LG02	3	m14-m15, m100-m101, m153-m154
LG03	5	m85-m86, m116-m117, m118-m119, m125-m126, m144-m145
LG04	4	m5-m6, m14-m15, m91-m92, m114-m115
LG05	5	m14-m15, m25-m26, m31-m32, m32-m33, m64-m65
LG06	4	m16-m17, m44-m45, m54-m55, m106-m107
LG07	4	m19-m20, m27-m28, m59-m60, m60-m61
LG08	4	m4-m5, m8-m9, m9-m10, m54-m55
LG09	5	m21-m22, m22-m23, m30-m31, m60-m61, m63-m64
LG10	4	m18-m19, m24-m25, m32-m33, m68-m69

Table 3.4: Marker intervals ≥ 5 cM in HI33 (paternal parent)

Linkage Group	Total	Marker Intervals in HI33 (paternal parent) ≥ 5 cM
LG01	4	m11-m12, m28-m29, m90-m91, m106-m107
LG02	4	m70-m71, m96-m97, m101-m102, m104-m105
LG03	4	m74-m75, m75-m76, m84-m85, m92-m93
LG04	3	m27-m28, m64-m65, m108-m109
LG05	7	m19-m20, m22-m23, m23-m24, m33-m34, m34-m35, m96-m97, m103-m104
LG06	4	m2-m3, m38-m39, m73-m74, m79-m80
LG07	4	m29-m30, m33-m34, m60-m61, m63-m64
LG08	3	m2-m3, m59-m60, m62-m63
LG09	4	m41-m42, m74-m75, m75-m76, m76-m77
LG10	3	m17-m18, m44-m45, m113-m114

GBS Tags as Comparative Markers

Of the 7480 total tags present in the final catalog, 3163 (42.3%) returned at least one hit in the *Sorghum bicolor* genome at an e-value threshold 1e-6 and 3229 (43.2%) returned at least one hit in the *Setaria italica* genome at the same threshold. A total of 2776 tags (37.1%) returned a hit in both sorghum and Setaria. Plots of the distributions of paspalum-tags in the sorghum and Setaria genomes indicate a non-random distribution, with a greater tag presence in the

chromosome arms, while centromeric regions have fewer markers per Mb; the pericentromeric regions of sorghum are larger than those of Setaria and are visible as regions depleted for paspalum-tags (Figures 3.4 and 3.5).

Paspalum – Sorghum and Paspalum – Setaria Comparative Maps

In the maternal map, 427 loci putatively orthologous to sorghum and 420 loci putatively orthologous to Setaria were identified (Table 3.5). In the paternal map, 378 loci putatively orthologous to sorghum and 386 loci putatively orthologous to Setaria were identified (Table 3.6 and Table 3.7). Of the markers identified in the maternal parent that had a hit in sorghum, 87.3% were located in syntenic positions while 82.2% of the markers that had a hit in Setaria were syntenic. This trend was observed in the paternal parent as well; 86.4% of the paspalum-sorghum markers were syntenic along with 83.7% of the paspalum-Setaria markers. The majority of the syntenic markers were collinear, with 83.8% and 78.9% of the paspalum-sorghum and paspalum-Setaria markers respectively in the maternal parent. In the paternal parent 82.0% and 77.8% of the paspalum-sorghum and paspalum-Setaria markers were collinear, respectively. In general, the second best hit with BLAST was only used rarely, 7 times total in the maternal parent-sorghum comparison and 6 times total in the maternal parent-Setaria comparison. The second best hit was used slightly more often in the paternal parent: 10 times in total in the sorghum comparison and 11 times in total in the Setaria comparison.

Each paspalum linkage group largely corresponded to a single sorghum chromosome (Supplementary Figure 1), and paspalum linkage groups were therefore numbered according to their synteny with sorghum. A known inversion on LG 4 spanning the region 57.15 to 63.72 Mb

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
>Chr01	15	16	20	21	10	21	12	17	9	10	14	8	11	15	6	6	6	11	12	14	8	5	1	2	4	2	1	4	2	1	1	3							
>Chr02	6	6	5	2	9	5	1	11	3	10	3	1	6	7	8	3	2	5	6	3	3	1	2	5		1		2	2	1									
>Chr03	7	8	8	7	13	16	13	11	12	6	6	8	2	3	2	10	4	2	3	3	2			2		2	1				1	2					3	1	1
>Chr04	8	12	20	18	12	7	15	15	9	4	3	3	7	1		2	4		1	2															2			3	
>Chr05	4	5	9		4	5	1	2	5			2	1	1	3	3	3	2	2		4		1								2				1				
>Chr06	1	9	3	4	4	2	6	2	1	2	1				2	1		1								1	1						1	1			1	3	3
>Chr07	8	19	14	4	7	15	7	7	9	1		5	5		1		1		3			1		1			1	3			1					1	1	1	
>Chr08	13	8	5	6	5	4	2	2		2			1		1	1	1	1													2			2					
>Chr09	11	10	11	7	5	7	6	5	4	4	4	1		1	1		1									1													
>Chr10	12	14	4	11	16	14	7	5	4	10	3	7	4	1	8	5		3	1	2	1	1		1		1											1		
Grand Total	105	108	103	80	86	96	70	77	57	49	34	35	37	29	32	31	22	25	28	24	18	8	4	11	4	7	4	10	4	2	5	7	1	3	3	1	5	9	4

	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	Grand Total
>Chr01				1	1		1	3	4	4	10	8	2	10	13	10	8	8	6	13	15	20	18	15	11	10	18	19	9	11	18	15	19	18	11					617
>Chr02	1			1			8	2	3		3	1	2	2	5	7	3	5	7	11	5	8	5	6	17	24	11	12	29	14	12	15	10	18	21	18	12	5	9	431
>Chr03						1	1	1	2		1	3	9	6	3		15	7	5	12	11	8	9	11	19	12	15	16	20	11	16	18	4	6	19	3				423
>Chr04		1	1	2	1	2	1		4	2	2	5	5	9	12	16	12	10	8	12	8	16	9	10	3	24	21	15	13										372
>Chr05			1	1		1	1	1	1	2	5	1	3		2	3	5	3	4	7	5	7																	113
>Chr06		1	2	3	3	5	5	8	3	10	17	16	15	12	15	15	7	22	18	15	15	19	22	1																299
>Chr07	1		2						2		9	3	3	3	6	4	8	7		13	15	7	6	13	9	4														231
>Chr08	2	2	1			1	9		6	9	2	8	8	3	7	2	7																							123
>Chr09		3		9		3	2	5	7	12	13	8	10	22	14	13	12	15	17	11	4																			259
>Chr10		1	1	2		5		1	4		3	8	3	11	4	9	11	12	10	20	18	9																		268
Grand Total	4	8	8	19	5	18	28	21	36	39	65	61	60	78	81	79	88	89	75	114	96	94	69	56	59	74	65	62	71	36	46	48	33	42	51	21	12	5	9	3163

Figure 3.4: Heat map showing the location and distribution of paspalum-tags in sorghum

Tags were identified to have sequence homology to the sorghum genome by blastn; centromeric regions for each chromosome are indicated with grey boxes and follow Paterson, Bowers et al. (2009). Tags per Mb and per chromosome (with totals) are provided.

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
>scaffold_9	24	17	26	17	15	15	12	21	14	11	12	13	11	11	14	13	11	6	2	6	6	7	5	3	1		3		1
>scaffold_5	11	1	7	5	11	8	13	12	11	15	14	10	9	13	4	4	5			3	1	1	4	5	4	3	10	3	15
>scaffold_2	10	6	8	11	4	11	5	7	9	6	8	7	4		7	10	4	2	5	2	2	3	6	5	12	7	9	5	5
>scaffold_3	25	17	17	11	17	10	13	10	10	12	14	12	10	7	11	7	6	19	4	6	13	11	11	6	2	9	1	1	3
>scaffold_1	12	8	7	13	22	6	10	19	16	8	4	5	7	1		2	1			1		1	3	7	4	3	6	7	8	8
>scaffold_7	1	3	7	5	2		1	9	4	2	2	6	4		1	1	3	7	3	11	11	7	26	10	17	12	9	11	16
>scaffold_4	19	11	6	18	12	11	7	2	8	10	7	5	5	12	1	3	3	2	2	2	2		1	1	4	1	5	2	2
>scaffold_6	13	4	14	12	8	3	12	19	3	4			1	2	4	1	5		5	3	1	9	2	5	1	5	2	9	8
>scaffold_8	9	6	6	3	9	8	5	1	3		3	1	6	1	7		1	1		1	1	1	2	1		3	2	1	2
Grand Total	141	73	98	95	100	72	78	100	78	68	64	59	57	47	49	41	39	37	22	34	38	42	64	40	44	46	48	40	60

	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	Grand Total
>scaffold_9	2	1	1	5	3	3	6	16	3		5	17	13	11	9	9	6	15	21	24	21	16	17	19	18	12	17	16	24	21	648
>scaffold_5	10	11	9	14	8	8	9	10	17	14	17	10	19	18	21	16	17	16	6												452
>scaffold_2	8	11	8	4	4	4	10	20	18	13	18	20	16	12	13	17	23	15	19	7	6										446
>scaffold_3		1	1		2	8	1	2	1	2			2	6	3	8	4	6	13	8	6	9									378
>scaffold_1	16	14	15	3	10	8	11	12	10	12	31	21	22	1																	375
>scaffold_7	17	9	23	9	8	12	15																								284
>scaffold_4	1	6	13	5	7	6	10	16	15	13	20	2																			278
>scaffold_6	5	2	11	9	6	13	20																								221
>scaffold_8	2	5	5	3	3	7	2	4	5	3	6	1																			130
Grand Total	61	60	86	52	51	69	84	80	69	57	97	71	72	48	46	50	50	52	59	39	33	25	17	19	18	12	17	16	24	21	3229

Figure 3.5: Heat map showing the location and distribution of paspalum-tags in Setaria

Tags were identified to have sequence homology to the Setaria genome by blastn; centromeric regions for each chromosome are indicated with grey boxes and follow Bennetzen, Schmutz et al. (2012). Tags per Mb and per chromosome (with totals) are provided.

Table 3.5: Setaria genomic rearrangements identified in the paspalum genetic maps

Paspalum Linkage Group	Rearrangement type	Setaria Linkage Group	Rearrangement interval in Setaria (Mb)	Detected in mother? Y/N	Observed interval (Mb)	Markers in the Interval/ (Collinear markers)	Detected detected in father? Y/N	Observed interval (Mb)	Markers in the Interval/ (Collinear markers)
LG1	inversion	LG9	20.09-22.44 Mb	N	n/a	n/a	Y	20.13-22.05 Mb	9 (5)
LG2	inversion	LG2	not detected	N	n/a	n/a	Y	31.41-32.26 Mb	6 (3)
LG3	inversion	LG5	2.18-5.54 Mb, 5.62-13.72 Mb	Y	2.70-13.09 Mb	35 (11)	Y	4.86-12.46 Mb	21 (9)
LG3	inversion	LG5	31.66-32.25 Mb	Y	31.85-32.06 Mb	3 (3)	N	n/a	n/a
LG4	inversion	LG1	0.52-10.39 Mb	Y	1.25-8.90 Mb	30 (9)	Y	1.25-10.26 Mb	38 (15)
LG4*	inversion*	LG1	not detected	Y	3.14-3.41 Mb*	4 (2)	N	n/a	n/a
LG6	inversion	LG7	6.89-9.19 Mb	Y	6.31-10.11 Mb	2 (2)	Y	9.45-11.88 Mb	6 (2)
LG6	translocation	LG3	n/a	Y	0.07-2.05 Mb	18 (13)	N	n/a	n/a
LG6*	inversion*	LG3	1.25-2.01 Mb	Y	1.30-1.80 Mb*	5 (3)	N	n/a	n/a
LG7	inversion	LG6	0.91-8.06 Mb	Y	2.35-7.54 Mb	22 (11)	Y	3.94-7.93 Mb	26 (14)
LG8	inversion	LG3	40.88-46.59 Mb	Y	41.13-46.45 Mb	15 (5)	Y	44.40-46.45 Mb	7 (4)
LG8	inversion	LG3	48.84-49.68 Mb	N	n/a	n/a	Y	49.17-49.64 Mb	3 (2)
LG9	inversion	LG3	7.92-29.34 Mb	Y	8.08-22.05 Mb	50 (21)	Y	9.45-23.70 Mb	51 (21)
LG 10	inversion	LG4	2.60-5.84 Mb	Y	2.62-5.57 Mb	9 (4)	Y	3.26-5.33 Mb	11 (5)
LG 10	inversion	LG4	30.21-32.34 Mb	Y	31.26-35.60 Mb	19 (7)	Y	31.50-36.37 Mb	26 (4)
LG 10	inversion	LG4	36.44-38.61 Mb	Y	37.10-37.67 Mb	7 (2)	Y	36.46-37.68 Mb	10 (4)

* Indicates that a rearrangement was detected nested within another rearrangement.

in sorghum (Devos et al. 2015) and encompassing 22 markers (13 with hits in sorghum) was identified between the maternal paspalum map and the sorghum genome sequence. Another known inversion on LG 7 spanning the region 58.36 to 64.31 Mb in sorghum (Devos et al. 2015) and encompassing 11 markers (4 with hits in sorghum) was identified between the paternal paspalum map and the sorghum genome sequence. A much greater number of rearrangements were identified between the paspalum maps and Setaria (Table 3.5). No paspalum-specific rearrangements were identified.

A comparison by linkage group of the percentage of GBS tags in the maternal and paternal paspalum maps with a putative ortholog in the sorghum and Setaria genomes showed that relatively fewer comparative tags were identified in paspalum LG5 than the other linkage groups (Figure 3.6).

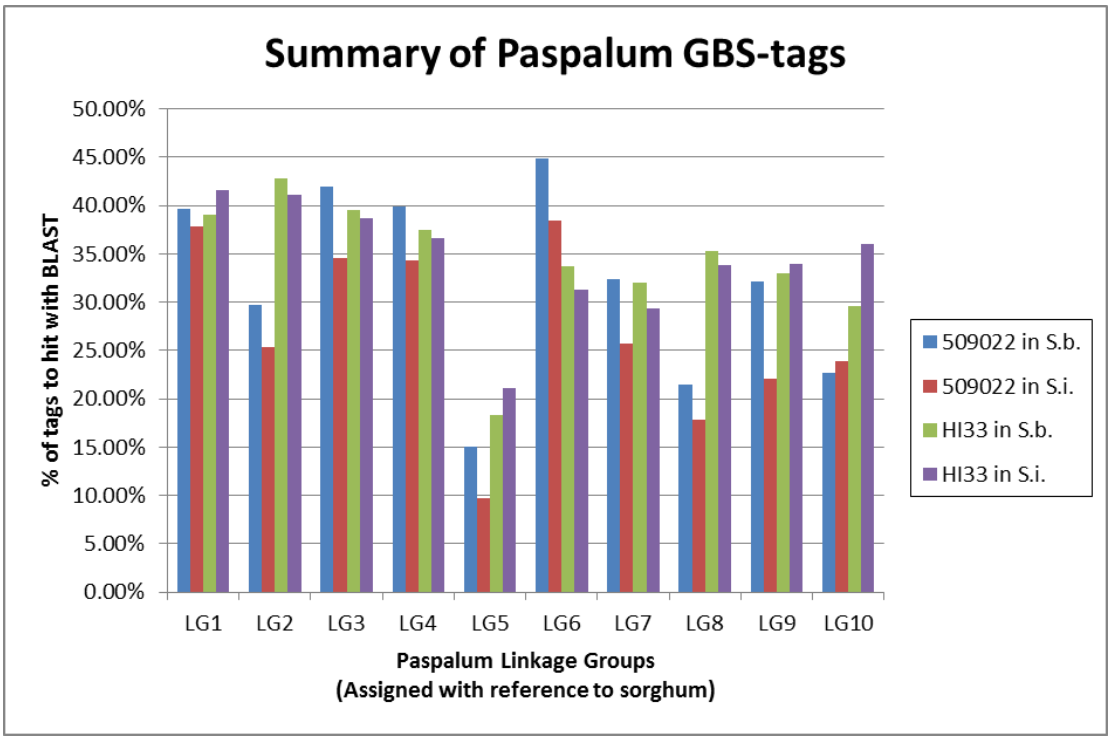

Figure 3.6: Percentage of paspalum tags in both reference genomes

Table 3.6: Comparative tags in 509022

Linkage Groups	Markers in 509022	Tags with hits in sorghum	% of Tags sorghum	Tags with hits in Setaria	% of Tags Setaria
LG1	209	83	39.71%	79	37.80%
LG2	185	55	29.73%	57	25.41%
LG3	162	68	41.98%	66	34.57%
LG4	128	51	39.84%	47	34.38%
LG5	113	17	15.04%	17	9.73%
LG6	138	62	44.93%	60	38.41%
LG7	74	24	32.43%	21	25.68%
LG8	56	12	21.43%	16	17.86%
LG9	109	35	32.11%	29	22.02%
LG10	88	20	22.73%	28	23.86%
TOTAL	**1262**	**427**	-	**420**	-

Table 3.7: Comparative tags in HI33

Linkage Groups	Markers in HI33	Tags with hits in sorghum	% of Tags sorghum	Tags with hits in Setaria	% of Tags Setaria
LG1	197	77	39.09%	82	41.62%
LG2	112	48	42.86%	46	41.07%
LG3	119	47	39.50%	46	38.66%
LG4	112	42	37.50%	41	36.61%
LG5	104	19	18.27%	22	21.15%
LG6	80	27	33.75%	25	31.25%
LG7	75	24	32.00%	22	29.33%
LG8	68	24	35.29%	23	33.82%
LG9	100	33	33.00%	34	34.00%
LG10	125	37	29.60%	45	36.00%
TOTAL	**1092**	**333**	-	**332**	-

Discussion

GBS as a highly robust marker system

The GBS approach described by Elshire et al. (2011) results in large numbers of missing data and low sequence coverage of SNP variants. Both problems can be alleviated by reducing the fragment pool that is sequenced so that much higher sequence coverage can be obtained without increasing costs. While this reduced the number of markers obtained, this is not a problem for mapping in biparental populations where the number of mappable markers is limited by the number of recombination events in the population. We therefore used a combination of three

restriction enzymes, *Pst*I, *Msp*I and *Nde*I, to generate the fragment pool. The methylation-sensitive restriction enzyme *Pst*I was used in combination with the 6-bp cutter *Nde*I to generate fragments for amplification. The use of a third enzyme allowed *Pst*I – *Nde*I fragments that carried a *Msp*I site to be cut thereby preventing their amplification and further reducing the fragment pool for sequencing. In general, a survey of sequence content of out reads indicated that 10% contained the *Msp*I sequence, and most of these reads (~97%) were not utilized by Ustacks. Though other enzyme combinations could have resulted in more sequence tags being available, it was expected that we would be recombination event limited. Therefore we prioritized attaining ample read depth over increased marker number to ensure the reliable calling of allele states and elected to employ the more conservative three enzyme system.

Distribution of SNPs in the GBS-tags

The distribution of SNP positions averaged across the final tag set illustrated that polymorphisms occur at any single position in the tag more or less randomly, the only exceptions being positions 130 and 131. These positions represent the transition point between the forward and reverse reads after they have been merged. The elevated SNP rate at these two positions is an artifact caused by the joining of non-overlapping paired reads into a contiguous sequence during the GBS read analysis. The joining step was introduced into the GBS pipeline because not all software packages that were used to generate a reference sequence from the GBS-tags (no whole genome sequence assembly was available for seashore paspalum) can deal with paired end reads. The joining of non-overlapping reads in the reference does not affect subsequent read alignment or SNP calling except when a deletion is present in the aligned read relative to the reference. A single base deletion in the forward read will cause the last base of the forward read to align with the first base of the reverse read in the joined reference tags, leading to a spurious SNP call at

position 131. Similarly, a single base deletion in the reverse read will lead to a spurious SNP call at position 130. Only positions 130 and 131 are affected because our SNP filtering pipeline includes a step that removes consecutive SNPs. For example, a 2-bp deletion in a forward read would lead to spurious SNPs at positions 131 and 132, but these SNPs would not pass the SNP filter. Since the number of SNPs appearing in excess of what is expected at these positions is on the order of ~25 per site, or less than 1% of our total dataset, this issue will have little impact on our mapping analyses.

GBS-Tags are Enriched for Genic Regions

The paspalum sequence tags were queried against both the *Sorghum bicolor* and *Setaria italica* genomes and the average level of sequence similarity between the paspalum sequence and both sets of BLAST results was ~90%, suggesting that many of these tags lie in genic sequence. Each of these estimates was derived from over 3100 sequences which had hits in one or both genomes (out of 7480 total tags). This strongly suggests that about 40% of the GBS tags contain highly conserved sequence consistent with a genic origin. This number is probably an underrepresentation because paspalum tags derived from promoter regions and introns, which are less conserved than exons, will likely not identify orthologs in related species. In the maternal map, ~33% of the total markers mapped hit a region in the sorghum or Setaria genome and, of these, ~87% and ~84% were on syntenic chromosomes. In the paternal map ~35% of the total markers hit in the sorghum or Setaria genome and ~82% and ~80%, respectively, were on syntenic chromosomes. This shows a high level of gene order conservation between Paspalum and other Panicoideae species.

This genic distribution of tags is not unexpected, *Pst*I is sensitive to 3-methylcytosine in a CHG sequence context which is abundant in repetitive DNA and rare in gene bodies in plants (Feng et

al. 2010). Additionally, much of the repetitive DNA of a genome is located in the pericentromeric region, which is larger in sorghum than in Setaria. The difference in genome size, ~423 Mb for Setaria and ~730 Mb for sorghum, is mostly attributed to variation in the amount of pericentromeric repeats and directly contributes to the differences in tag coverage in these regions (Figures 3.4 and 3.5). Although the seashore paspalum genome is relatively small (~593 Mb; Chapter 2), use of a non-methylation sensitive restriction endonuclease would have led to a greater proportion of the resulting sequence to be difficult or impossible to assemble and/or to not segregate as a single copy sequence. In that case, a greater proportion of the total read pool would have consisted of tags with limited utility for genetic map construction.

Comparisons Between the Maternal and Parent Maps

The genetic maps that were produced for each parent resolved all of the expected chromosomes. However, 15% more tags were present in the map of the mother genotype than in the father. One possible explanation for this is that the father has higher levels of homozygosity. Indeed, of the 5085 polymorphisms identified, 3222 (63.4%) were called as an 'H' in maternal line 509022, and 2707 (53.2%) were called as an 'H' in paternal line HI33. The homozygous ('A' or 'B') calls numbered 1608 (31.6%) in the mother and 2331 (45.8%) in the father. This difference in homozygosity could also relate to the average size, if not the number, of inter-marker intervals which were found to be greater than 5 cM. The total number of marker intervals with distances greater than 5 cM is very similar in HI33 and 509022 (40 vs. 42), however the sum of the distances contained in these intervals is greater in the paternal parent compared to the maternal parent (307.2 cM vs. 265.1 cM). The average distance between this subset of markers (those at least 5 cM apart) was 7.7 cM in the paternal parent and 6.3 cM in the maternal parent (~20% less in the maternal parent). It is also possible that the latter differences are caused by variation in the

level and distribution of recombination in the maternal and paternal parents, and/or male and female gametogenesis (Busso et al. 1995; Liu et al. 1996).

The number of regions exhibiting segregation distortion (with at least three adjacent distorted markers) was greater in the father than in the mother (18 vs 10) (Supplementary Table 3.3 and 3.4); however the total number of markers comprising these intervals was surprisingly consistent (173 in 509022 and 174 in HI33). In terms of the locations where distortion was identified, stretches of distorted markers on LG 01, LG 03, and LG9 were shared between the parents; the other distorted regions were unique to each parent. Since the meiosis we mapped occurred in each parent separately, a region observed to be distorted in both parents is a good indicator of a potential segregation distortion locus (SDL). (Figure 3.2 and 3.3).

The comparative maps have utility in assessing the genome coverage of the paspalum genetic maps, and identifying chromosomal rearrangements between paspalum and sequenced grass genomes. In general, it appears that our GBS-tag set covered the paspalum genome reasonably well, on average for each sorghum chromosome, a paspalum sequence tag was present within ~250,000 bp of the end of an assembled chromosome, in Setaria the average distance was ~80,000 bp. (I will confirm on the near side) Comparing the genomic coverage of the paternal and maternal maps illustrates the lower limit for reliable detection of rearrangements. On LG 3 in the maternal parent, for example, a known translocation in Setaria was identified and was composed of 18 markers (13 comparative tags) despite spanning only 2Mb. This translocated segment was not detected in the paternal parent and among the sixteen rearrangements we detected, the threshold for detection seemed to be near that ~2Mb mark. Of the five rearrangements which were not detected in both parents, the largest has been identified as occupying 2.35 Mb in Setaria, with the others ranging from 2 Mb down to ~0.6 Mb (Table

3.5). In general, the ability to detect these smaller rearrangements is limited by the number of markers we were able to integrate into our maps which also provide a comparative data point in one of the other genomes, and hence will depend on the chromosomal location (distal or pericentromeric) of these rearrangements. Secondarily, there could also be limitations in the marker coverage in our maps if regions in the homologous chromosomes of either parent are homozygous. In this case, there will be no segregation and thus no markers mapping in that interval.

Conclusions

Utilizing a GBS-approach for genetic marker development makes it possible to generate and utilize thousands of genetic markers with relative ease, compared to older methods. The triple-enzyme combination employed here produced a quantity of sequence tags that were sufficiently resolvable with the number of recombination events expected from a mapping population of this size. Additionally, the distribution of these markers was determined to be genome wide, but highly enriched for the genic regions of the paspalum genome. As a consequence of the conserved nature of this sequence between grass relatives, comparative mapping with other genomes proved to have utility in further aiding in the ordering of ambiguously placed markers. Future genotyping efforts in seashore paspalum and other species with similar genome sizes would likely benefit from the omission of a third enzyme (*Msp*I here) and/or by replacing the secondary enzyme (*Nde*I here) with an enzyme that will cut more frequently, such as *Msp*I. The result of one or both of these changes would be to produce a larger fragment pool that would provide increased mapping resolution if larger numbers of genotypes were to be used. Retaining *Pst*I as the primary cutter is recommended because it localizes tags into and near genes. This ensures that a large proportion of sequence tags have sufficient sequence similarity to related

species to allow for the leveraging of those genomes, though this may become less important once the paspalum genome is finalized, it will remain important for other orphan crops or other species without reference genomes.

Furthermore, the genetic maps generated here provide a valuable illustration of the structure of the paspalum genome and confirm that seashore paspalum is largely collinear with sorghum, despite representing the more distant Paspaleae clade. Additionally, the equivalent sequence similarities between Paspalum and both sorghum and Setaria suggest that either outgroup would provide good sequence references for paspalum gene models, and perhaps utilizing both simultaneously would be better than either one in isolation.

References

Bai, H., Y. Cao, J. Quan, L. Dong, Z. Li, Y. Zhu, L. Zhu, Z. Dong and D. Li (2013). "Identifying the genome-wide sequence variations and developing new molecular markers for genetics research by re-sequencing a landrace cultivar of foxtail millet." PLoS ONE 8(9): e73514.

Bennetzen, J. L., J. Schmutz, H. Wang, R. Percifield, J. Hawkins, A. C. Pontaroli, M. Estep, L. Feng, J. N. Vaughn, J. Grimwood, J. Jenkins, K. Barry, E. Lindquist, U. Hellsten, S. Deshpande, X. Wang, X. Wu, T. Mitros, J. Triplett, X. Yang, C.-Y. Ye, M. Mauro-Herrera, L. Wang, P. Li, M. Sharma, R. Sharma, P. C. Ronald, O. Panaud, E. A. Kellogg, T. P. Brutnell, A. N. Doust, G. A. Tuskan, D. Rokhsar and K. M. Devos (2012). "Reference genome sequence of the model plant *Setaria*." Nat Biotech 30(6): 555-561.

Catchen, J. M., A. Amores, P. Hohenlohe, W. Cresko and J. H. Postlethwait (2011). "Stacks: building and genotyping loci de novo from short-read sequences." G3 (Bethesda) 1(3): 171-182.

Causse, M., N. Desplat, L. Pascual, M.-C. Le Paslier, C. Sauvage, G. Bauchet, A. Bérard, R. Bounon, M. Tchoumakov, D. Brunel and J.-P. Bouchet (2013). "Whole genome resequencing in tomato reveals variation associated with introgression and breeding events." BMC Genomics 14(1): 1-14.

Chen, Z., M. Newman, K. Wook, M. Wang and P. Raymer (2005). "Molecular characterization of genetic diversity in the USDA seashore paspalum germplasm collection." ITS Research Journal 10: 543-549.

DePristo, M. A., E. Banks, R. Poplin, K. V. Garimella, J. R. Maguire, C. Hartl, A. A. Philippakis, G. del Angel, M. A. Rivas, M. Hanna, A. McKenna, T. J. Fennell, A. M. Kernytsky, A. Y. Sivachenko, K. Cibulskis, S. B. Gabriel, D. Altshuler and M. J. Daly (2011). "A framework for variation discovery and genotyping using next-generation DNA sequencing data." Nat Genet 43(5): 491-498.

Duncan, R. and R. Carrow (2000). Seashore Paspalum: the Environmental Turfgrass, Sleeping Bear Press.

Elshire, R. J., J. C. Glaubitz, Q. Sun, J. A. Poland, K. Kawamoto, E. S. Buckler and S. E. Mitchell (2011). "A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species." PLoS ONE 6(5): e19379.

Faircloth, B. and T. Glenn (2014). Protocol: preparation of an AMPure XP substitute (AKA Serapure). doi:10.6079/J9MW2F26.

Feng, S., S. J. Cokus, X. Zhang, P.-Y. Chen, M. Bostick, M. G. Goll, J. Hetzel, J. Jain, S. H. Strauss, M. E. Halpern, C. Ukomadu, K. C. Sadler, S. Pradhan, M. Pellegrini and S. E. Jacobsen (2010). "Conservation and divergence of methylation patterning in plants and animals." Proceedings of the National Academy of Sciences 107(19): 8689-8694.

Flowers, T. J. (2004). "Improving crop salt tolerance." Journal of Experimental Botany 55(396).

Grattapaglia, D. and R. Sederoff (1994). "Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers." Genetics 137(4): 1121-1137.

Harris-Shultz, K., P. Raymer, B. E. Scheffler and R. S. Arias (2013). "Development and characterization of seashore paspalum SSR markers." Crop Science 53(6).

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S. E. Lincoln and L. Newburg (1987). "MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations." Genomics 1(2): 174-181.

Langmead, B. and S. L. Salzberg (2012). "Fast gapped-read alignment with Bowtie 2." Nat Meth 9(4): 357-359.

Liu, Z. W., R. L. Jarret, R. R. Duncan and S. Kresovich (1994). "Genetic-relationships and variation among ecotypes of seashore paspalum (*Paspalum vaginatum*) determined by random amplified polymorphic DNA markers." Genome 37(6): 1011-1017.

Liu, Z. W., R. L. Jarret, S. Kresovich and R. R. Duncan (1995). "Characterization and analysis of simple sequence repeat (SSR) loci in seashore paspalum (*Paspalum vaginatum* Swartz)." Theoretical and Applied Genetics 91(1): 47-52.

Magoč, T. and S. L. Salzberg (2011). "FLASH: fast length adjustment of short reads to improve genome assemblies." Bioinformatics 27(21): 2957-2963.

Paterson, A. H., J. E. Bowers, R. Bruggmann, I. Dubchak, J. Grimwood, H. Gundlach, G. Haberer, U. Hellsten, T. Mitros, A. Poliakov, J. Schmutz, M. Spannagl, H. Tang, X. Wang, T. Wicker, A. K. Bharti, J. Chapman, F. A. Feltus, U. Gowik, I. V. Grigoriev, E. Lyons, C. A. Maher, M. Martis, A. Narechania, R. P. Otillar, B. W. Penning, A. A.

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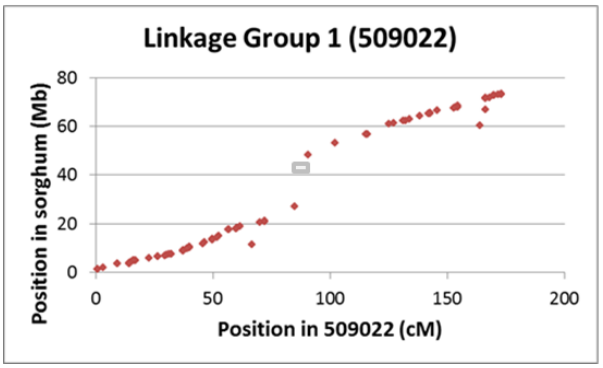
Poland, J. A., P. J. Brown, M. E. Sorrells and J.-L. Jannink (2012). "Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach." PLoS ONE 7(2): e32253.

Stam, P. (1993). "Construction of integrated genetic linkage maps by means of a new computer package: Join Map." The Plant Journal 3(5): 739-744.

Unamba, C. I. N., A. Nag and R. K. Sharma (2015). "Next generation sequencing technologies: The doorway to the unexplored genomics of non-model plants." Frontiers in Plant Science 6: 1074.

Xu, X., X. Liu, S. Ge, J. D. Jensen, F. Hu and X. Li (2012). "Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes." Nat Biotechnol 30.

Zhou, Z., Y. Jiang, Z. Wang, Z. Gou, J. Lyu, W. Li, Y. Yu, L. Shu, Y. Zhao, Y. Ma, C. Fang, Y. Shen, T. Liu, C. Li, Q. Li, M. Wu, M. Wang, Y. Wu, Y. Dong, W. Wan, X. Wang, Z. Ding, Y. Gao, H. Xiang, B. Zhu, S.-H. Lee, W. Wang and Z. Tian (2015). "Resequencing 302 wild and cultivated accessions identifies genes related to domestication and improvement in soybean." Nat Biotech 33(4): 408-414.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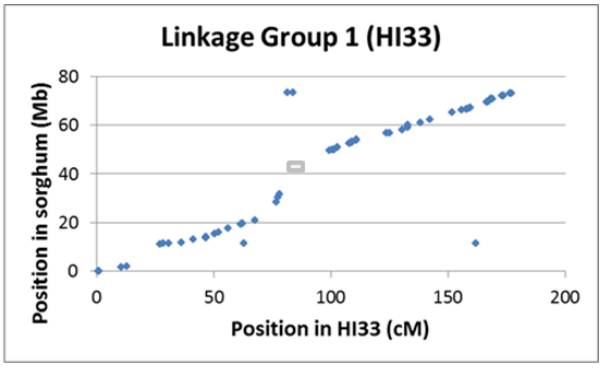
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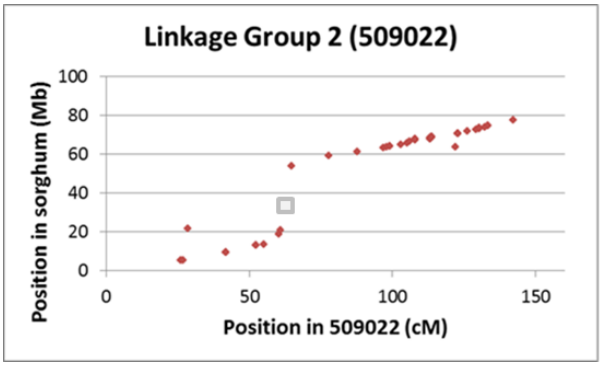
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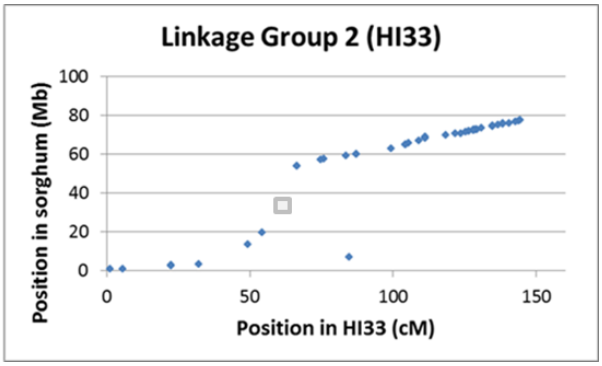
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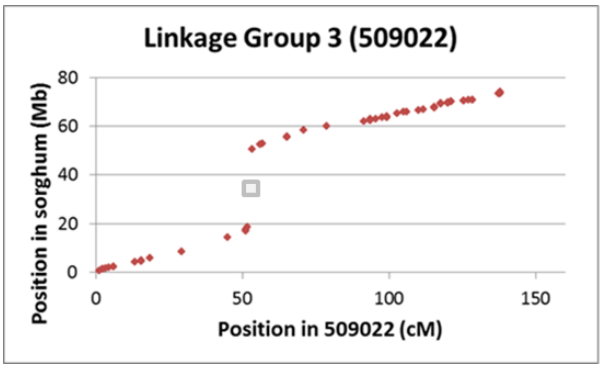
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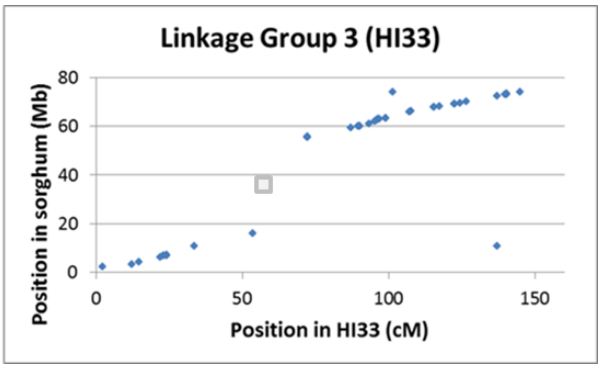
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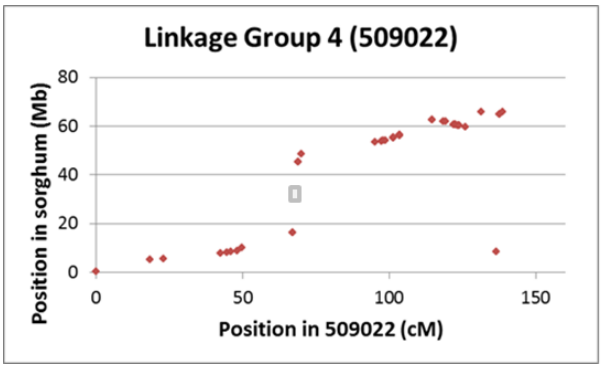
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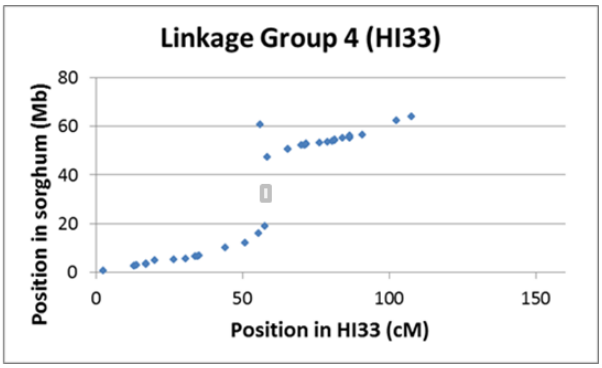
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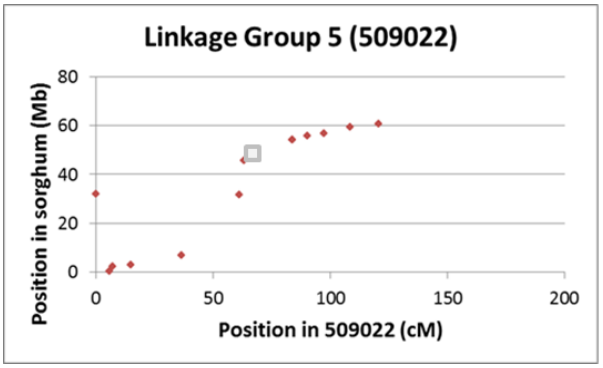
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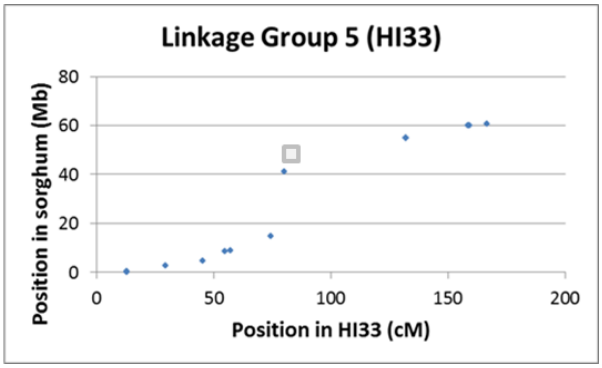
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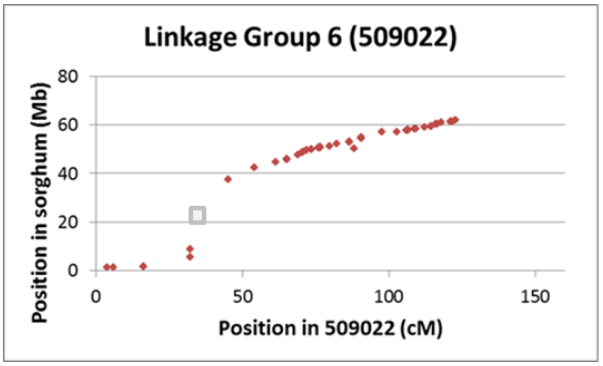
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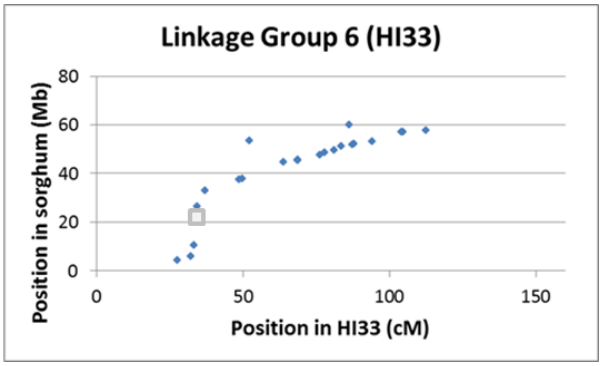
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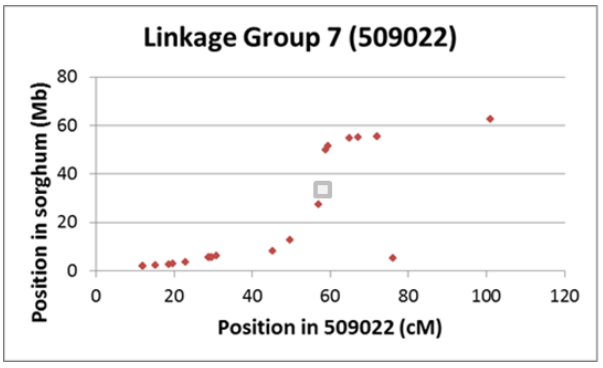
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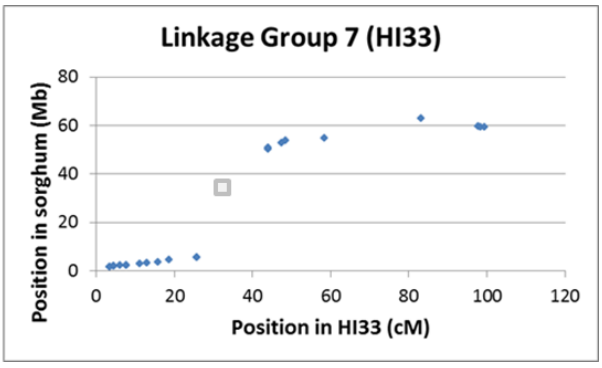
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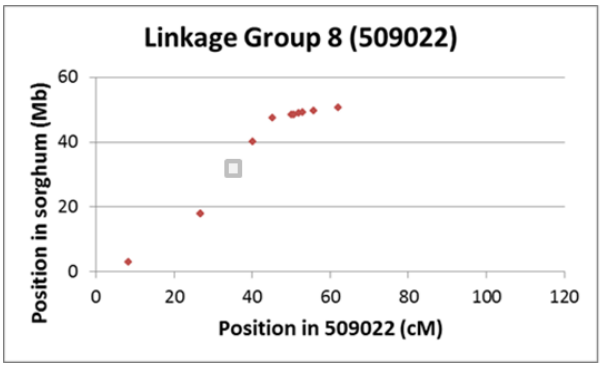
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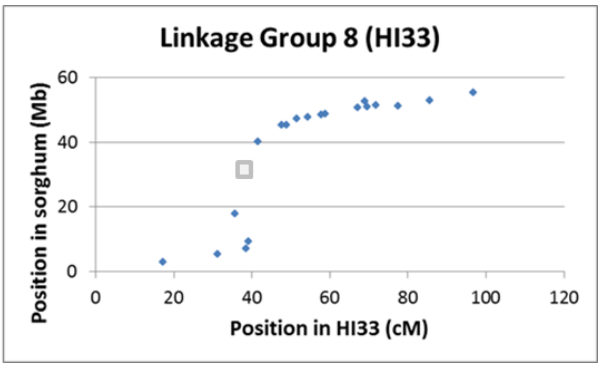
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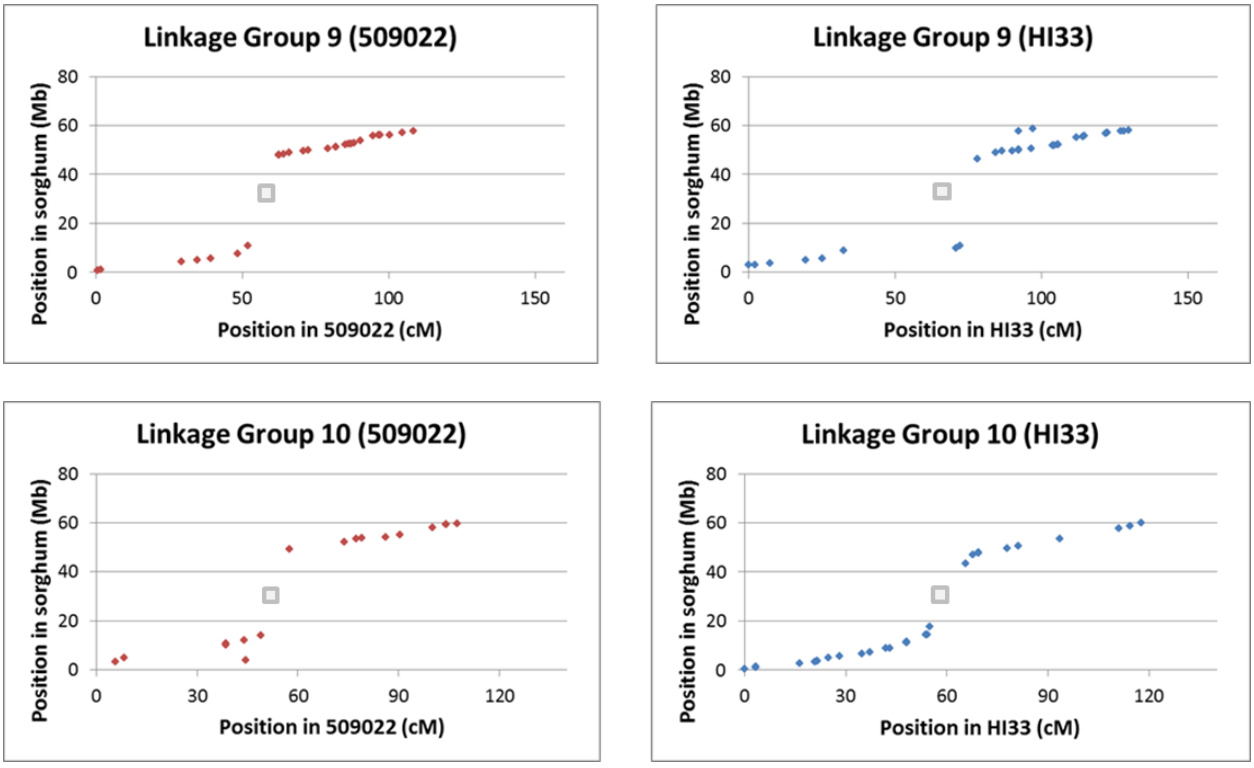

Supplementary Figure 3.1: Dot plots illustrating Paspalum-sorghum genome comparisons

Syntenic paspalum marker positions (cM) with orthologs in sorghum (Mb) are plotted for each parent, the sorghum centromere positions are indicted with grey boxes.

Supplementary Table 3.1: GBS-tag barcodes

Plate 1						Plate 2						Plate 3		
Barcode #	Well	Barcode Seq	Barcode #	Well	Barcode Seq	Barcode #	Well	Barcode Seq	Barcode #	Well	Barcode Seq	Barcode #	Well	Barcode Seq
1	A01	ATTAT	49	E01	GGTGCACATT	97	A01	CATCACAAG	145	E01	CAAGCCAATT	193	A01	CTCTCCAG
2	A02	CACCA	50	E02	CTCTCGCAT	98	A02	CGCAGACACT	146	E02	CCATAGA	194	A02	TAATTG
3	A03	TGCTT	51	E03	CAGAGGT	99	A03	CAATA	147	E03	ATCTGCAACA	195	A03	ATCTCGT
4	A04	GAAGTG	52	E04	GCGTACAAT	100	A04	TTATCCAT	148	E04	CGTCTG	196	A04	GACAACT
5	A05	TCTTGG	53	E05	ACGCGCG	101	A05	AACTT	149	E05	GTCCTGCCA	197	A05	CTCGCAA
6	A06	ACAACT	54	E06	GTCGCCT	102	A06	CTGTTG	150	E06	ACGCTAA	198	A06	TGGACACT
7	A07	CTAAGCA	55	E07	AATAACCAA	103	A07	TAAGACA	151	E07	GCCAGACATT	199	A07	TGTCAAT
8	A08	TAGCCAA	56	E08	AATGAACGA	104	A08	CTGTGT	152	E08	TGACGT	200	A08	TCCTGCT
9	A09	GTTCACA	57	E09	CGTCGCCACT	105	A09	AATCGGAGG	153	E09	AACGG	201	A09	GAACTT
10	A10	GCCTACCT	58	E10	ATGGCAA	106	A10	GTAACG	154	E10	GCGCGAG	202	A10	ATGCT
11	A11	TGACGCCA	59	E11	GAAGCA	107	A11	GGAGAGCAT	155	E11	GATTGGAAGA	203	A11	ATTCCAA
12	A12	CAGATA	60	E12	AACGTGCCT	108	A12	TACAAG	156	E12	GGCGGACGA	204	A12	GACACACT
13	B01	TAGCGGAT	61	F01	CCTCG	109	B01	TCCAG	157	F01	CAATCAT	205	B01	CGCGT
14	B02	TATTCGCAT	62	F02	CTCAT	110	B02	TGTGGA	158	F02	GGCACGCAT	206	B02	CATACGCG
15	B03	ATAGAT	63	F03	ACGGTACT	111	B03	TAGTCCAT	159	F03	AAGGA	207	B03	CTATCACT
16	B04	CCGAACA	64	F04	GCGCCG	112	B04	GGATTG	160	F04	TCTGA	208	B04	CTGAACCA
17	B05	GGAAGACAT	65	F05	CAAGT	113	B05	GAGTCACAAT	161	F05	GTTACA	209	B05	TCTCCGT
18	B06	GGCTTA	66	F06	TCCGAG	114	B06	TCAGTAAT	162	F06	ATAGG	210	B06	TGTACA
19	B07	AACGCACATT	67	F07	TAGATGA	115	B07	TGAGA	163	F07	GTGCG	211	B07	AAGCAACT
20	B08	GAGCGACAT	68	F08	TGGCCAG	116	B08	CGCGCCG	164	F08	ACTGAG	212	B08	ACCGA
21	B09	CCTTGCCATT	69	F09	GCACGAT	117	B09	TGGAGCCT	165	F09	GAGACG	213	B09	GTAAG
22	B10	GGTATA	70	F10	TTGCTG	118	B10	TCCTCACAT	166	F10	GCTCA	214	B10	TGATCGCT
23	B11	GGTGT	71	F11	CGCAACCAGT	119	B11	CCATG	167	F11	AATAAGAGT	215	B11	TGCGG
24	B12	GGATA	72	F12	TCACTG	120	B12	TTCAGCCAGT	168	F12	ATCGTACGT	216	B12	ACTAA
25	C01	GCGCTCA	73	G01	ACAGT	121	C01	AACTGAAG	169	G01	ACATCACCG	217	C01	GAGGTCCT
26	C02	ACTGCGAT	74	G02	GGAGTCAAG	122	C02	TGGATA	170	G02	GTGTT	218	C02	TAGCTAT
27	C03	TTCGTT	75	G03	TGAAT	123	C03	CGTGACCT	171	G03	TTACT	219	C03	CAGCGCAAGA
28	C04	ATATAA	76	G04	CATAT	124	C04	GACGTGA	172	G04	ATCTTA	220	C04	GCTCGCCAT
29	C05	TGGCAACAGA	77	G05	GTGACACAT	125	C05	CGGTTGCAT	173	G05	GCGGA	221	C05	TGTACCAG
30	C06	CTCGTCG	78	G06	TATGT	126	C06	TCACA	174	G06	CCTGCCA	222	C06	TGTACGCA
31	C07	AATTAG	79	G07	CAGTGCCATT	127	C07	AATGCAG	175	G07	TTACACA	223	C07	TTGGCGCT
32	C08	GGAACGA	80	G08	ACAACCAACT	128	C08	CTAACA	176	G08	GCGCACT	224	C08	CATGG
33	C09	ACTGCT	81	G09	TGCAGA	129	C09	GATGGCCAT	177	G09	CTTATCA	225	C09	ACTACAAT
34	C10	CGTGGACAGT	82	G10	CATCTGCCG	130	C10	TCGTA	178	G10	ACGATA	226	C10	GACTAACT
35	C11	TGGCACAGA	83	G11	GGACAG	131	C11	CGCTCACACA	179	G11	GAGCAA	227	C11	ATGGTGA
36	C12	GCAAGCCAT	84	G12	ATCTGT	132	C12	TGAAGCAACT	180	G12	AATGTA	228	C12	TATTGCAG
37	D01	CGCACCAATT	85	H01	GAATGCAATA	133	D01	GATTCA	181	H01	TTGCGCT	229	D01	ATCTGACT
38	D02	CTCGCGG	86	H02	TAGCAG	134	D02	ATAGCGT	182	H02	ATTAACAATT	230	D02	GTCACGA
39	D03	AACTGG	87	H03	ATCCG	135	D03	CTTCAGA	183	H03	CCTTCG	231	D03	AACGACCACA
40	D04	ATGAGCAA	88	H04	CTTAG	136	D04	GACGGCA	184	H04	TGTATACAG	232	D04	CGCCTCAT
41	D05	CTTGA	89	H05	TTATTACAT	137	D05	TGTGACAAGA	185	H05	ATGATACG	233	D05	CTTATG
42	D06	GCGTCCT	90	H06	GCCAACAAGA	138	D06	GTCGT	186	H06	GGTATGAAT	234	D06	TAGAG
43	D07	ACCAGGA	91	H07	TGCCGCAT	139	D07	CCGTGA	187	H07	CCGTCACAGT	235	D07	GGCAT
44	D08	CCACTCA	92	H08	CGTGTCA	140	D08	GGCCTG	188	H08	GGTAAGCA	236	D08	CCGACG
45	D09	TCACGGAAG	93	H09	CAACCACACA	141	D09	TGCAA	189	H09	CCGTACCACT	237	D09	TGGTCAAG
46	D10	TATCA	94	H10	GCTCCGA	142	D10	GTATTGACT	190	H10	CAGTAA	238	D10	ACCAAG
47	D11	ATATCGCCA	95	H11	TCAGAGAT	143	D11	TGTTACG	191	H11	CTTCCGCAA	239	D11	GTTCGGT
48	D12	CTCTA	96	H12	CGTTCA	144	D12	ACAACGCAT	192	H12	GTACGGACG	240	D12	GCCGCAAT

Supplementary Table 3.2: Adapter and primer sequences

Oligonucleotide Name	Oligonucleotide Sequence
Barcoded Adapter, top (*Pst* I)	5'-CACGACGCTCTTCCGATCT**[BARCODE]**TGCA-3'
Barcoded Adapter, bottom (*Pst* I)	3'-GTGCTGCGAGAAGGCTAGA**[BARCODE]**-5'
Common Adapter, top (*Nde* I)	5'-TAAGATCGGAAGAGCGGGGACTTTAAGC-3'
Common Adapter, bottom (*Nde* I)	5'-GATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT-3'
GBS_PCR_1 (barcode adapter-specific forward primer)	5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'
GBS_PCR_2 (common adapter-specific reverse primer)	5'-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAA-3'

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
1	1	**230_180_109**	0	81	100	0	1.99	-								
1	2	**994_148_75**	0.6	80	101	0	2.44	-	>Chr01	73430789			>scaffold_9	58666780		
1	3	**W_8076_132_103**	0.6	79	100	2	2.46	-	>Chr01	73048169			>scaffold_9	58495590		
1	4	**3062_174_268**	0.6	80	101	0	2.44	-								
1	5	**12092_176_147**	1.7	80	101	0	2.44	-	>Chr01	72954375						
1	6	**6259_172_37**	2.3	73	93	15	2.41	-								
1	7	**9091_177_89**	3.4	74	104	3	5.06	**	>Chr01	72767437			>scaffold_9	58312198		
1	8	**10400_154_165**	4	78	103	0	3.45	*	>Chr01	72651561			>scaffold_9	58243175		
1	9	**10834_171_110**	5.7	77	104	0	4.03	**	>Chr01	71917772			>scaffold_9	57581940		
1	10	**9749_174_187**	7.3	68	101	12	6.44	**								
1	11	**670_152_119**	7.3	74	107	0	6.02	**	>Chr01	71655907			>scaffold_9	57384885		
1	12	**10606_176_13**	7.3	71	105	5	6.57	**	>Chr01	71449468			>scaffold_9	57239939		
1	13	**13593_181_35**	7.3	74	106	1	5.69	**								
1	14	**210_154_97**	7.3	74	107	0	6.02	**	>Chr01	66968395			>scaffold_9	53774899		
1	15	**13622_135_155**	9.5	74	107	0	6.02	**								
1	16	**8240_170_182**	9.5	64	95	22	6.04	**					>scaffold_9	56307420		
1	17	**6100_149_248**	9.5	74	107	0	6.02	**								
1	18	**9580_173_11**	9.5	74	107	0	6.02	**	>Chr01	60244187			>scaffold_9	47613240		
1	19	**8129_157_90**	10.7	66	94	21	4.9	**								
1	20	**3844_154_159**	16.2	70	106	5	7.36	***								
1	21	**12666_165_32**	17.9	69	112	0	10.22	****								
1	22	**4557_155_135**	19	71	110	0	8.4	****	>Chr01	68409448			>scaffold_9	54935365		
1	23	**8318_146_57**	19	70	110	1	8.89	****	>Chr01	67906463			>scaffold_9	54538657		
1	24	**5944_174_54**	20.1	71	110	0	8.4	****	>Chr01	67691351						
1	25	**12530_166_185**	20.7	72	109	0	7.56	***								
1	26	**9618_178_24**	20.7	72	109	0	7.56	***	>Chr01	67601851			>scaffold_9	54279899		
1	27	**4959_174_131**	21.3	71	110	0	8.4	****								
1	28	**6513_177_132**	21.9	69	108	4	8.59	****								
1	29	**1332_175_183**	21.9	72	109	0	7.56	***					>scaffold_9	54208628		
1	30	**10645_174_102**	26.9	75	106	0	5.31	**					>scaffold_9	53647316		
1	31	**1025_144_201**	28	75	106	0	5.31	**	>Chr01	66481999			>scaffold_9	53298214		
1	32	**11490_145_137**	28	75	106	0	5.31	**	>Chr06	27683551						
1	33	**15570_138_263**	28.6	76	105	0	4.65	**								
1	34	**11226_176_136**	28.6	75	105	1	5	**								
1	35	**3172_169_245**	29.2	75	106	0	5.31	**								
1	36	**4728_143_35**	29.8	75	104	2	4.7	**								
1	37	**9738_176_196**	30.9	72	96	13	3.43	*	>Chr01	65408064			>scaffold_9	52301593		
1	38	**1721_181_93**	30.9	78	103	0	3.45	*	>Chr01	65324826			>scaffold_9	52249865		
1	39	**12949_138_212**	31.5	77	104	0	4.03	**	>Chr01	65271236			>scaffold_9	52173626		
1	40	**13249_151_33**	32.6	75	106	0	5.31	**								
1	41	**4271_132_199**	32.6	75	105	1	5	**								
1	42	**10787_160_151**	32.6	73	104	4	5.43	**								
1	43	**9045_185_23**	34.2	76	104	1	4.36	**								
1	44	**760_181_153**	35.3	76	105	0	4.65	**								
1	45	**7327_149_133**	35.3	76	105	0	4.65	**	>Chr01	64221641			>scaffold_9	51143192		
1	46	**7012_175_70**	37.5	74	107	0	6.02	**								
1	47	**12797_177_87**	39.7	76	105	0	4.65	**	>Chr01	62969812						
1	48	**3455_173_85**	41.4	75	106	0	5.31	**	>Chr01	62454087	>Chr03	66914216	>scaffold_9	49720087		
1	49	**3181_168_95**	42.5	77	104	0	4.03	**	>Chr01	62210860			>scaffold_9	49483806		
1	50	**3354_185_43**	42.5	77	104	0	4.03	**								
1	51	**3133_150_87**	43.6	77	104	0	4.03	**								
1	52	**10724_173_57**	46.4	78	103	0	3.45	*	>Chr01	61318725			>scaffold_9	48707000		
1	53	3100_160_209	48.6	81	99	1	1.8	-	>Chr01	60971766			>scaffold_9	48415416		
1	54	12147_181_100	50.8	83	95	3	0.81	-								
1	55	12579_151_56	51.4	83	98	0	1.24	-								
1	56	8476_163_139_R	54.7	83	98	0	1.24	-								
1	57	7832_161_149_R	54.7	83	93	5	0.57	-								
1	58	**W_10033_155_36_R**	55.3	82	96	3	1.1	-								
1	59	3733_181_130	56.4	81	99	1	1.8	-	>Chr04	56612187						
1	60	10372_157_42_R	57	81	100	0	1.99	-								
1	61	2698_182_69_R	57	81	100	0	1.99	-								
1	62	13460_162_98_R	57.6	80	100	1	2.22	-	>Chr01	56819460			>scaffold_9	43810179		
1	63	11758_146_192_R	58.3	77	98	6	2.52	-	>Chr01	56869184			>scaffold_9	43868927		
1	64	4808_150_25_R	60.4	80	100	1	2.22	-								
1	65	9494_167_118	62.6	82	92	7	0.57	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
1	66	11943_161_42_R	67	75	94	12	2.14	-								
1	67	5026_138_263_R	67	79	100	2	2.46	-					>scaffold_9	41642394		
1	68	**13723_150_229_R**	71.4	79	102	0	2.92	*	>Chr01	53091496			>scaffold_9	40335286		
1	69	**9552_144_79_R**	71.4	79	102	0	2.92	*					>scaffold_9	40238974		
1	70	**10910_172_195_R**	71.4	78	102	1	3.2	*					>scaffold_9	40165209		
1	71	**4294_174_205_R**	72	78	103	0	3.45	*								
1	72	**8985_148_270**	73.7	75	106	0	5.31	**								
1	73	**W_8716_170_121**	73.7	75	106	0	5.31	**								
1	74	**1188_178_85_R**	78.1	77	104	0	4.03	**								
1	75	**5792_164_83**	82	82	97	2	1.26	-								
1	76	**W_12226_168_26_R**	83.1	81	98	2	1.61	-	>Chr01	48421716			>scaffold_9	35459429	>scaffold_3	25155026
1	77	13557_175_72_R	83.1	82	99	0	1.6	-								
1	78	4097_167_152_R	88.6	84	97	0	0.93	-								
1	79	4939_175_40	88.6	80	92	9	0.84	-	>Chr01	27169925			>scaffold_9	21478383		
1	80	10549_182_216	88.6	84	96	1	0.8	-								
1	81	2725_186_84_R	88.6	84	97	0	0.93	-								
1	82	10553_169_69_R	88.6	82	96	3	1.1	-								
1	83	12183_180_86_R	88.6	84	97	0	0.93	-								
1	84	9551_175_209_R	88.6	83	97	1	1.09	-								
1	85	**5629_181_86_R**	89.7	63	86	32	3.55	*								
1	86	11042_142_101_R	93	67	87	27	2.6	-								
1	87	7399_180_144_R	93	84	90	7	0.21	-								
1	88	6928_175_159_R	93	88	93	0	0.14	-								
1	89	13353_166_24_R	98.6	79	88	14	0.49	-								
1	90	4852_149_48_R	99.7	82	88	11	0.21	-								
1	91	6637_183_173	99.7	82	90	9	0.37	-					>scaffold_9	17652906		
1	92	6020_166_203	101.4	91	90	0	0.01	-	>Chr02	66660246						
1	93	12343_177_137	101.4	91	89	1	0.02	-	>Chr01	21140772						
1	94	10238_160_49	101.4	91	90	0	0.01	-	>Chr01	20966553	>super_73	30806	>scaffold_9	16831158	>scaffold_2	12328458
1	95	8673_180_201_R	101.4	91	90	0	0.01	-	>Chr01	20966082	>super_73	30574				
1	96	9368_156_93	103.1	91	88	2	0.05	-	>Chr04	55058050						
1	97	9356_180_42	103.7	90	89	2	0.01	-	>Chr01	20653872			>scaffold_9	16656342		
1	98	**8914_144_176_R**	103.7	91	66	24	3.98	**								
1	99	8439_165_14	105.3	84	82	15	0.02	-								
1	100	4519_186_200	105.3	89	91	1	0.02	-								
1	101	931_176_146_R	105.9	89	92	0	0.05	-								
1	102	6030_139_29_R	107.1	88	91	2	0.05	-	>Chr01	11623643			>scaffold_5	11050587	>scaffold_6	15044136
1	103	10467_153_233	112	91	89	1	0.02	-					>scaffold_9	14892380		
1	104	13577_165_104	112	91	89	1	0.02	-	>Chr01	18858836			>scaffold_9	14851277		
1	105	701_183_187	112.6	90	90	1	0	-								
1	106	11260_146_120	112.6	91	90	0	0.01	-								
1	107	6485_153_21	113.2	92	89	0	0.05	-	>Chr01	18389914			>scaffold_9	14429673	>scaffold_5	25365832
1	108	13556_181_224	113.8	91	89	1	0.02	-	>Chr01	18135123			>scaffold_9	14203325		
1	109	7335_187_76_R	113.8	91	90	0	0.01	-	>Chr01	18107752			>scaffold_9	14181193		
1	110	2570_144_176_R	116	92	88	1	0.09	-								
1	111	5754_179_49_R	116	93	88	0	0.14	-								
1	112	11305_150_80_R	116.6	73	73	35	0	-	>Chr01	17694667			>scaffold_9	13877769		
1	113	5800_188_180_R	117.2	93	88	0	0.14	-	>Chr01	17682859			>scaffold_9	13866936		
1	114	5701_145_40_R	121.1	92	89	0	0.05	-								
1	115	8363_143_153_R	121.1	92	89	0	0.05	-								
1	116	5419_147_111_R	121.1	91	89	1	0.02	-								
1	117	10909_177_108_R	121.1	92	88	1	0.09	-								
1	118	14360_148_15_R	121.1	92	89	0	0.05	-	>Chr01	14974606			>scaffold_9	12242263		
1	119	8134_155_38	121.7	93	88	0	0.14	-	>Chr01	14392489			>scaffold_9	11845427		
1	120	11654_174_126	122.3	92	89	0	0.05	-								
1	121	10423_156_100_R	123.4	84	82	15	0.02	-								
1	122	8732_166_117	123.4	89	75	17	1.2	-					>scaffold_9	11450587		
1	123	4962_184_85	124	93	88	0	0.14	-	>Chr01	13919011						
1	124	3847_178_89_R	124	93	88	0	0.14	-	>Chr01	13875210			>scaffold_9	11301049		
1	125	8185_181_176_R	124	93	86	2	0.27	-	>Chr01	13573296	>Chr08	46115203	>scaffold_9	11195843		
1	126	**W_6733_157_5_R**	124	91	83	7	0.37	-	>Chr01	13378992						
1	127	5580_135_235_R	126.2	89	92	0	0.05	-								
1	128	11034_160_255_R	127.3	87	94	0	0.27	-								
1	129	4092_187_58_R	127.3	85	94	2	0.45	-								
1	130	7366_162_162_R	127.3	87	94	0	0.27	-	>Chr01	12353784			>scaffold_9	10184422		

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
1	131	7940_179_134_R	127.9	88	93	0	0.14	-	>Chr01	11801664			>scaffold_9	9741360	>scaffold_5	44711912
1	132	546_175_185_R	130.7	87	94	0	0.27	-								
1	133	2126_170_106	131.8	87	94	0	0.27	-								
1	134	9371_133_7	131.8	86	94	1	0.36	-								
1	135	3236_177_230	131.8	87	93	1	0.2	-								
1	136	14087_170_95	132.9	89	92	0	0.05	-								
1	137	6990_167_194_R	133.5	71	82	28	0.79	-								
1	138	3454_170_135	133.5	88	93	0	0.14	-								
1	139	2971_134_178	133.5	88	93	0	0.14	-								
1	140	8418_141_87	133.5	88	93	0	0.14	-	>Chr01	10605345	>Chr04	41871710	>scaffold_9	8711871		
1	141	2231_150_41	133.5	88	93	0	0.14	-	>Chr01	10366898			>scaffold_9	8458813		
1	142	7160_135_271	133.5	88	93	0	0.14	-	>Chr01	10363301			>scaffold_9	8453960		
1	143	5284_177_8_R	133.5	88	93	0	0.14	-	>Chr01	10039521			>scaffold_9	8143919		
1	144	4747_134_34	133.5	88	93	0	0.14	-								
1	145	9971_176_69_R	134.6	90	91	0	0.01	-	>Chr01	9816438			>scaffold_9	7951364		
1	146	9684_151_51_R	134.6	90	91	0	0.01	-	>Chr01	9815127			>scaffold_9	7950008		
1	147	9370_153_73	136.3	80	79	22	0.01	-								
1	148	10354_170_107_R	136.3	87	93	1	0.2	-	>Chr01	9184247			>scaffold_9	7425709		
1	149	9417_178_258_R	136.3	86	94	1	0.36	-					>scaffold_9	7319919		
1	150	7278_164_179_R	136.3	87	94	0	0.27	-	>Chr01	8943757						
1	151	13900_146_216_R	136.3	87	94	0	0.27	-	>Chr01	8802831			>scaffold_9	7126157		
1	152	12013_156_195	136.3	79	87	15	0.39	-								
1	153	8933_181_140	139.6	87	94	0	0.27	-								
1	154	5859_186_213_R	140.7	87	93	1	0.2	-	>Chr03	68337716						
1	155	8329_169_115_R	140.7	82	92	7	0.57	-								
1	156	2989_171_153_R	140.7	87	94	0	0.27	-								
1	157	8538_155_169	140.7	87	94	0	0.27	-								
1	158	482_182_80_R	141.3	86	95	0	0.45	-	>Chr01	7722198						
1	159	1355_151_9_R	141.3	85	95	1	0.56	-								
1	160	8592_164_55_R	141.3	86	95	0	0.45	-	>Chr01	7523801			>scaffold_9	6091525		
1	161	13178_152_249_R	142.4	88	93	0	0.14	-	>Chr01	7435603						
1	162	8103_183_178_R	143	85	92	4	0.28	-								
1	163	8859_166_186_R	143.6	79	89	13	0.6	-	>Chr01	7332471			>scaffold_9	5905035		
1	164	6494_154_228_R	143.6	86	95	0	0.45	-								
1	165	3724_137_141_R	144.2	87	93	1	0.2	-								
1	166	517_157_175_R	144.2	87	94	0	0.27	-	>Chr01	6942912						
1	167	10548_183_89_R	144.8	88	93	0	0.14	-								
1	168	653_152_66_R	145.4	79	82	20	0.06	-					>scaffold_9	5452413		
1	169	6590_160_63	147.1	86	95	0	0.45	-	>Chr01	6698130			>scaffold_9	5371913		
1	170	8898_166_21	147.1	86	95	0	0.45	-					>scaffold_9	5294479		
1	171	7549_171_5_R	147.1	84	94	3	0.56	-	>Chr01	6576119			>scaffold_9	5268985		
1	172	13924_165_227_R	148.2	86	95	0	0.45	-								
1	173	7727_176_18_R	149.3	86	95	0	0.45	-								
1	174	7018_175_8	151	89	92	0	0.05	-	>Chr01	5978478			>scaffold_9	4672264		
1	175	2664_183_112	154.3	85	96	0	0.67	-								
1	176	438_173_131	154.3	83	96	2	0.94	-								
1	177	5342_167_5	154.3	84	96	1	0.8	-								
1	178	7332_184_26	154.3	85	96	0	0.67	-								
1	179	12823_162_34	154.9	84	97	0	0.93	-								
1	180	748_145_227	154.9	84	97	0	0.93	-								
1	181	7118_164_89	154.9	84	97	0	0.93	-								
1	182	3189_147_81	155.7	74	76	31	0.03	-								
1	183	3604_168_91	156.5	83	97	1	1.09	-	>Chr01	5086573			>scaffold_9	3800339		
1	184	10104_158_21	157.1	84	97	0	0.93	-	>Chr01	5086280			>scaffold_9	3799973		
1	185	8764_163_146	157.1	83	95	3	0.81	-								
1	186	4910_156_39	157.4	83	97	1	1.09	-								
1	187	13217_155_106	157.7	82	98	1	1.42	-	>Chr01	4905195			>scaffold_9	3669031	>scaffold_3	26950968
1	188	11472_147_59	157.7	82	98	1	1.42	-	>Chr01	4884440	>Chr06	2304920	>scaffold_9	3655114		
1	189	1523_173_123	157.7	83	98	0	1.24	-								
1	190	13941_134_65	158.8	83	98	0	1.24	-	>Chr01	4377208			>scaffold_9	3288495		
1	191	12017_162_84	159.4	82	98	1	1.42	-	>Chr01	3861323			>scaffold_9	2918456		
1	192	1145_160_123	159.4	82	99	0	1.6	-					>scaffold_9	2873164		
1	193	16862_148_94	159.4	82	99	0	1.6	-	>Chr01	3770085	>Chr02	6792823				
1	194	13502_171_80	159.4	81	99	1	1.8	-	>Chr01	3744195			>scaffold_9	2828593		
1	195	9283_163_75	159.4	74	87	20	1.05	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
1	196	844_152_42	161.3	83	69	29	1.29	-								
1	197	1004_174_106	163.2	83	95	3	0.81	-								
1	198	10623_164_116	164.3	81	100	0	1.99	-								
1	199	12105_148_166	164.3	81	100	0	1.99	-	>Chr01	3733091			>scaffold_9	2821439		
1	200	10566_150_222	164.3	81	100	0	1.99	-					>scaffold_9	2741139		
1	201	3873_182_131	164.3	80	100	1	2.22	-	>Chr01	3628288			>scaffold_9	2734011		
1	202	13103_173_77	164.9	81	97	3	1.44	-								
1	203	5132_161_130	165.5	81	100	0	1.99	-								
1	204	8724_163_169	166.6	81	100	0	1.99	-								
1	205	**11286_169_21_R**	170.5	75	105	1	5	**	>Chr01	1869715			>scaffold_9	1374367		
1	206	**9718_169_153_R**	172.7	75	105	1	5	**	>Chr01	1369186						
1	207	**5187_186_50_R**	172.7	76	105	0	4.65	**	>Chr01	1212796			>scaffold_9	642583		
1	208	**6153_158_64_R**	172.7	76	105	0	4.65	**								
1	209	**8911_159_104**	173.9	74	99	8	3.61	*								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
2	1	10322_148_153	0	93	88	0	0.14	-								
2	2	1334_135_102	2.2	89	92	0	0.05	-								
2	3	**W_9613_134_145**	3.3	78	84	19	0.22	-								
2	4	11628_158_82	3.3	89	92	0	0.05	-	>Chr09	5537087			>scaffold_7	19121021	>scaffold_5	30484553
2	5	8574_156_168	4.4	89	92	0	0.05	-								
2	6	**W_8827_149_234**	4.4	88	92	1	0.09	-								
2	7	8780_176_113	4.4	88	91	2	0.05	-								
2	8	11301_150_158	4.4	89	92	0	0.05	-	>Chr04	466391			>scaffold_1	353536		
2	9	6073_182_164	6.6	89	92	0	0.05	-								
2	10	8976_175_116	7.2	90	91	0	0.01	-								
2	11	3554_134_149	7.2	89	91	1	0.02	-								
2	12	14633_149_228	7.2	90	91	0	0.01	-								
2	13	7787_167_32	7.2	89	91	1	0.02	-								
2	14	5750_179_62	7.2	90	91	0	0.01	-								
2	15	13742_146_142	13.1	91	81	9	0.58	-					>scaffold_2	9176726		
2	16	9514_171_67	17.8	88	92	1	0.09	-								
2	17	8660_173_164	20	89	92	0	0.05	-	>Chr01	62062231			>scaffold_4	4100015	>scaffold_9	49342030
2	18	9831_139_240	22.8	86	85	10	0.01	-								
2	19	335_185_259	23.4	85	96	0	0.67	-								
2	20	1816_178_1	23.4	85	96	0	0.67	-								
2	21	5554_153_90	24	85	91	5	0.2	-								
2	22	6953_180_212	25.1	88	93	0	0.14	-								
2	23	7883_148_43	25.1	88	93	0	0.14	-								
2	24	6041_161_44	26.2	86	95	0	0.45	-								
2	25	8108_166_210	26.2	86	95	0	0.45	-	>Chr06	53655280	>Chr02	5502950	>scaffold_2	5063928		
2	26	5027_165_179	26.2	84	95	2	0.68	-					>scaffold_2	5064244		
2	27	10653_185_49	26.8	85	96	0	0.67	-	>Chr02	5545771			>scaffold_2	5083226		
2	28	10261_160_211	28.5	84	97	0	0.93	-	>Chr02	21701370						
2	29	13969_179_194	28.5	80	96	5	1.45	-								
2	30	5203_136_266	28.5	84	97	0	0.93	-								
2	31	5937_166_25	30.7	84	97	0	0.93	-								
2	32	3290_182_26	30.7	83	96	2	0.94	-								
2	33	7134_169_23	31.3	85	96	0	0.67	-								
2	34	11599_162_14	31.3	85	96	0	0.67	-								
2	35	852_155_110	34.1	77	86	18	0.5	-								
2	36	61_155_174	35.2	82	94	5	0.82	-								
2	37	11637_160_170	35.2	84	96	1	0.8	-								
2	38	8953_165_7	35.2	74	84	23	0.63	-								
2	39	11935_159_82	36.8	86	93	2	0.27	-								
2	40	4269_136_40	36.8	81	81	19	0	-								
2	41	723_180_56	36.8	87	94	0	0.27	-								
2	42	9455_170_108	38.5	86	95	0	0.45	-								
2	43	5106_179_36	38.5	86	95	0	0.45	-								
2	44	13118_145_176	39.6	88	93	0	0.14	-								
2	45	4665_181_24	39.6	88	92	1	0.09	-								
2	46	2957_152_159	39.6	86	86	9	0	-								
2	47	1335_152_46	41.8	82	87	12	0.15	-								
2	48	6458_140_100	41.8	88	93	0	0.14	-	>Chr10	54716078			>scaffold_4	31885522		
2	49	3440_157_59	41.8	88	93	0	0.14	-								
2	50	5930_182_246	41.8	88	93	0	0.14	-								
2	51	11603_149_144	41.8	88	92	1	0.09	-	>Chr02	9362829			>scaffold_2	8619311		
2	52	3322_176_52	41.8	88	92	1	0.09	-	>Chr02	9458558	>Chr01	1984699	>scaffold_373	519	>scaffold_9	1289493
2	53	9330_170_119	41.8	87	89	5	0.02	-	>Chr02	9554503			>scaffold_2	8742232		
2	54	5771_151_190	41.8	88	92	1	0.09	-								
2	55	13350_138_43	42.9	88	92	1	0.09	-								
2	56	10131_152_209	44	88	93	0	0.14	-								
2	57	3882_184_173	44	88	88	5	0	-								
2	58	13846_157_159	44.6	89	92	0	0.05	-								
2	59	1112_163_81	45.2	84	82	15	0.02	-								
2	60	8558_168_117	45.8	89	92	0	0.05	-								
2	61	13368_160_83	45.8	88	91	2	0.05	-								
2	62	1384_181_71	45.8	89	92	0	0.05	-								
2	63	4_145_130	45.8	89	89	3	0	-								
2	64	6993_138_54	47.1	83	75	23	0.41	-								
2	65	1532_171_30	49.1	91	90	0	0.01	-	>Chr03	15675011						

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
2	66	8788_183_201	49.1	90	88	3	0.02	-								
2	67	7107_179_117	49.1	91	89	1	0.02	-					>scaffold_2	9659311		
2	68	12600_153_144	49.1	91	90	0	0.01	-								
2	69	12423_175_55	50.2	91	90	0	0.01	-								
2	70	10726_168_33	52.4	93	88	0	0.14	-	>Chr02	13023114	>Chr01	4674289	>scaffold_2	10112221		
2	71	13579_153_34	52.4	93	88	0	0.14	-	>Chr02	13023776			>scaffold_2	10112879		
2	72	11752_177_189	52.4	93	87	1	0.2	-								
2	73	14293_162_100	52.4	93	88	0	0.14	-					>scaffold_7	2046515	>scaffold_3	18548248
2	74	4439_157_254	53	91	89	1	0.02	-								
2	75	13365_151_207_R	55.2	92	88	1	0.09	-	>Chr02	13730762						
2	76	1680_163_172_R	55.8	93	88	0	0.14	-								
2	77	11016_158_216_R	57.5	85	87	9	0.02	-								
2	78	8578_148_6_R	57.5	90	91	0	0.01	-								
2	79	4212_164_108	59.7	90	91	0	0.01	-								
2	80	7912_161_82	59.7	90	90	1	0	-								
2	81	1778_165_58_R	59.7	84	84	13	0	-								
2	82	8617_174_53_R	59.7	90	91	0	0.01	-					>scaffold_2	15059791		
2	83	7943_175_49_R	60.3	91	90	0	0.01	-	>Chr04	58570945			>scaffold_2	15168852		
2	84	9180_158_144_R	60.3	91	90	0	0.01	-	>Chr02	18937108			>scaffold_2	15309846		
2	85	10381_155_14_R	60.9	92	89	0	0.05	-								
2	86	**W_3681_144_73_R**	60.9	91	89	1	0.02	-								
2	87	3359_158_121_R	60.9	92	89	0	0.05	-	>Chr07	35603007			>scaffold_4	30759214		
2	88	727_165_174_R	60.9	92	89	0	0.05	-	>Chr02	20842317			>scaffold_2	15588135		
2	89	5300_174_72_R	60.9	92	89	0	0.05	-					>scaffold_7	22216432		
2	90	3675_179_122_R	62	94	87	0	0.27	-								
2	91	9923_178_83	63.7	93	88	0	0.14	-								
2	92	5422_136_73	64.3	92	76	13	1.52	-								
2	93	10496_176_261	64.9	93	88	0	0.14	-	>Chr02	53906209						
2	94	1462_161_177	67.7	96	85	0	0.67	-								
2	95	523_183_117	70.5	93	88	0	0.14	-								
2	96	10474_170_52	71.6	95	86	0	0.45	-								
2	97	9868_162_172	74.4	94	87	0	0.27	-								
2	98	8677_179_80	77.1	91	87	3	0.09	-								
2	99	10157_160_261	77.8	92	87	2	0.14	-	>Chr02	58966846			>scaffold_2	30415074		
2	100	6006_146_91	77.8	85	82	14	0.05	-								
2	101	3687_158_129	85	87	94	0	0.27	-								
2	102	980_167_3	87.8	86	95	0	0.45	-	>Chr02	61080088						
2	103	3878_163_110	87.8	85	94	2	0.45	-								
2	104	10013_138_238	89.5	87	94	0	0.27	-								
2	105	8372_168_270	89.5	87	93	1	0.2	-								
2	106	8360_162_118	89.5	87	94	0	0.27	-								
2	107	12393_157_86	92.3	83	97	1	1.09	-								
2	108	15609_148_131	92.3	84	97	0	0.93	-								
2	109	5105_174_155	95.1	80	100	1	2.22	-								
2	110	**5015_180_80_R**	96.2	75	97	9	2.81	*								
2	111	8374_158_126_R	96.8	77	94	10	1.69	-	>Chr02	63296085			>scaffold_2	35292192	>scaffold_9	56861934
2	112	4653_180_103	97.9	81	99	1	1.8	-	>Chr02	63540016						
2	113	**7750_167_62_R**	99	69	95	17	4.12	**								
2	114	697_156_76_R	99	80	101	0	2.44	-	>Chr02	63884417			>scaffold_2	36259880		
2	115	783_161_129_R	99	80	101	0	2.44	-	>Chr02	63906636			>scaffold_2	36279255		
2	116	2401_185_176_R	99.6	77	94	10	1.69	-					>scaffold_2	36304305	>scaffold_6	33137019
2	117	10921_156_146_R	100.2	73	94	14	2.64	-								
2	118	**1293_156_89_R**	101.3	74	98	9	3.35	*								
2	119	**5142_183_252_R**	103	79	102	0	2.92	*	>Chr02	64866923						
2	120	711_182_131_R	104.1	81	100	0	1.99	-								
2	121	11304_170_58	104.1	81	100	0	1.99	-								
2	122	7630_151_12	104.1	81	100	0	1.99	-								
2	123	7857_140_30	104.1	81	100	0	1.99	-								
2	124	**W_253_138_21_R**	105.2	78	99	4	2.49	-					>scaffold_2	37892409		
2	125	65_181_150	105.2	81	100	0	1.99	-	>Chr02	65823242			>scaffold_2	38292602		
2	126	4158_164_14_R	106	69	87	25	2.08	-	>Chr02	66443651			>scaffold_2	38956643		
2	127	2018_181_92_R	106.8	80	100	1	2.22	-								
2	128	10045_168_165	106.8	80	97	4	1.63	-								
2	129	7967_164_132	106.8	80	101	0	2.44	-								
2	130	**9197_176_144_R**	107.4	71	94	16	3.21	*								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
2	131	3066_153_232_R	108	79	101	1	2.69	-	>Chr02	67129852			>scaffold_2	39722462		
2	132	8237_179_134_R	108	79	101	1	2.69	-	>Chr02	67300878						
2	133	6738_151_77_R	108	79	101	1	2.69	-	>Chr02	67561365	>Chr01	55594893	>scaffold_2	40208325	>scaffold_3	7258941
2	134	4790_169_230_R	108	80	101	0	2.44	-	>Chr02	67654907			>scaffold_1	39966222		
2	135	9201_152_220_R	108	80	101	0	2.44	-	>Chr02	67655304						
2	136	9836_185_157_R	108	79	100	2	2.46	-								
2	137	**9899_168_142_R**	108	77	101	3	3.24	*								
2	138	13445_175_21	109.5	74	89	18	1.38	-								
2	139	950_179_130	112.5	77	97	7	2.3	-								
2	140	**2812_160_68_R**	113.1	78	102	1	3.2	*								
2	141	**11403_160_76_R**	113.1	72	97	12	3.7	*								
2	142	**8940_176_176_R**	113.1	78	102	1	3.2	*	>Chr02	67883823			>scaffold_2	40569845		
2	143	8155_162_88_R	113.1	79	101	1	2.69	-	>Chr02	68268919			>scaffold_2	40985095		
2	144	**153_135_9_R**	113.1	79	102	0	2.92	*	>Chr06	59473077	>Chr02	68318543	>scaffold_2	41031612		
2	145	**5434_174_65_R**	113.1	79	102	0	2.92	*	>Chr08	235084			>scaffold_1	3942772		
2	146	11582_170_137_R	113.7	80	101	0	2.44	-	>Chr02	68613621			>scaffold_2	41330859		
2	147	12517_148_16_R	113.7	80	101	0	2.44	-	>Chr02	68788324			>scaffold_2	41514538		
2	148	7952_186_92_R	113.7	80	101	0	2.44	-	>Chr02	68841253			>scaffold_2	41585817		
2	149	**3774_154_147_R**	113.7	78	100	3	2.72	*								
2	150	1526_163_97_R	115.4	83	97	1	1.09	-								
2	151	3850_174_13_R	115.7	69	81	31	0.96	-								
2	152	6449_171_99	116	83	97	1	1.09	-								
2	153	788_176_162	116.6	83	98	0	1.24	-								
2	154	5617_147_207_R	122.1	78	96	7	1.86	-	>Chr07	62755780	>Chr02	63437380	>scaffold_2	43078620	>scaffold_6	32441986
2	155	11087_142_71	122.1	85	96	0	0.67	-								
2	156	975_188_110_R	122.7	83	96	2	0.94	-	>Chr02	70615313			>scaffold_2	43279042		
2	157	4239_167_217_R	122.7	84	97	0	0.93	-	>Chr02	70617801			>scaffold_2	43281551		
2	158	11014_159_143_R	122.7	84	97	0	0.93	-	>Chr02	70620969			>scaffold_2	43284696		
2	159	7989_150_109_R	122.7	84	97	0	0.93	-	>Chr02	70750032			>scaffold_2	43397554		
2	160	8690_153_81	126	88	93	0	0.14	-	>Chr02	71873663			>scaffold_2	44478225		
2	161	11351_171_117	129.3	82	99	0	1.6	-	>Chr02	72674677			>scaffold_2	45136088		
2	162	9174_187_12_R	130.4	84	97	0	0.93	-								
2	163	5197_180_163_R	130.4	83	97	1	1.09	-								
2	164	131_169_154_R	130.4	84	97	0	0.93	-	>Chr02	73197480			>scaffold_2	45602650		
2	165	5469_167_3_R	130.4	84	97	0	0.93	-	>Chr02	73221077			>scaffold_2	45641584		
2	166	12468_173_125_R	130.4	84	97	0	0.93	-	>Chr02	73349708			>scaffold_2	45787439		
2	167	15257_166_130	130.4	84	97	0	0.93	-	>Chr02	73446861			>scaffold_2	45865857		
2	168	138_173_127_R	130.4	84	97	0	0.93	-								
2	169	12524_151_17_R	131.5	84	97	0	0.93	-					>scaffold_2	44535194		
2	170	13530_182_155_R	132.1	83	98	0	1.24	-	>Chr02	73701424			>scaffold_2	46079822		
2	171	1930_167_115	133.2	81	100	0	1.99	-	>Chr02	74485505			>scaffold_2	46760478		
2	172	12152_168_256_R	133.2	81	99	1	1.8	-								
2	173	10389_172_183	133.2	81	100	0	1.99	-								
2	174	12908_179_97	133.2	81	100	0	1.99	-								
2	175	15668_164_269	133.2	81	100	0	1.99	-								
2	176	10951_135_54	133.2	81	100	0	1.99	-	>Chr02	74653407			>scaffold_2	46876667		
2	177	13149_136_171_R	133.8	80	101	0	2.44	-								
2	178	13883_175_19	134.5	72	91	18	2.21	-								
2	179	11203_171_122	137.5	82	92	7	0.57	-								
2	180	13595_166_171	139.4	79	97	5	1.84	-					>scaffold_5	42637022	>scaffold_2	47721814
2	181	1026_136_127	140	81	96	4	1.27	-								
2	182	9958_180_147_R	142.2	81	95	5	1.11	-								
2	183	13740_160_114_R	142.2	81	100	0	1.99	-					>scaffold_2	48877964		
2	184	15613_156_243	142.2	81	100	0	1.99	-	>Chr02	77543844			>scaffold_2	49084777		
2	185	4807_161_44_R	142.2	78	95	8	1.67	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
3	1	2870_133_109_R	0	86	94	1	0.36	-								
3	2	2276_150_72_R	0	86	95	0	0.45	-								
3	3	10123_173_98	0	85	95	1	0.56	-					>scaffold_5	47132974		
3	4	11058_132_236_R	0	86	95	0	0.45	-	>Chr03	73980778	>Chr09	322625	>scaffold_5	46984262	>scaffold_3	4430660
3	5	13273_165_249_R	0	86	94	1	0.36	-	>Chr03	73907548			>scaffold_7	22966715		
3	6	8084_170_103_R	0	86	94	1	0.36	-	>Chr03	73580090			>scaffold_5	46763062		
3	7	4967_136_229_R	0	86	95	0	0.45	-	>Chr03	73562136	>Chr09	59111302				
3	8	5157_163_179_R	0	86	95	0	0.45	-	>Chr03	73493670			>scaffold_5	46691796		
3	9	11621_142_64	0.6	85	96	0	0.67	-								
3	10	9476_172_215	0.6	85	95	1	0.56	-	>Chr03	73228812			>scaffold_5	46468427		
3	11	7726_161_47	4.5	85	94	2	0.45	-								
3	12	12576_145_261	8.9	82	81	18	0.01	-								
3	13	4397_155_24_R	9.5	88	89	4	0.01	-	>Chr03	70941383			>scaffold_5	44642593		
3	14	13336_158_1_R	9.5	88	90	3	0.02	-	>Chr03	70895041			>scaffold_5	44594777		
3	15	8016_176_172_R	10.1	76	87	18	0.74	-								
3	16	10612_156_258	10.1	88	93	0	0.14	-								
3	17	5232_155_175_R	10.8	73	83	25	0.64	-	>Chr03	70894184			>scaffold_5	44593776		
3	18	9626_138_64_R	12.3	88	93	0	0.14	-	>Chr03	70538970			>scaffold_5	44250675		
3	19	8864_165_183_R	12.3	87	93	1	0.2	-	>Chr03	70518667			>scaffold_5	44231141		
3	20	7340_145_66	13.4	90	90	1	0	-								
3	21	10178_175_122	16.7	88	93	0	0.14	-	>Chr03	70018729			>scaffold_5	43738529		
3	22	4340_164_186	16.7	87	93	1	0.2	-	>Chr03	69985150	>Chr10	13605714	>scaffold_5	43704160		
3	23	8042_167_202_R	16.7	88	93	0	0.14	-	>Chr03	69974613			>scaffold_5	43696939		
3	24	2763_165_66_R	17.3	78	90	13	0.86	-								
3	25	11488_177_153	17.9	86	95	0	0.45	-	>Chr03	69659023			>scaffold_5	43394699		
3	26	4057_166_13	17.9	86	95	0	0.45	-	>Chr03	69615258	>Chr09	49742894	>scaffold_5	43365014		
3	27	437_157_92	18.5	78	90	13	0.86	-								
3	28	8868_158_166_R	20.2	88	93	0	0.14	-	>Chr03	69423202	>Chr09	49936213	>scaffold_5	43244031	>scaffold_3	20053480
3	29	13763_136_8_R	20.2	88	92	1	0.09	-	>Chr03	69245532			>scaffold_5	43075147		
3	30	4237_172_128_R	20.2	88	92	1	0.09	-								
3	31	9165_186_1_R	20.2	88	93	0	0.14	-								
3	32	10361_174_39_R	22.4	88	93	0	0.14	-	>Chr03	67824196			>scaffold_9	9403294		
3	33	6704_163_55_R	22.4	88	93	0	0.14	-	>Chr03	67814366			>scaffold_9	9394422		
3	34	409_172_85_R	22.4	88	93	0	0.14	-	>Chr03	67677617			>scaffold_5	41668859	>scaffold_4	36533578
3	35	10719_186_204	24.6	86	94	1	0.36	-								
3	36	13108_170_165	26.3	85	96	0	0.67	-	>Chr03	66968282						
3	37	148_182_3	26.3	84	95	2	0.68	-								
3	38	811_166_168	27.8	77	88	16	0.73	-	>Chr03	66559262			>scaffold_5	40489123		
3	39	10404_184_7	28.5	85	96	0	0.67	-								
3	40	2067_177_81	28.5	83	94	4	0.68	-								
3	41	**11786_178_64**	31.8	79	102	0	2.92	*	>Chr03	65873428			>scaffold_5	39872466		
3	42	**313_151_82**	32.9	76	104	1	4.36	**	>Chr03	65747051			>scaffold_5	39744198		
3	43	1163_170_120	35.1	79	101	1	2.69	-	>Chr03	65131196						
3	44	**839_168_12**	37.3	79	102	0	2.92	*								
3	45	4204_178_136	37.9	80	101	0	2.44	-								
3	46	12929_170_78	38.5	80	100	1	2.22	-					>scaffold_1	41993102		
3	47	13150_143_83	38.5	81	100	0	1.99	-								
3	48	9250_171_256	38.5	81	100	0	1.99	-								
3	49	2885_148_145	38.5	81	89	11	0.38	-								
3	50	1323_170_196	38.5	81	100	0	1.99	-								
3	51	11894_151_62	38.5	81	99	1	1.8	-	>Chr03	64056743			>scaffold_5	38203136		
3	52	5907_183_84	38.5	81	100	0	1.99	-	>Chr03	63928067			>scaffold_5	38079936		
3	53	4364_155_21_R	38.5	81	99	1	1.8	-	>Chr03	63793749			>scaffold_5	37933789		
3	54	4941_181_154_R	38.5	81	100	0	1.99	-	>Chr03	63732222			>scaffold_5	37872661		
3	55	6343_172_142	38.5	81	100	0	1.99	-	>Chr03	63644164			>scaffold_5	37770377		
3	56	2002_178_208	38.5	81	100	0	1.99	-	>Chr03	63632998						
3	57	7110_180_267	38.5	81	99	1	1.8	-	>Chr06	61158961			>scaffold_3	708954		
3	58	10931_161_176	40.2	80	101	0	2.44	-	>Chr03	63562326			>scaffold_5	37670514		
3	59	13737_136_51	40.2	80	101	0	2.44	-								
3	60	**W_8127_142_20**	40.8	80	99	2	2.02	-								
3	61	**9906_147_78_R**	41.9	70	92	19	2.99	*								
3	62	11097_165_210_R	42.5	82	99	0	1.6	-	>Chr03	63081806			>scaffold_5	37169963		
3	63	**W_5735_149_140_R**	43.6	78	97	6	2.06	-								
3	64	8837_180_36_R	43.6	81	99	1	1.8	-								
3	65	**W_5998_168_136_R**	43.9	76	96	9	2.33	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
3	66	896_142_246_R	44.2	81	100	0	1.99	-	>Chr03	62983598						
3	67	794_162_106_R	44.2	79	99	3	2.25	-	>Chr03	62229692			>scaffold_5	36238662		
3	68	8887_142_181_R	44.2	81	100	0	1.99	-								
3	69	11252_176_227_R	44.2	81	99	1	1.8	-								
3	70	5389_184_50_R	45.3	81	95	5	1.11	-								
3	71	7999_171_218_R	45.9	82	95	4	0.95	-								
3	72	13899_142_199	46.5	82	98	1	1.42	-	>Chr03	61825203			>scaffold_5	35878898		
3	73	3899_181_255_R	49.8	79	102	0	2.92	*								
3	74	3492_136_105_R	50.4	63	94	24	6.12	**					>scaffold_5	35397298		
3	75	386_178_222	51.5	78	103	0	3.45	*								
3	76	12747_170_100_R	52.6	78	103	0	3.45	*								
3	77	**W_4947_144_99_R**	53.7	77	82	22	0.16	-								
3	78	10671_167_41	54.8	72	102	7	5.17	**								
3	79	1516_168_129_R	58.1	75	105	1	5	**								
3	80	2863_162_24	58.7	77	104	0	4.03	**	>Chr05	58986972			>scaffold_8	36776253		
3	81	5625_133_147	58.7	77	104	0	4.03	**					>scaffold_5	34118412		
3	82	3845_134_49_R	58.7	76	103	2	4.07	**								
3	83	11297_177_174	59.3	76	105	0	4.65	**	>Chr03	60151722	>Chr10	992153	>scaffold_7	19105550	>scaffold_5	33935715
3	84	10630_169_82_R	60.4	73	104	4	5.43	**								
3	85	5707_148_125_R	61.5	64	94	23	5.7	**								
3	86	8087_146_136_R	66.5	76	96	9	2.33	-								
3	87	**W_6771_142_166_R**	67.1	67	97	17	5.49	**								
3	88	13947_176_260_R	67.1	84	97	0	0.93	-	>Chr03	58335842			>scaffold_5	31373378	>scaffold_2	46307892
3	89	1765_164_11_R	67.7	85	96	0	0.67	-								
3	90	**W_3576_175_107_R**	67.7	84	92	5	0.36	-								
3	91	11355_183_94_R	69.9	83	98	0	1.24	-								
3	92	7331_187_131_R	69.9	83	98	0	1.24	-								
3	93	955_135_34_R	69.9	83	98	0	1.24	-								
3	94	13413_179_89_R	71	85	96	0	0.67	-								
3	95	13631_149_154_R	72.7	84	97	0	0.93	-					>scaffold_8	33611448		
3	96	6018_164_193_R	72.7	70	87	24	1.84	-	>Chr03	55703347			>scaffold_5	28920731		
3	97	14163_159_23_R	72.7	84	97	0	0.93	-	>Chr03	55488048			>scaffold_5	28504373		
3	98	954_173_48_R	73.3	83	98	0	1.24	-					>scaffold_5	28214722		
3	99	11271_167_154_R	73.9	74	95	12	2.61	-								
3	100	6880_184_89_R	75.6	83	97	1	1.09	-								
3	101	134_179_65_R	78.4	80	97	4	1.63	-								
3	102	7361_170_207_R	80.1	81	100	0	1.99	-								
3	103	8125_184_25_R	80.1	81	100	0	1.99	-								
3	104	3185_171_185_R	81.2	81	100	0	1.99	-	>Chr03	52803146			>scaffold_5	31851006		
3	105	10615_173_96_R	81.8	78	101	2	2.96	*	>Chr03	52667010			>scaffold_5	31916594		
3	106	5042_144_61_R	81.8	66	84	31	2.16	-					>scaffold_5	32059275		
3	107	6202_175_148_R	84.6	81	100	0	1.99	-								
3	108	7836_184_260_R	84.6	81	100	0	1.99	-	>Chr03	50580940	>Chr09	59173874	>scaffold_5	25348811	>scaffold_3	8122108
3	109	4060_171_162	86.3	84	65	32	2.42	-								
3	110	454_143_57_R	86.3	83	97	1	1.09	-								
3	111	15456_158_141_R	86.3	84	97	0	0.93	-	>Chr02	54632904						
3	112	13389_143_27	86.3	84	97	0	0.93	-	>Chr03	18496528			>scaffold_5	15742604		
3	113	5778_164_191_R	86.3	84	97	0	0.93	-	>Chr07	59490528			>scaffold_6	35423851		
3	114	6832_154_137	86.9	85	96	0	0.67	-	>Chr03	17592670			>scaffold_5	15318869		
3	115	3093_171_54	86.9	85	96	0	0.67	-	>Chr03	16899709						
3	116	1525_167_252	87.5	86	95	0	0.45	-								
3	117	9216_155_217_R	93	80	101	0	2.44	-	>Chr03	14364327			>scaffold_5	2696812		
3	118	9389_166_34_R	93.6	81	100	0	1.99	-								
3	119	**8608_144_42_R**	98.6	78	103	0	3.45	*								
3	120	**7710_181_148_R**	99.2	77	104	0	4.03	**								
3	121	**8781_139_191_R**	100.3	77	100	4	2.99	*								
3	122	**7953_159_49_R**	100.9	78	103	0	3.45	*								
3	123	**W_603_180_112_R**	102.6	76	104	1	4.36	**								
3	124	**9845_171_106_R**	102.6	72	99	10	4.26	**	>Chr02	72317499			>scaffold_3	31522031		
3	125	**4153_138_11_R**	103.2	76	105	0	4.65	**								
3	126	**10215_159_113_R**	108.7	71	101	9	5.23	**								
3	127	**12668_182_202_R**	108.7	78	103	0	3.45	*	>Chr03	8504957			>scaffold_5	6760004		
3	128	**9341_157_167_R**	108.7	78	103	0	3.45	*								
3	129	**6222_176_183_R**	110.4	75	106	0	5.31	**								
3	130	**4401_162_177_R**	112.6	75	106	0	5.31	**					>scaffold_6	25046155		

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
3	131	**5550_177_74_R**	116.5	76	105	0	4.65	**								
3	132	**6452_181_2_R**	118.7	78	103	0	3.45	*								
3	133	**10508_161_116_R**	119.4	68	102	11	6.8	***	>Chr03	6039576			>scaffold_5	8817569		
3	134	**10638_179_72_R**	120.8	75	101	5	3.84	*								
3	135	3613_170_96_R	122.5	81	100	0	1.99	-								
3	136	13058_140_208_R	122.5	81	100	0	1.99	-								
3	137	9735_161_37_R	122.5	80	99	2	2.02	-	>Chr03	4895483						
3	138	2703_148_42_R	122.5	80	100	1	2.22	-					>scaffold_5	9897252		
3	139	3753_182_30_R	122.5	81	100	0	1.99	-	>Chr03	4769937			>scaffold_5	10020074		
3	140	13903_162_34_R	122.5	81	100	0	1.99	-	>Chr03	4769192			>scaffold_5	10020934		
3	141	9817_158_70_R	122.5	81	100	0	1.99	-								
3	142	10121_179_92_R	123.6	79	99	3	2.25	-								
3	143	9598_167_95_R	124.7	80	99	2	2.02	-	>Chr03	4315119	>Chr01	9130342	>scaffold_5	10424995		
3	144	4894_159_31_R	124.7	81	100	0	1.99	-					>scaffold_5	10467023		
3	145	6476_175_154	131.9	80	79	22	0.01	-								
3	146	9973_139_198	131.9	85	93	3	0.36	-								
3	147	6093_170_140	131.9	88	93	0	0.14	-								
3	148	10466_178_243	131.9	87	93	1	0.2	-								
3	149	15626_176_95	131.9	88	93	0	0.14	-	>Chr03	2492278			>scaffold_5	12466270		
3	150	6523_180_23	131.9	88	93	0	0.14	-	>Chr03	2450851			>scaffold_5	12546806		
3	151	7254_184_226	133.6	89	92	0	0.05	-	>Chr03	1992441			>scaffold_5	13089871		
3	152	8968_167_269	134.7	91	89	1	0.02	-	>Chr03	1812998						
3	153	9878_170_122	134.7	91	90	0	0.01	-								
3	154	1210_168_10_R	134.7	86	86	9	0	-	>Chr02	7084963	>Chr01	3675851				
3	155	4483_152_96_R	135.7	91	87	3	0.09	-								
3	156	1657_148_81_R	135.7	77	78	26	0.01	-								
3	157	1240_150_159	135.7	91	89	1	0.02	-								
3	158	12971_167_77	135.7	90	90	1	0	-	>Chr03	1467743						
3	159	5691_168_69	135.7	89	81	11	0.38	-	>Chr03	1356835						
3	160	10595_174_123	136.8	90	86	5	0.09	-	>Chr09	3848528	>Chr05	9914505	>scaffold_5	614461		
3	161	3996_168_151	136.8	91	90	0	0.01	-	>Chr03	776901			>scaffold_5	631036	>scaffold_4	24815634
3	162	6681_160_193	137.9	90	90	1	0	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
4	1	9432_135_187	0	92	89	0	0.05	-	>Chr04	229793	>Chr10	549705	>scaffold_1	212191	>scaffold_4	430753
4	2	3754_173_125	2.8	89	90	2	0.01	-								
4	3	13741_145_261	5	87	94	0	0.27	-					>scaffold_1	8901998		
4	4	13289_152_86_R	6.7	83	93	5	0.57	-								
4	5	4003_134_90	8.9	92	85	4	0.28	-								
4	6	10003_135_119_R	16	75	82	24	0.31	-								
4	7	8679_169_131_R	17.3	90	89	2	0.01	-								
4	8	**W_2490_180_90**	17.9	89	88	4	0.01	-								
4	9	271_181_107_R	18.5	91	89	1	0.02	-	>Chr04	4844731			>scaffold_3	24955692	>scaffold_1	6321801
4	10	3547_181_131_R	18.5	88	90	3	0.02	-								
4	11	9300_135_201	22.9	91	88	2	0.05	-	>Chr04	5316023			>scaffold_1	5917433		
4	12	10108_134_240_R	26.8	96	85	0	0.67	-								
4	13	11069_167_58_R	26.8	81	77	23	0.1	-								
4	14	13958_142_17_R	30.7	91	86	4	0.14	-								
4	15	13462_147_141_R	36.3	95	86	0	0.45	-								
4	16	10657_171_34_R	36.9	95	85	1	0.56	-								
4	17	5992_186_156_R	36.9	94	83	4	0.68	-								
4	18	3088_171_146_R	38.6	62	77	42	1.62	-								
4	19	12477_172_7_R	42.5	98	83	0	1.24	-								
4	20	1892_173_37_R	42.5	98	83	0	1.24	-	>Chr04	7610329			>scaffold_1	3857439		
4	21	12515_177_111	43.6	97	83	1	1.09	-								
4	22	10437_180_7_R	44.7	98	83	0	1.24	-	>Chr04	7942308			>scaffold_1	3140687		
4	23	**W_11376_135_262**	45.3	97	82	2	1.26	-								
4	24	57_168_206_R	45.3	97	79	5	1.84	-								
4	25	13429_168_157	45.9	98	83	0	1.24	-	>Chr04	8314751	>Chr10	50028066	>scaffold_1	3414184	>scaffold_4	36147255
4	26	4921_135_101_R	46.5	95	84	2	0.68	-								
4	27	1717_159_120_R	47.1	98	83	0	1.24	-								
4	28	**W_11379_157_59_R**	48.2	90	85	6	0.14	-	>Chr04	8666813			>scaffold_1	2900354		
4	29	8966_167_41_R	49.9	92	84	5	0.36	-	>Chr04	9733247			>scaffold_1	2134889		
4	30	2928_183_252	52.1	97	84	0	0.93	-								
4	31	422_145_55_R	52.7	88	75	18	1.04	-								
4	32	**12059_168_145**	55.5	89	51	41	10.31	****	>Chr09	54254862			>scaffold_1	1251307	>scaffold_3	14035955
4	33	10571_181_173	57.2	100	81	0	1.99	-								
4	34	7537_173_207	57.2	100	81	0	1.99	-								
4	35	11397_138_37	57.2	99	80	2	2.02	-								
4	36	7349_158_51	59.1	93	77	11	1.51	-								
4	37	10741_144_77_R	60.1	81	80	20	0.01	-								
4	38	3619_132_27_R	62.2	99	82	0	1.6	-								
4	39	10396_163_232_R	62.2	99	82	0	1.6	-								
4	40	**W_2099_161_51_R**	64.4	82	81	18	0.01	-	>Chr10	6073952						
4	41	11074_177_22	67.2	96	82	3	1.1	-	>Chr03	5326771						
4	42	12504_181_237	67.2	96	85	0	0.67	-	>Chr04	16092928	>Chr01	9668395	>scaffold_1	11543768		
4	43	3956_166_242	67.2	96	85	0	0.67	-	>Chr04	16094269	>Chr01	9669493	>scaffold_1	11542768	>scaffold_9	7834101
4	44	12777_155_55	67.2	96	85	0	0.67	-								
4	45	12808_159_186_R	67.2	95	84	2	0.68	-								
4	46	11677_178_160	67.2	96	84	1	0.8	-	>Chr08	51598088						
4	47	**5650_168_84**	68.3	95	69	17	4.12	**								
4	48	13288_159_15	68.9	95	86	0	0.45	-					>scaffold_9	51146363	>scaffold_7	33643837
4	49	6633_176_145	68.9	95	81	5	1.11	-								
4	50	12123_177_169	68.9	95	86	0	0.45	-								
4	51	694_182_217_R	68.9	95	86	0	0.45	-	>Chr04	44951973			>scaffold_1	22821428		
4	52	2594_151_49_R	68.9	94	85	2	0.45	-	>Chr04	45087187			>scaffold_1	22858580		
4	53	9345_160_174_R	68.9	95	86	0	0.45	-								
4	54	10037_176_192_R	68.9	91	86	4	0.14	-								
4	55	249_153_156_R	70	95	86	0	0.45	-	>Chr04	48184424			>scaffold_1	24024153		
4	56	6442_141_117	70	94	85	2	0.45	-								
4	57	7618_180_189_R	71.7	89	81	11	0.38	-								
4	58	2929_138_55_R	72.8	88	87	6	0.01	-								
4	59	10316_169_85	74.5	95	84	2	0.68	-								
4	60	6162_173_247_R	75.1	94	87	0	0.27	-	>Chr06	5291719			>scaffold_1	325584		
4	61	1135_186_62	75.1	94	86	1	0.36	-								
4	62	9362_166_97_R	75.7	93	87	1	0.2	-								
4	63	13565_145_125_R	79.3	91	89	1	0.02	-	>Chr05	58839136						
4	64	6881_153_179_R	83.5	78	73	30	0.17	-								
4	65	5397_171_258_R	83.5	93	88	0	0.14	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
4	66	7924_161_91_R	85.2	91	89	1	0.02	-								
4	67	12086_158_37	86.5	70	90	21	2.5	-								
4	68	11795_136_8_R	88.5	90	89	2	0.01	-					>scaffold_4	601127		
4	69	10819_142_193	91.3	91	90	0	0.01	-								
4	70	4353_167_225	95.2	92	89	0	0.05	-								
4	71	1600_137_84_R	95.2	90	86	5	0.09	-								
4	72	4762_160_92	95.2	92	89	0	0.05	-	>Chr04	53323554			>scaffold_1	28383191		
4	73	8648_165_145	97.4	94	87	0	0.27	-								
4	74	5666_167_174_R	97.4	94	87	0	0.27	-	>Chr04	53653885			>scaffold_1	28831103		
4	75	3092_170_151_R	98	95	86	0	0.45	-	>Chr04	53839416			>scaffold_1	29017403		
4	76	724_169_181	98	95	86	0	0.45	-								
4	77	9144_172_181	98.6	93	87	1	0.2	-	>Chr04	53863365	>Chr09	58628268	>scaffold_1	29039601	>scaffold_3	8539901
4	78	12750_167_103	99.7	94	87	0	0.27	-	>Chr03	73865257						
4	79	8045_173_96	100.8	88	84	9	0.09	-	>Chr09	3848528	>Chr05	9914516	>scaffold_5	614460		
4	80	12322_177_109	101.4	93	88	0	0.14	-	>Chr04	54921321			>scaffold_1	30026089		
4	81	10587_137_102_R	101.4	93	88	0	0.14	-	>Chr04	55056846			>scaffold_1	30193263		
4	82	9816_153_256_R	101.4	93	88	0	0.14	-								
4	83	10541_169_66	101.4	88	80	13	0.38	-								
4	84	10688_133_170_R	102.5	92	88	1	0.09	-	>Chr02	72137974			>scaffold_1	30819331	>scaffold_7	24309781
4	85	3799_173_109	103.6	95	86	0	0.45	-	>Chr04	55827846			>scaffold_1	31401432		
4	86	542_154_141	103.6	95	86	0	0.45	-	>Chr04	56052414			>scaffold_1	31552854		
4	87	4389_159_125	103.6	95	86	0	0.45	-	>Chr04	56086053			>scaffold_1	31573165		
4	88	13898_162_90	103.6	95	86	0	0.45	-	>Chr04	56087807			>scaffold_1	31574720		
4	89	5236_184_178	106.9	90	88	3	0.02	-	>Chr07	255602						
4	90	5098_133_62	108.6	90	87	4	0.05	-								
4	91	7660_179_16	108.6	92	89	0	0.05	-								
4	92	**W_2804_142_30**	114.7	95	79	7	1.47	-	>Chr04	62415639			>scaffold_1	33763052		
4	93	7525_180_112	114.7	96	82	3	1.1	-	>Chr04	62404524			>scaffold_1	33769849	>scaffold_6	19472548
4	94	607_169_209	116.9	101	80	0	2.44	-								
4	95	**6370_162_4**	118.6	102	79	0	2.92	*								
4	96	**8810_174_2**	118.6	102	78	1	3.2	*	>Chr04	61609743			>scaffold_1	34708262		
4	97	**11910_180_194**	119.2	103	78	0	3.45	*	>Chr04	61557932			>scaffold_1	34764138	>scaffold_6	666639
4	98	**6628_154_20**	122	104	77	0	4.03	**								
4	99	**2961_157_135**	122	103	77	1	3.76	*					>scaffold_1	35673015		
4	100	**5214_146_35**	122	104	77	0	4.03	**	>Chr04	60511804			>scaffold_1	35831101		
4	101	**5167_178_110**	122.6	105	74	2	5.37	**								
4	102	**2363_170_123**	122.6	105	76	0	4.65	**								
4	103	**13583_150_161**	122.6	105	76	0	4.65	**	>Chr04	60404858			>scaffold_1	35927176		
4	104	**985_176_138**	122.6	105	76	0	4.65	**	>Chr04	60404479			>scaffold_1	35927511	>scaffold_2	37797512
4	105	**6444_181_42**	122.6	98	68	15	5.42	**	>Chr04	60321756			>scaffold_1	36030508		
4	106	**11669_160_8**	122.6	105	76	0	4.65	**	>Chr04	60320052			>scaffold_1	36032701		
4	107	**10601_141_262**	123.2	104	77	0	4.03	**	>Chr04	60127735			>scaffold_1	36178157		
4	108	**9608_183_85**	123.2	104	75	2	4.7	**								
4	109	**10551_173_172**	123.8	103	78	0	3.45	*	>Chr04	60009757	>Chr10	19422037	>scaffold_1	36281440	>scaffold_4	16477378
4	110	**4455_183_93**	123.8	103	78	0	3.45	*	>Chr04	60000052			>scaffold_1	36288923		
4	111	**9086_132_183**	123.8	98	71	12	4.31	**								
4	112	**2745_158_82**	124.9	98	71	12	4.31	**								
4	113	9002_184_1	126	101	79	1	2.69	-	>Chr04	59538456						
4	114	64_172_75	126	101	80	0	2.44	-								
4	115	2904_163_61_R	131.5	101	80	0	2.44	-	>Chr04	65567814						
4	116	10332_135_153_R	134.6	99	80	2	2.02	-								
4	117	1062_181_184_R	136.5	98	80	3	1.82	-	>Chr04	8302868			>scaffold_1	39247355	>scaffold_4	39572536
4	118	8213_181_63_R	137.6	87	78	16	0.49	-								
4	119	12775_175_67_R	137.6	98	83	0	1.24	-	>Chr04	64670006			>scaffold_1	39641923		
4	120	1859_141_135_R	137.6	98	83	0	1.24	-	>Chr04	64699515			>scaffold_1	39663500		
4	121	10200_183_223_R	138.2	97	84	0	0.93	-								
4	122	10530_155_66_R	138.8	96	81	4	1.27	-	>Chr04	65473441						
4	123	7116_137_134_R	141	92	81	8	0.7	-								
4	124	11897_137_87_R	142.1	94	83	4	0.68	-					>scaffold_1	40818667	>scaffold_8	23361063
4	125	11128_169_113_R	143.8	95	86	0	0.45	-								
4	126	13758_165_186_R	144.4	95	85	1	0.56	-								
4	127	12113_151_131_R	145	94	81	6	0.97	-								
4	128	9053_171_265_R	145	96	84	1	0.8	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
5	1	16239_151_42_R	0	76	88	17	0.88	-	>Chr05	31885536						
5	2	10940_156_260	1.8	96	85	0	0.67	-								
5	3	7962_168_267	1.8	96	85	0	0.67	-								
5	4	920_133_104	3.5	94	79	8	1.3	-								
5	5	7714_177_185_R	4.1	91	85	5	0.2	-								
5	6	7898_157_268	5.8	91	90	0	0.01	-								
5	7	12598_155_59_R	5.8	90	90	1	0	-	>Chr05	294154	>Chr08	334096				
5	8	**W_9863_168_163**	7.2	87	74	20	1.05	-	>Chr05	2446607	>Chr07	11845670				
5	9	5089_173_1	10.8	96	84	1	0.8	-								
5	10	14345_149_216_R	11.4	97	84	0	0.93	-	>Chr08	1329561			>scaffold_7	34763112	>scaffold_8	1208824
5	11	3471_143_126	12	96	76	9	2.33	-								
5	12	11334_154_86	14.8	95	86	0	0.45	-	>Chr08	3116974	>Chr05	3127753	>scaffold_8	2025563	>scaffold_7	35119056
5	13	8954_184_232_R	15.4	69	75	37	0.25	-								
5	14	7613_149_132_R	15.4	96	85	0	0.67	-								
5	15	13709_146_153_R	20.4	91	89	1	0.02	-	>Chr04	57564238	>Chr10	7783339	>scaffold_1	38284037	>scaffold_4	7720469
5	16	12584_146_191_R	20.4	88	89	4	0.01	-					>scaffold_8	2484874		
5	17	1658_162_198	20.4	91	90	0	0.01	-								
5	18	6816_135_149_R	23.2	94	87	0	0.27	-								
5	19	**W_3438_178_64_R**	23.2	93	87	1	0.2	-								
5	20	**W_7752_168_87_R**	24.3	93	87	1	0.2	-								
5	21	15860_138_170_R	24.3	94	87	0	0.27	-								
5	22	**W_8672_168_64**	25.4	94	85	2	0.45	-								
5	23	11341_154_20	25.4	96	85	0	0.67	-								
5	24	7186_171_55	26	97	84	0	0.93	-								
5	25	3069_175_138_R	26.6	98	83	0	1.24	-								
5	26	1341_175_107_R	33.8	88	85	8	0.05	-								
5	27	8863_151_253	36.6	100	81	0	1.99	-								
5	28	2922_143_25	36.6	96	78	7	1.86	-								
5	29	8210_146_150	36.6	87	68	26	2.33	-	>Chr05	6787827	>Chr08	5616159	>scaffold_8	5063315		
5	30	1273_173_268	36.6	100	81	0	1.99	-								
5	31	8083_173_164	37.2	99	82	0	1.6	-	>super_10	393937			>scaffold_8	14744425		
5	32	1960_147_51_R	42.7	93	81	7	0.83	-								
5	33	13896_166_118	48.7	96	83	2	0.94	-	>Chr08	1170720			>scaffold_6	26346579		
5	34	3592_178_25	51.6	95	81	5	1.11	-								
5	35	13892_146_108	51.6	96	84	1	0.8	-								
5	36	13540_144_56	52.7	96	84	1	0.8	-					>scaffold_7	7098161		
5	37	11391_170_197_R	55.5	94	86	1	0.36	-								
5	38	1842_165_13_R	55.5	88	84	9	0.09	-					>scaffold_9	17293522		
5	39	10120_170_29_R	55.5	94	85	2	0.45	-								
5	40	**W_10427_146_239_R**	56.6	91	86	4	0.14	-								
5	41	7909_161_15	56.6	93	81	7	0.83	-								
5	42	10382_149_192	56.6	92	84	5	0.36	-								
5	43	2913_169_59	58.2	95	83	3	0.81	-								
5	44	1669_160_179	58.8	84	80	17	0.1	-								
5	45	2444_140_119	59.3	93	88	0	0.14	-								
5	46	**624_186_16**	59.9	94	64	23	5.7	**								
5	47	3650_177_133_R	60.5	93	88	0	0.14	-	>Chr07	21772109						
5	48	9802_177_180_R	60.8	88	83	10	0.15	-								
5	49	3403_178_14_R	61.1	86	85	10	0.01	-								
5	50	5414_167_70_R	61.1	85	86	10	0.01	-	>Chr05	31700972	>Chr08	50195731				
5	51	3099_154_40_R	62.2	72	84	25	0.92	-								
5	52	8357_158_64	63.3	86	80	15	0.22	-								
5	53	391_145_155_R	63.3	92	89	0	0.05	-								
5	54	10280_164_79	63.3	91	86	4	0.14	-	>Chr05	45650313	>Chr03	72023085	>scaffold_8	25591511	>scaffold_5	45518975
5	55	8023_179_166	63.3	92	88	1	0.09	-					>scaffold_1	16649429		
5	56	10164_179_159	63.3	92	88	1	0.09	-								
5	57	2797_137_54_R	63.4	78	82	21	0.1	-								
5	58	6342_145_124	64	79	71	31	0.43	-								
5	59	6941_173_209	68.3	91	90	0	0.01	-								
5	60	11368_175_3_R	68.3	90	89	2	0.01	-								
5	61	3575_165_73	69.4	89	91	1	0.02	-								
5	62	7645_184_206	69.4	88	90	3	0.02	-								
5	63	9123_165_158	72.2	88	92	1	0.09	-								
5	64	1715_186_107	72.2	88	93	0	0.14	-								
5	65	8773_170_200	80	94	85	2	0.45	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
5	66	**W_1392_144_63**	80	90	79	12	0.72	-								
5	67	3923_170_112	80.6	97	84	0	0.93	-								
5	68	4083_188_149	80.6	97	84	0	0.93	-								
5	69	8463_162_178	80.6	97	84	0	0.93	-								
5	70	10778_180_113	80.6	97	82	2	1.26	-								
5	71	**W_1024_145_17**	82.7	83	71	27	0.94	-								
5	72	172_156_104_R	83.4	96	83	2	0.94	-								
5	73	12940_171_150_R	84	95	86	0	0.45	-	>Chr05	54269161			>scaffold_8	30158503		
5	74	5588_161_188_R	84	94	86	1	0.36	-	>Chr05	54307507						
5	75	7251_187_79_R	84	95	86	0	0.45	-								
5	76	13696_153_189_R	85.3	79	81	21	0.02	-								
5	77	3776_171_59	88.5	95	85	1	0.56	-								
5	78	12654_156_46	90.2	92	89	0	0.05	-	>Chr05	55642750			>scaffold_8	31729976		
5	79	7023_183_52	91.9	91	89	1	0.02	-								
5	80	8586_185_21_R	91.9	87	87	7	0	-								
5	81	3077_171_94_R	91.9	91	89	1	0.02	-								
5	82	1379_161_84_R	92.5	87	78	16	0.49	-								
5	83	11875_162_79	93.1	91	90	0	0.01	-								
5	84	12466_176_76_R	94.2	91	89	1	0.02	-								
5	85	8316_177_134_R	94.2	88	85	8	0.05	-								
5	86	1613_174_191_R	96.4	93	88	0	0.14	-								
5	87	7433_138_94	97.5	89	90	2	0.01	-	>Chr05	56855489			>scaffold_8	32997541		
5	88	**W_3520_167_65**	97.5	91	88	2	0.05	-								
5	89	9054_161_122	100.8	92	80	9	0.84	-								
5	90	5322_168_37	101.9	93	88	0	0.14	-								
5	91	14421_142_95	101.9	93	88	0	0.14	-								
5	92	4490_164_90	101.9	93	88	0	0.14	-								
5	93	7291_165_119	101.9	91	83	7	0.37	-								
5	94	4430_175_110_R	104.7	88	91	2	0.05	-								
5	95	6486_172_135_R	105.8	80	80	21	0	-								
5	96	**W_15464_138_2**	107.5	90	87	4	0.05	-								
5	97	4395_178_10	108.6	90	85	6	0.14	-								
5	98	675_175_169_R	108.6	91	90	0	0.01	-	>Chr05	59366428			>scaffold_8	37355652		
5	99	**W_13981_164_162_R**	110.8	76	87	18	0.74	-								
5	100	11147_178_130_R	110.8	76	88	17	0.88	-								
5	101	4624_179_195	111.4	92	89	0	0.05	-								
5	102	10616_181_151_R	111.4	92	85	4	0.28	-								
5	103	**W_7524_167_134_R**	112	84	81	16	0.05	-								
5	104	**W_7306_139_103**	114.1	89	88	4	0.01	-								
5	105	85_157_33_R	114.8	87	91	3	0.09	-								
5	106	4730_183_173_R	115.9	90	91	0	0.01	-								
5	107	556_145_259_R	117.6	89	92	0	0.05	-								
5	108	10756_137_143	120.8	85	86	10	0.01	-	>Chr05	60591168			>scaffold_8	39013359	>scaffold_3	48996469
5	109	6220_183_124	121.4	88	91	2	0.05	-								
5	110	1628_143_245	121.4	87	90	4	0.05	-					>scaffold_1	6848574		
5	111	**W_9296_159_247**	121.4	90	90	1	0	-								
5	112	11835_171_84	122.5	90	91	0	0.01	-								
5	113	10739_177_71	124.4	90	72	19	2	-								

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
6	1	8377_160_211	0	79	94	8	1.3	-								
6	2	6609_179_103	0	82	98	1	1.42	-								
6	3	5517_172_26	3.8	70	85	26	1.45	-	>Chr06	1215965			>scaffold_4	33794423		
6	4	5657_172_171_R	6.1	84	94	3	0.56	-	>Chr06	1271948			>scaffold_7	2048149		
6	5	7240_177_159_R	7.3	87	94	0	0.27	-								
6	6	**W_3214_165_142**	7.3	85	94	2	0.45	-								
6	7	5031_163_178	7.3	87	94	0	0.27	-					>scaffold_7	2157683		
6	8	3271_158_52_R	7.3	74	84	23	0.63	-								
6	9	3854_137_70	11.5	75	67	39	0.45	-								
6	10	12915_186_156_R	14.5	90	89	2	0.01	-								
6	11	176_150_45	14.5	90	91	0	0.01	-	>Chr09	4019500			>scaffold_7	2707648		
6	12	4085_132_115	16.2	91	90	0	0.01	-	>Chr06	1750335			>scaffold_7	2939918		
6	13	15753_139_69	16.2	91	90	0	0.01	-	>Chr06	1750859			>scaffold_7	2940341		

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
6	14	215_168_179_R	16.2	91	90	0	0.01	-	>Chr10	5750285	>Chr08	43611125	>scaffold_9	33119874		
6	15	11015_177_39	16.2	91	90	0	0.01	-	>Chr05	2979048	>Chr07	51425132	>scaffold_8	1735187	>scaffold_7	35261137
6	16	2296_177_99_R	16.8	89	91	1	0.02	-								
6	17	5275_167_14_R	23.5	90	91	0	0.01	-					>scaffold_7	3632885		
6	18	10843_157_100	23.5	90	91	0	0.01	-								
6	19	9153_172_192	24.1	89	89	3	0	-	>Chr04	3694908			>scaffold_1	7588454	>scaffold_24	2311
6	20	13183_153_71	25.2	88	92	1	0.09	-								
6	21	4104_163_202	25.2	89	91	1	0.02	-								
6	22	2440_161_6	25.2	79	90	12	0.72	-								
6	23	11116_165_4	25.2	87	92	2	0.14	-								
6	24	**W_10476_158_96**	25.8	77	81	23	0.1	-								
6	25	9781_147_116_R	28.2	77	86	18	0.5	-								
6	26	10091_143_10	30.1	83	64	34	2.46	-								
6	27	11027_164_123	31.8	82	64	35	2.22	-								
6	28	9705_169_110	32.1	89	86	6	0.05	-								
6	29	3631_181_154	32.3	89	91	1	0.02	-								
6	30	2043_179_228	32.3	90	91	0	0.01	-	>Chr06	5768364			>scaffold_7	10114197		
6	31	4300_161_242	32.3	89	90	2	0.01	-	>Chr06	8941368			>scaffold_7	6308733	>scaffold_1	19862178
6	32	3063_183_28_R	32.3	89	90	2	0.01	-								
6	33	9578_162_220	32.3	89	89	3	0	-								
6	34	11076_169_140	32.3	88	86	7	0.02	-								
6	35	10393_176_141	32.3	90	91	0	0.01	-								
6	36	2255_179_70	32.3	89	90	2	0.01	-								
6	37	177_158_28	32.3	90	91	0	0.01	-								
6	38	8651_150_114	33.1	82	73	26	0.52	-								
6	39	7815_155_65	34.2	82	71	28	0.79	-								
6	40	8915_150_29	36.1	88	68	25	2.56	-								
6	41	7126_137_60	36.8	90	91	0	0.01	-								
6	42	897_135_109_R	36.8	90	91	0	0.01	-								
6	43	2529_163_94_R	37.4	91	90	0	0.01	-	>Chr09	1472436			>scaffold_3	4011394		
6	44	7644_165_14_R	37.4	80	86	15	0.22	-								
6	45	11513_173_78	42.4	81	95	5	1.11	-	>Chr04	4709242			>scaffold_1	6435285		
6	46	13957_173_99	43.5	86	88	7	0.02	-								
6	47	433_166_67	44.1	81	93	7	0.83	-								
6	48	**W_8629_177_163_R**	45.2	86	94	1	0.36	-								
6	49	8641_174_102	45.2	84	91	6	0.28	-								
6	50	13069_182_64_R	45.2	87	93	1	0.2	-	>Chr06	37670143						
6	51	13106_153_143_R	47	82	95	4	0.95	-								
6	52	2539_176_166_R	51.3	85	93	3	0.36	-								
6	53	15603_140_40	52.4	90	91	0	0.01	-								
6	54	238_134_116_R	54.2	89	90	2	0.01	-	>Chr06	42304794			>scaffold_7	19036192		
6	55	3879_168_5_R	61.4	81	84	16	0.05	-	>Chr06	44591842			>scaffold_7	19664195		
6	56	10873_137_76_R	63.4	73	76	32	0.06	-								
6	57	9363_159_10_R	65.2	88	92	1	0.09	-	>Chr06	45792322			>scaffold_7	20130339		
6	58	**W_8786_176_102_R**	65.2	88	90	3	0.02	-	>Chr06	45922790			>scaffold_7	20220235		
6	59	9948_162_102_R	65.2	85	92	4	0.28	-								
6	60	8482_182_125_R	68	92	89	0	0.05	-								
6	61	132_165_123_R	69.1	94	86	1	0.36	-								
6	62	10950_156_59_R	69.1	94	87	0	0.27	-	>Chr06	47674520			>scaffold_7	21448156		
6	63	2562_184_21_R	70.2	94	87	0	0.27	-					>scaffold_7	21755186		
6	64	3442_181_195_R	70.2	90	85	6	0.14	-								
6	65	9779_167_170_R	70.2	92	87	2	0.14	-								
6	66	8701_158_190_R	70.2	88	82	11	0.21	-	>Chr06	48729836			>scaffold_7	22106489		
6	67	9036_147_79_R	70.2	93	87	1	0.2	-								
6	68	1048_156_246_R	70.8	79	86	16	0.3	-								
6	69	6636_164_23_R	70.8	95	86	0	0.45	-	>Chr06	49001520			>scaffold_7	22333483		
6	70	175_175_16_R	71.9	93	88	0	0.14	-								
6	71	4526_181_142_R	71.9	90	87	4	0.05	-	>Chr06	49545263			>scaffold_7	22815255		
6	72	245_173_110	71.9	93	88	0	0.14	-	>Chr06	49552997			>scaffold_7	22828997		
6	73	870_168_100_R	73.6	90	91	0	0.01	-					>scaffold_7	23163638		
6	74	15305_173_197	73.6	87	87	7	0	-	>Chr06	49979181			>scaffold_7	23200057	>scaffold_6	19882049
6	75	7255_134_185	73.6	88	91	2	0.05	-	>Chr06	49987208			>scaffold_7	23207869		
6	76	1915_147_199	74.2	82	80	19	0.02	-								
6	77	6294_181_114	75.9	90	91	0	0.01	-	>Chr06	50437186						
6	78	10314_145_231	75.9	90	91	0	0.01	-	>Chr06	50477099			>scaffold_7	23645953		

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
6	79	684_178_242	76.5	89	92	0	0.05	-	>Chr06	50686245			>scaffold_6	14041987		
6	80	6430_176_259	76.5	89	92	0	0.05	-								
6	81	4611_183_211	76.5	89	92	0	0.05	-								
6	82	3024_182_77	76.5	89	92	0	0.05	-	>Chr06	50843103						
6	83	2355_169_82_R	76.5	76	89	16	1.02	-	>Chr06	50946535						
6	84	13752_177_50	76.5	89	91	1	0.02	-	>Chr06	50991943			>scaffold_7	24234564		
6	85	10014_169_207	76.5	89	92	0	0.05	-	>Chr06	51027766			>scaffold_7	24293371		
6	86	5401_145_186	77.6	77	75	29	0.03	-								
6	87	1055_166_154_R	79.8	87	94	0	0.27	-	>Chr06	51376052			>scaffold_7	24568787	>scaffold_8	8054225
6	88	11361_143_168_R	80.4	70	83	28	1.1	-								
6	89	5329_176_208_R	80.4	85	95	1	0.56	-								
6	90	4099_153_124	82.1	87	94	0	0.27	-	>Chr06	52181833			>scaffold_7	25378489		
6	91	6798_143_116_R	84.8	71	79	31	0.43	-								
6	92	10830_139_50_R	85.5	89	92	0	0.05	-								
6	93	487_183_47_R	86.6	91	90	0	0.01	-	>Chr06	52826164			>scaffold_7	26005880		
6	94	10369_167_63_R	86.6	83	87	11	0.09	-								
6	95	1579_174_216_R	86.6	91	89	1	0.02	-	>Chr06	53111404			>scaffold_7	26229759		
6	96	15221_145_234_R	87.7	89	92	0	0.05	-	>Chr03	5246411			>scaffold_7	26409813		
6	97	10658_172_77_R	88.3	90	91	0	0.01	-								
6	98	10080_151_19_R	88.3	89	91	1	0.02	-	>Chr06	50323507						
6	99	11030_174_170_R	90.5	84	85	12	0.01	-								
6	100	4998_160_7_R	90.5	90	91	0	0.01	-	>Chr06	54491342			>scaffold_7	27334358		
6	101	4375_170_155_R	90.5	90	91	0	0.01	-	>Chr06	54585915						
6	102	4892_175_137_R	90.5	89	90	2	0.01	-	>Chr06	54740809			>scaffold_7	27546346		
6	103	9644_164_14_R	90.5	90	91	0	0.01	-	>Chr06	54855614			>scaffold_7	27670210		
6	104	**W_2196_175_64_R**	90.5	89	91	1	0.02	-	>Chr06	54961127			>scaffold_5	42791398		
6	105	5472_170_144_R	90.5	90	91	0	0.01	-								
6	106	628_155_48_R	91.2	72	80	29	0.42	-								
6	107	8236_169_271_R	97.7	95	86	0	0.45	-	>Chr06	56977037			>scaffold_7	29644599		
6	108	5951_163_102_R	100.5	86	85	10	0.01	-								
6	109	10918_164_35_R	102.7	94	87	0	0.27	-	>Chr06	57018542			>scaffold_7	29686149		
6	110	7393_181_267_R	106	94	87	0	0.27	-	>Chr06	57666043						
6	111	10915_149_139_R	106.6	92	88	1	0.09	-								
6	112	11387_162_182_R	106.6	92	87	2	0.14	-								
6	113	13087_178_216_R	106.6	93	88	0	0.14	-	>Chr06	57755848			>scaffold_2	49182464	>scaffold_8	554239
6	114	5019_133_245_R	106.6	90	87	4	0.05	-	>Chr06	58015692			>scaffold_7	30626628		
6	115	9147_188_142	108.8	92	87	2	0.14	-								
6	116	3519_185_171	108.8	89	83	9	0.21	-	>Chr06	58346114	>Chr07	6462747	>scaffold_7	31001854	>scaffold_6	3336264
6	117	11225_138_267_R	109.4	92	89	0	0.05	-	>Chr06	58396634			>scaffold_7	31049261		
6	118	1080_180_135_R	109.4	92	89	0	0.05	-								
6	119	3148_181_230	111.1	89	92	0	0.05	-								
6	120	1321_170_53	112.2	89	89	3	0	-	>Chr06	59102504			>scaffold_7	31574507		
6	121	13398_177_249_R	114.4	92	88	1	0.09	-	>Chr06	59405419			>scaffold_3	2053297		
6	122	5881_143_214	114.4	93	88	0	0.14	-	>Chr06	59406891	>Chr01	56404951	>scaffold_3	2052170	>scaffold_9	43434473
6	123	8368_159_24_R	114.4	93	88	0	0.14	-	>Chr06	59513392			>scaffold_3	1295468		
6	124	9266_179_168_R	114.4	91	88	2	0.05	-								
6	125	11291_148_161	114.4	93	88	0	0.14	-								
6	126	5508_139_171	116.1	92	89	0	0.05	-	>Chr06	60251927			>scaffold_3	1799994		
6	127	9466_159_246	116.7	91	90	0	0.01	-	>Chr06	60252852			>scaffold_3	1800815		
6	128	8367_146_27	116.7	87	88	6	0.01	-								
6	129	10213_183_208	117.8	91	90	0	0.01	-	>Chr06	60955479			>scaffold_3	868288		
6	130	13518_178_93	117.8	91	90	0	0.01	-								
6	131	14287_166_57	121.1	97	84	0	0.93	-	>Chr06	61189095			>scaffold_3	683064		
6	132	12898_157_240	121.1	97	84	0	0.93	-	>Chr06	61195605			>scaffold_3	677272		
6	133	4255_178_213	121.1	97	84	0	0.93	-	>Chr06	61239524			>scaffold_3	642766		
6	134	9660_182_29	121.1	97	84	0	0.93	-	>Chr06	61286163			>scaffold_3	603594		
6	135	6961_185_114	121.7	98	83	0	1.24	-	>Chr06	61189439						
6	136	11601_141_79	122.8	100	81	0	1.99	-					>scaffold_3	103506		
6	137	7497_149_196_R	122.8	100	81	0	1.99	-	>Chr06	61980783	>Chr07	398459	>scaffold_3	77610	>scaffold_6	424970
6	138	12923_175_214	122.8	100	81	0	1.99	-					>scaffold_3	65460	>scaffold_5	43171556

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
7	1	10241_168_8_R	0	86	81	14	0.15	-								
7	2	3987_154_149_R	0.6	94	87	0	0.27	-								
7	3	7249_184_62_R	2.8	92	88	1	0.09	-								
7	4	9593_182_103_R	2.8	92	89	0	0.05	-								
7	5	3537_158_119_R	7.2	92	89	0	0.05	-								
7	6	11337_185_149_R	7.2	92	89	0	0.05	-								
7	7	3047_162_98_R	10	95	86	0	0.45	-								
7	8	5786_157_80_R	12.2	90	85	6	0.14	-								
7	9	1564_178_123	12.2	94	86	1	0.36	-	>Chr07	1738649			>scaffold_6	7540023		
7	10	6209_180_169	12.2	88	77	16	0.73	-	>Chr07	1739150			>scaffold_6	7539518		
7	11	4468_165_210	12.2	95	86	0	0.45	-	>Chr07	1745156			>scaffold_6	7533450		
7	12	9712_150_62	15.5	76	72	33	0.11	-								
7	13	10188_142_126_R	15.5	91	90	0	0.01	-	>Chr03	65949041			>scaffold_1	37643032	>scaffold_5	39908708
7	14	1144_178_8	15.5	91	90	0	0.01	-	>Chr07	1993989			>scaffold_6	7417243	>scaffold_1	6072292
7	15	10711_171_196_R	18.8	89	92	0	0.05	-	>Chr07	2440766			>scaffold_6	6948709		
7	16	8865_169_32_R	19.9	89	91	1	0.02	-	>Chr07	2809033			>scaffold_6	6532367		
7	17	3852_135_86_R	22.4	72	87	22	1.42	-								
7	18	13873_160_47	23.2	90	90	1	0	-								
7	19	8153_135_16_R	23.2	91	90	0	0.01	-	>Chr07	3481163			>scaffold_6	5597368		
7	20	654_160_94_R	28.7	91	88	2	0.05	-								
7	21	42_166_120_R	29	82	83	16	0.01	-	>Chr07	5267131	>Chr03	15426491	>scaffold_6	3983093	>scaffold_7	24969707
7	22	11228_169_241_R	29.3	94	87	0	0.27	-	>Chr01	49371624	>Chr07	5314458	>scaffold_6	3976532		
7	23	484_184_84_R	29.9	95	86	0	0.45	-	>Chr07	5401586			>scaffold_6	3916731		
7	24	7137_145_201_R	29.9	95	86	0	0.45	-								
7	25	2967_184_209_R	29.9	95	86	0	0.45	-	>Chr07	5412624	>Chr05	60201759	>scaffold_8	38729602		
7	26	13839_145_13_R	31.1	92	83	6	0.46	-	>Chr07	5812263						
7	27	772_167_7	34.8	94	87	0	0.27	-								
7	28	7720_150_94_R	41.5	92	83	6	0.46	-								
7	29	1090_168_79	42.6	96	85	0	0.67	-								
7	30	258_140_41_R	45.4	91	90	0	0.01	-	>Chr07	7877150			>scaffold_6	2348485	>scaffold_3	6940848
7	31	**W_1236_132_139**	46	92	89	0	0.05	-								
7	32	7589_170_180	47.7	89	91	1	0.02	-								
7	33	6902_165_27	49.9	89	92	0	0.05	-	>Chr07	12461200			>scaffold_6	9107011		
7	34	**W_6465_174_175**	50.5	90	90	1	0	-								
7	35	11737_167_67	51.5	84	72	25	0.92	-								
7	36	**9241_150_73**	53.9	90	69	22	2.77	*								
7	37	532_168_112	56.7	89	92	0	0.05	-								
7	38	14379_170_179	56.7	89	92	0	0.05	-								
7	39	3225_169_10	56.7	89	92	0	0.05	-								
7	40	2549_148_124	56.7	89	92	0	0.05	-								
7	41	11430_162_163	56.7	89	92	0	0.05	-								
7	42	650_160_45	56.7	89	91	1	0.02	-								
7	43	10545_161_213	56.7	89	92	0	0.05	-								
7	44	11770_185_130	57.3	86	93	2	0.27	-								
7	45	10908_150_134	57.3	88	93	0	0.14	-	>Chr07	27269032	>Chr02	45955157	>scaffold_6	18791049	>scaffold_2	23957563
7	46	2378_132_153	57.9	89	91	1	0.02	-								
7	47	**W_5083_179_209**	59	89	90	2	0.01	-	>Chr07	49529814			>scaffold_6	21928275		
7	48	766_174_156	59	84	89	8	0.14	-								
7	49	1338_157_231	59.6	90	91	0	0.01	-	>Chr07	51353785						
7	50	2631_174_82	63.3	88	88	5	0	-								
7	51	12610_158_159	65.1	92	89	0	0.05	-	>Chr07	54361896						
7	52	**W_943_139_84**	67.3	89	90	2	0.01	-	>Chr07	54703404						
7	53	11373_177_56	69	89	92	0	0.05	-								
7	54	8256_132_12	72.3	93	88	0	0.14	-	>Chr07	55264895			>scaffold_6	28234543		
7	55	12788_164_3	72.3	93	87	1	0.2	-								
7	56	8829_174_222	72.3	93	88	0	0.14	-	>Chr07	55282026			>scaffold_6	28244434		
7	57	3302_174_230	72.3	93	87	1	0.2	-								
7	58	3804_174_47	72.3	93	88	0	0.14	-								
7	59	11478_147_122	76.2	90	91	0	0.01	-	>Chr07	4989154			>scaffold_6	4261333		
7	60	12794_163_151	82.6	89	91	1	0.02	-								
7	61	13971_153_191	88.4	94	86	1	0.36	-								
7	62	11111_162_163	91.7	92	89	0	0.05	-								
7	63	4474_155_268	95.6	91	89	1	0.02	-								
7	64	5084_176_80	98.4	92	89	0	0.05	-					>scaffold_6	32831375		
7	65	13722_183_187	99	91	90	0	0.01	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
7	66	5624_136_111	100.1	80	72	29	0.42	-								
7	67	9711_175_7	101.2	91	90	0	0.01	-	>Chr07	62158496			>scaffold_6	32973990		
7	68	**W_12181_138_236**	103.4	93	86	2	0.27	-								
7	69	2528_178_30	104.5	95	86	0	0.45	-								
7	70	4171_180_110	104.5	95	86	0	0.45	-								
7	71	4776_166_60_R	106	85	76	20	0.5	-								
7	72	7388_182_121	106.7	93	88	0	0.14	-								
7	73	4040_164_196_R	106.7	93	88	0	0.14	-								
7	74	**1289_168_140**	109.3	93	62	26	6.2	**								

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
8	1	8350_182_213	0	86	94	1	0.36	-								
8	2	12441_158_58_R	0	87	93	1	0.2	-								
8	3	8843_161_187	0	87	93	1	0.2	-								
8	4	6475_140_162	0	87	94	0	0.27	-								
8	5	11893_133_267_R	5.5	87	94	0	0.27	-								
8	6	11605_168_175_R	8.3	82	99	0	1.6	-	>Chr08	2874256	>Chr04	3479821	>scaffold_7	35543903	>scaffold_8	854146
8	7	8908_186_120_R	8.3	81	99	1	1.8	-								
8	8	6070_158_31_R	8.3	82	99	0	1.6	-								
8	9	10362_133_165_R	13.3	85	96	0	0.67	-								
8	10	1727_152_46_R	21.1	85	96	0	0.67	-								
8	11	7520_183_173_R	22.2	87	93	1	0.2	-					>scaffold_2	24388597		
8	12	8419_137_103_R	22.8	86	95	0	0.45	-								
8	13	13430_140_110_R	22.8	85	95	1	0.56	-								
8	14	9669_141_228_R	22.8	86	95	0	0.45	-					>scaffold_5	614450		
8	15	11520_139_158	23.4	85	96	0	0.67	-								
8	16	11303_156_211	26.7	81	96	4	1.27	-	>Chr10	9695360	>Chr08	17891314	>scaffold_3	5584915		
8	17	11411_155_10	26.7	84	96	1	0.8	-	>Chr10	9694948	>Chr08	17891773	>scaffold_3	5585368		
8	18	12760_137_57_R	26.7	85	96	0	0.67	-	>Chr02	73445907			>scaffold_2	45864931		
8	19	11632_166_195	26.7	81	90	10	0.47	-								
8	20	7756_148_51_R	29.5	82	99	0	1.6	-								
8	21	6496_174_26_R	30.1	83	98	0	1.24	-								
8	22	11300_177_39_R	30.7	84	97	0	0.93	-					>scaffold_7	32651709		
8	23	4704_176_212_R	33.5	83	98	0	1.24	-								
8	24	4891_167_126_R	37.4	84	97	0	0.93	-								
8	25	11482_136_250	40.2	85	96	0	0.67	-	>Chr08	40107145			>scaffold_3	46450691		
8	26	6846_181_168_R	40.8	81	94	6	0.97	-					>scaffold_3	45958852		
8	27	2206_132_265	41.9	69	74	38	0.17	-								
8	28	3692_173_175_R	42.5	85	96	0	0.67	-								
8	29	11444_175_65_R	43.6	85	96	0	0.67	-								
8	30	6203_181_158	45.3	82	99	0	1.6	-								
8	31	3858_175_42	45.3	82	99	0	1.6	-	>Chr08	47382402			>scaffold_4	6340841		
8	32	13437_147_194_R	45.9	81	100	0	1.99	-								
8	33	6124_181_38_R	47	83	98	0	1.24	-								
8	34	4520_165_103	50.2	82	93	6	0.69	-	>Chr08	48449309						
8	35	8098_142_68_R	50.8	86	95	0	0.45	-								
8	36	13776_166_119_R	50.8	85	95	1	0.56	-								
8	37	4461_143_262	50.8	86	95	0	0.45	-	>Chr08	48489630			>scaffold_3	42586790		
8	38	7774_149_7	51.9	84	96	1	0.8	-	>Chr08	48837596			>scaffold_3	42315184	>scaffold_5	11179658
8	39	8691_148_73	53	85	93	3	0.36	-	>Chr08	49160602			>scaffold_3	41129260		
8	40	4359_145_76	55.1	87	84	10	0.05	-								
8	41	8221_135_91_R	55.8	89	91	1	0.02	-								
8	42	1302_142_120_R	55.8	89	85	7	0.09	-								
8	43	10064_169_112_R	55.8	89	92	0	0.05	-	>Chr08	49657027			>scaffold_3	46646203		
8	44	319_161_5	58.6	88	93	0	0.14	-								
8	45	9465_166_195_R	61.4	89	90	2	0.01	-								
8	46	3423_165_163_R	61.4	76	79	26	0.06	-								
8	47	4472_155_104_R	61.4	85	82	14	0.05	-								
8	48	16105_153_157_R	62	90	89	2	0.01	-								
8	49	3375_188_89_R	62	90	91	0	0.01	-	>Chr08	50700501			>scaffold_3	47457873		
8	50	13495_163_69_R	63.1	90	88	3	0.02	-					>scaffold_3	47633356		
8	51	5082_135_6	64.2	90	91	0	0.01	-								
8	52	14105_160_10_R	67.5	94	87	0	0.27	-								
8	53	15516_140_184_R	67.5	91	87	3	0.09	-								
8	54	6807_169_62_R	67.5	87	82	12	0.15	-								
8	55	5129_135_121	81.1	94	87	0	0.27	-								
8	56	10340_168_174	83.3	92	89	0	0.05	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
9	1	5830_176_121_R	0	93	88	0	0.14	-								
9	2	9775_155_76_R	0.6	94	87	0	0.27	-								
9	3	11037_174_118_R	0.6	94	87	0	0.27	-								
9	4	9642_180_223_R	0.6	94	87	0	0.27	-								
9	5	8381_184_33_R	0.6	93	87	1	0.2	-								
9	6	10114_169_75	0.6	94	87	0	0.27	-	>Chr09	883561						
9	7	5113_185_173	0.6	91	87	3	0.09	-								
9	8	9198_168_226_R	0.6	81	76	24	0.16	-								
9	9	6811_134_12_R	0.6	85	83	13	0.02	-								
9	10	9366_138_64	0.6	94	87	0	0.27	-								
9	11	9806_167_84_R	0.6	90	85	6	0.14	-	>Chr04	64577197			>scaffold_1	39549332		
9	12	3363_140_159	0.6	93	87	1	0.2	-								
9	13	4050_175_64_R	1.7	92	89	0	0.05	-								
9	14	13506_167_187_R	1.7	90	85	6	0.14	-	>Chr09	1016717						
9	15	8512_148_116	2.3	90	84	7	0.21	-								
9	16	4622_159_82	3.7	78	81	22	0.06	-								
9	17	3523_168_36_R	7.3	96	85	0	0.67	-								
9	18	11861_164_104_R	8.4	96	85	0	0.67	-								
9	19	3268_144_25_R	9	95	86	0	0.45	-								
9	20	7823_176_161_R	9	95	86	0	0.45	-								
9	21	9121_147_54	10.4	83	70	28	1.1	-								
9	22	7474_172_9	17.4	90	91	0	0.01	-								
9	23	9585_187_64	24.1	92	89	0	0.05	-					>scaffold_9	29771216		
9	24	8734_179_119	24.7	91	90	0	0.01	-								
9	25	5749_167_128	24.7	90	85	6	0.14	-								
9	26	3115_156_186	24.7	91	90	0	0.01	-								
9	27	1016_175_33	24.7	91	90	0	0.01	-								
9	28	6747_163_44	24.7	91	90	0	0.01	-								
9	29	8947_160_116	25.3	92	75	14	1.73	-								
9	30	9396_171_42	29.1	88	90	3	0.02	-	>Chr09	4438873	>Chr07	62441067	>scaffold_3	2215275		
9	31	8942_175_98	34.7	87	94	0	0.27	-	>Chr09	4913614			>scaffold_7	31902239		
9	32	4497_150_28	35.8	83	90	8	0.28	-								
9	33	2968_151_170	35.8	87	94	0	0.27	-								
9	34	7616_172_165	35.8	79	84	18	0.15	-								
9	35	939_176_119	37.4	86	95	0	0.45	-								
9	36	9412_165_240	39.1	87	94	0	0.27	-	>Chr09	5767735						
9	37	2867_176_88	40.8	88	92	1	0.09	-								
9	38	1923_169_4	45.2	86	95	0	0.45	-								
9	39	3544_160_5	48.5	83	88	10	0.15	-								
9	40	9402_160_6	48.5	84	97	0	0.93	-	>Chr09	7546861	>Chr03	3589256	>scaffold_3	7192388	>scaffold_5	11257828
9	41	12586_163_161	48.5	83	96	2	0.94	-								
9	42	6276_179_5	48.5	84	97	0	0.93	-	>Chr01	42989119	>Chr10	60731403				
9	43	9805_176_106	49.1	83	98	0	1.24	-								
9	44	10823_175_75	49.1	81	91	9	0.58	-					>scaffold_3	7631177	0	0
9	45	3417_176_205	49.1	82	97	2	1.26	-								
9	46	10905_184_175	49.1	83	90	8	0.28	-								
9	47	6830_168_129	51.9	82	97	2	1.26	-	>Chr09	10709336						
9	48	5465_159_23	52.5	83	98	0	1.24	-								
9	49	4381_148_1	52.5	83	98	0	1.24	-								
9	50	9567_150_25	54.7	87	94	0	0.27	-								
9	51	4311_176_53	57.5	86	95	0	0.45	-								
9	52	3357_177_199	57.5	86	91	4	0.14	-								
9	53	10903_182_201	59.7	90	91	0	0.01	-								
9	54	545_132_183	60.8	90	91	0	0.01	-								
9	55	10850_172_173	62.5	87	93	1	0.2	-	>Chr09	48077039			>scaffold_3	22048507		
9	56	5097_177_212	62.5	87	92	2	0.14	-	>Chr09	48077046			>scaffold_3	22048500		
9	57	3427_168_62	62.5	87	94	0	0.27	-	>Chr09	48110483						
9	58	692_179_220	64.2	90	90	1	0	-	>Chr09	48356961			>scaffold_3	21500924		
9	59	4571_148_185	65.9	89	92	0	0.05	-	>Chr09	48966981			>scaffold_3	21154310		
9	60	4578_184_210	65.9	89	92	0	0.05	-								
9	61	5283_159_179	70.9	96	85	0	0.67	-	>Chr09	49527552			>scaffold_3	20519556		
9	62	9512_179_106	72.6	99	82	0	1.6	-	>Chr09	50048008						
9	63	**1585_175_126**	73.7	101	78	2	2.96	*								
9	64	**1480_173_108**	79.2	103	78	0	3.45	*	>Chr09	50678525	>Chr03	68624662	>scaffold_3	19093677	>scaffold_5	42441471
9	65	**8239_185_100**	82	106	75	0	5.31	**	>Chr09	51187142			>scaffold_3	18531966		

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
9	66	**13416_172_223**	82	106	75	0	5.31	**	>Chr09	51197655	>Chr03	68069918	>scaffold_3	18518525		
9	67	**11204_176_82**	85.3	112	69	0	10.22	****								
9	68	**5936_179_182**	85.3	104	55	22	15.1	******	>Chr09	52355977	>Chr03	66800041				
9	69	**9122_179_97**	85.3	93	67	21	4.22	**								
9	70	**9590_141_132**	86.4	114	67	0	12.2	******	>Chr09	52409591			>scaffold_3	17047831		
9	71	**5911_164_127**	87	111	68	2	10.33	****	>Chr09	52592795			>scaffold_3	16311258		
9	72	**9161_153_12**	87	113	68	0	11.19	*****	>Chr09	52671212			>scaffold_3	16131645		
9	73	**9703_140_47**	88.1	113	68	0	11.19	*****								
9	74	**1758_169_99**	88.1	113	68	0	11.19	*****	>Chr09	52768341			>scaffold_3	16029089		
9	75	**1828_167_103**	90.3	113	68	0	11.19	*****	>Chr09	53809414			>scaffold_3	14520761		
9	76	**7117_169_150**	91.4	115	66	0	13.27	******								
9	77	**13325_161_105**	91.4	115	66	0	13.27	******					>scaffold_7	31486137		
9	78	**11005_167_126**	91.4	115	66	0	13.27	******								
9	79	**10234_133_69**	92	116	65	0	14.37	******								
9	80	**6916_176_77**	94.2	108	63	10	11.84	*****								
9	81	**3125_179_15**	94.2	114	67	0	12.2	******	>Chr07	1445698			>scaffold_6	7972416		
9	82	**11380_166_113**	94.8	113	65	3	12.94	******	>Chr09	55820890			>scaffold_3	12094566		
9	83	**1503_147_123**	96.5	105	54	22	16.36	*******	>Chr09	56106133			>scaffold_3	11783564		
9	84	**9486_183_184**	97.1	115	66	0	13.27	******	>Chr09	56106771			>scaffold_3	11782758		
9	85	**6270_161_89**	98.4	107	56	18	15.96	*******								
9	86	**7165_156_199**	100.4	113	68	0	11.19	*****	>Chr06	54609214						
9	87	**2443_157_7**	100.4	112	68	1	10.76	****								
9	88	**9227_161_150**	100.4	102	57	22	12.74	******	>Chr09	56236227			>scaffold_3	11643014		
9	89	**13265_171_93_R**	100.4	107	67	7	9.2	****								
9	90	**1741_137_77_R**	100.4	111	68	2	10.33	****								
9	91	**187_133_163_R**	103.7	113	68	0	11.19	*****								
9	92	**9947_171_51_R**	104.8	109	65	7	11.13	*****	>Chr09	57044038			>scaffold_3	10730926		
9	93	**14816_141_39**	105.4	109	66	6	10.57	****								
9	94	**4479_176_44**	106	112	65	4	12.48	******								
9	95	**11797_164_38_R**	108.5	94	58	29	8.53	****	>Chr09	57687274	>Chr03	58094896				
9	96	**1513_145_88_R**	109.3	110	70	1	8.89	****								
9	97	**11516_170_203_R**	109.3	111	70	0	9.29	****								
9	98	**5731_132_213_R**	110.9	113	67	1	11.76	*****								
9	99	**7794_175_163_R**	110.9	111	66	4	11.44	*****								
9	100	**13385_172_165_R**	112	110	68	3	9.91	****								
9	101	**7575_160_116_R**	112	112	69	0	10.22	****					>scaffold_3	8545220		
9	102	**4796_179_36_R**	112	112	69	0	10.22	****								
9	103	**10163_170_95_R**	112.6	111	66	4	11.44	*****								
9	104	**3656_151_43_R**	113.2	112	69	0	10.22	****					>scaffold_3	8195278		
9	105	**W_3250_135_108_R**	113.2	94	55	32	10.21	****	>Chr09	59206462	>Chr03	47891813	>scaffold_3	8078130	>scaffold_5	24285454
9	106	**13062_167_121_R**	113.2	112	69	0	10.22	****								
9	107	**11526_180_108_R**	113.2	112	69	0	10.22	****								
9	108	**10107_164_173_R**	113.5	108	66	7	10.14	****	>Chr07	960145						
9	109	**4848_149_7_R**	113.8	113	68	0	11.19	*****								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
10	1	3436_141_67_R	0	93	85	3	0.36	-								
10	2	9474_184_68_R	0	94	87	0	0.27	-								
10	3	1079_180_124_R	0	94	87	0	0.27	-								
10	4	14838_135_80_R	0	87	82	12	0.15	-	>Chr10	59637280			>scaffold_4	39365306		
10	5	164_150_120_R	2.2	91	89	1	0.02	-								
10	6	1238_174_39_R	3.3	85	80	16	0.15	-	>Chr10	59502616			>scaffold_4	39261362		
10	7	7842_158_89_R	3.3	94	86	1	0.36	-	>Chr10	59228168						
10	8	**W_5225_137_139_R**	3.3	94	86	1	0.36	-								
10	9	4142_160_134_R	3.9	93	88	0	0.14	-								
10	10	6627_175_219_R	5	93	88	0	0.14	-					>scaffold_5	45823766		
10	11	8878_166_8_R	5	83	82	16	0.01	-					>scaffold_4	37104922		
10	12	8503_173_35_R	6.1	79	87	15	0.39	-								
10	13	3205_176_34_R	7.2	88	86	7	0.02	-								
10	14	4199_173_72_R	7.2	91	88	2	0.05	-								
10	15	13674_156_249_R	7.2	92	88	1	0.09	-								
10	16	5278_162_74_R	7.2	93	88	0	0.14	-	>Chr10	58049070						
10	17	4234_179_28_R	8.9	94	87	0	0.27	-					>scaffold_4	37675160		
10	18	15298_134_209_R	8.9	94	87	0	0.27	-								
10	19	10544_133_86_R	16.9	71	73	37	0.03	-	>Chr10	55183498	>Chr04	5285165	>scaffold_4	31264692		
10	20	11699_171_114	18.9	91	80	10	0.71	-					>scaffold_4	31502900		
10	21	11613_179_60	20.1	93	87	1	0.2	-								
10	22	7708_172_131	21.2	94	87	0	0.27	-	>Chr10	54312822			>scaffold_4	32244859		
10	23	2787_184_221	21.2	94	87	0	0.27	-								
10	24	4547_178_8	21.2	94	87	0	0.27	-								
10	25	5486_155_170	27.8	84	83	14	0.01	-								
10	26	1103_176_246_R	28.4	97	84	0	0.93	-	>Chr10	53763532			>scaffold_4	32796165		
10	27	9132_159_180	30.1	96	85	0	0.67	-	>Chr02	18702957			>scaffold_2	15035504	>scaffold_11	13258
10	28	13205_142_127_R	30.1	96	84	1	0.8	-	>Chr10	53630422			>scaffold_4	32948772		
10	29	13592_159_23	31.8	95	86	0	0.45	-								
10	30	9937_142_135_R	33.5	91	88	2	0.05	-	>Chr10	52149821			>scaffold_4	34403633		
10	31	10781_184_212_R	35.2	91	90	0	0.01	-								
10	32	13835_180_111_R	35.2	91	90	0	0.01	-								
10	33	8361_174_187	41.2	87	88	6	0.01	-								
10	34	13098_163_107_R	43	85	88	8	0.05	-								
10	35	13891_149_124_R	43.6	90	91	0	0.01	-								
10	36	5891_133_267_R	43.6	90	91	0	0.01	-								
10	37	9627_182_103	44.2	89	88	4	0.01	-					>scaffold_4	35604838		
10	38	1733_170_219	44.2	91	90	0	0.01	-								
10	39	13123_170_177	46.4	90	89	2	0.01	-								
10	40	9136_188_54	48.1	88	91	2	0.05	-					>scaffold_5	14870847	>scaffold_2	45411194
10	41	8107_181_220	48.1	90	91	0	0.01	-								
10	42	9324_177_141_R	49.2	90	91	0	0.01	-								
10	43	7723_166_120_R	49.8	87	83	11	0.09	-	>Chr10	49311857						
10	44	5484_181_61	51.5	90	91	0	0.01	-								
10	45	6324_178_7_R	53.2	79	87	15	0.39	-								
10	46	7405_174_10	54.9	88	93	0	0.14	-								
10	47	11653_175_175	56	88	91	2	0.05	-								
10	48	**W_6085_133_44**	56.6	89	91	1	0.02	-								
10	49	436_175_137_R	57.2	88	93	0	0.14	-								
10	50	7649_147_65_R	58.3	88	93	0	0.14	-	>Chr10	14100396			>scaffold_4	13397989		
10	51	13224_174_153_R	61.1	89	92	0	0.05	-								
10	52	634_186_237_R	61.7	88	93	0	0.14	-								
10	53	10765_186_187	62.8	88	93	0	0.14	-	>Chr10	4040540			>scaffold_1	31869764	>scaffold_4	12780954
10	54	7802_151_119_R	63.4	75	88	18	1.04	-	>Chr10	12252679	>Chr04	59138774	>scaffold_4	12534131	>scaffold_1	36926369
10	55	4956_145_115_R	64	68	85	28	1.89	-								
10	56	7050_175_96	68.9	89	91	1	0.02	-	>Chr10	10781208	>Chr07	58636509	>scaffold_4	11477226		
10	57	10709_184_73	68.9	89	92	0	0.05	-								
10	58	8171_160_24_R	68.9	89	91	1	0.02	-								
10	59	7022_159_19	68.9	84	88	9	0.09	-								
10	60	6514_177_114_R	68.9	89	92	0	0.05	-	>Chr10	10284062			>scaffold_4	10975440		
10	61	4034_158_72_R	68.9	89	86	6	0.05	-								
10	62	9756_184_8_R	68.9	89	92	0	0.05	-								
10	63	12943_164_240_R	70	87	94	0	0.27	-					>scaffold_4	9774468		
10	64	10960_138_30_R	70	87	94	0	0.27	-								
10	65	11477_152_144_R	71.7	89	91	1	0.02	-					>scaffold_8	33611344		

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
10	66	10469_150_57	73.4	92	87	2	0.14	-								
10	67	10497_178_103	77.8	95	86	0	0.45	-								
10	68	2613_171_77	77.8	95	86	0	0.45	-								
10	69	10426_161_128	86.6	93	81	7	0.83	-								
10	70	**7801_143_122**	91.2	107	68	6	8.69	****								
10	71	**11940_160_100_R**	92.3	101	75	5	3.84	*								
10	72	**9858_139_180_R**	92.3	105	76	0	4.65	**								
10	73	**350_166_207_R**	95.1	110	71	0	8.4	****	>Chr09	54660897			>scaffold_3	13696210		
10	74	**4966_157_88_R**	95.1	110	71	0	8.4	****					>scaffold_4	2618733		
10	75	**7638_179_115_R**	95.1	109	71	1	8.02	****								
10	76	**8897_148_49**	97.3	112	69	0	10.22	****								
10	77	**2950_146_232**	98.4	114	67	0	12.2	******					>scaffold_4	3691966		
10	78	**4510_172_153_R**	99	115	66	0	13.27	******								
10	79	**8165_180_159_R**	99	111	65	5	12.02	*****	>Chr10	5041539	>Chr04	67809587	>scaffold_4	3794670	>scaffold_1	42019650
10	80	**8438_185_39_R**	101.2	107	66	8	9.72	****								
10	81	**4185_179_8_R**	101.2	113	68	0	11.19	*****								
10	82	**11310_162_25_R**	101.8	89	61	31	5.23	**	>Chr10	3222575			>scaffold_4	5570832		
10	83	**10079_162_54_R**	101.8	112	69	0	10.22	****	>Chr05	17265228			>scaffold_8	12330687		
10	84	**7488_180_179_R**	102.4	113	68	0	11.19	*****								
10	85	**622_181_56**	105.2	112	69	0	10.22	****					>scaffold_9	49710612		
10	86	**W_6917_142_107**	106	97	64	20	6.76	***								
10	87	**13698_175_55_R**	106.8	111	70	0	9.29	****								
10	88	**10818_174_96_R**	107.4	103	68	10	7.16	***								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
1	1	7437_169_63_R	0	84	77	20	0.3	-								
1	2	3772_174_106_R	0.6	99	78	4	2.49	-	>Chr01	195654			>scaffold_9	168460		
1	3	**13607_163_176_R**	1.2	104	77	0	4.03	**								
1	4	**1089_176_198_R**	1.2	95	73	13	2.88	*								
1	5	**9424_173_190_R**	1.2	102	70	9	5.95	**	>Chr01	199145			>scaffold_9	169177		
1	6	**1449_171_159**	5.1	105	76	0	4.65	**					>scaffold_9	426745		
1	7	6153_158_57_R	8.4	101	80	0	2.44	-								
1	8	4258_144_230_R	8.4	101	80	0	2.44	-					>scaffold_9	661276		
1	9	2740_166_190_R	10.6	99	82	0	1.6	-	>Chr01	1729742			>scaffold_9	1077208		
1	10	5137_174_42_R	12.8	97	84	0	0.93	-	>Chr01	2091394	>Chr02	9453192	>scaffold_9	1213141	>scaffold_2	8699894
1	11	1283_176_71	16.1	101	79	1	2.69	-								
1	12	8527_156_163	22.8	99	82	0	1.6	-								
1	13	7103_175_247_R	25	99	82	0	1.6	-								
1	14	10424_168_243_R	27.2	97	84	0	0.93	-								
1	15	15485_172_228_R	27.2	97	84	0	0.93	-	>Chr01	11124754	>Chr08	54837910	>scaffold_9	9093060	>scaffold_3	50246041
1	16	7229_182_157_R	28.3	95	86	0	0.45	-	>Chr01	11378713			>scaffold_9	9425377		
1	17	3200_142_215_R	29.4	97	84	0	0.93	-								
1	18	3213_164_128_R	30.7	87	83	11	0.09	-	>Chr01	11438924			>scaffold_9	9503835		
1	19	10505_162_122_R	33.3	67	87	27	2.6	-								
1	20	6013_177_171_R	34.4	90	82	9	0.37	-								
1	21	9482_155_119_R	34.4	96	84	1	0.8	-								
1	22	8282_153_206_R	35.5	96	85	0	0.67	-								
1	23	7940_179_191_R	36.1	97	84	0	0.93	-	>Chr01	11801664			>scaffold_9	9741360	>scaffold_5	44711912
1	24	7635_173_18_R	38.2	95	82	4	0.95	-								
1	25	6348_159_31_R	38.5	87	74	20	1.05	-								
1	26	660_155_97_R	38.8	95	79	7	1.47	-								
1	27	10852_173_12_R	39.9	98	83	0	1.24	-								
1	28	3920_167_74_R	41.1	95	74	12	2.61	-	>Chr01	13008795			>scaffold_9	10751984		
1	29	**11615_173_44_R**	46.6	101	75	5	3.84	*	>Chr01	13882192			>scaffold_9	11411443		
1	30	**4962_184_105_R**	46.6	104	77	0	4.03	**	>Chr01	13919011						
1	31	**3517_185_130**	46.6	103	76	2	4.07	**	>Chr01	14029403						
1	32	**9182_177_108**	46.6	104	77	0	4.03	**	>Chr01	14065006						
1	33	**6423_168_78_R**	46.6	104	77	0	4.03	**					>scaffold_9	11416494	>scaffold_3	47418164
1	34	**5701_145_45**	48.3	103	78	0	3.45	*								
1	35	**2061_182_255**	50.5	106	74	1	5.69	**								
1	36	**8936_175_234**	50.5	106	74	1	5.69	**								
1	37	**8115_165_222**	50.5	107	74	0	6.02	**	>Chr01	15247216			>scaffold_9	12347822		
1	38	**3080_170_181_R**	52.2	102	67	12	7.25	***	>Chr01	15893115			>scaffold_9	12753150		
1	39	**385_145_221_R**	54.4	108	73	0	6.77	***								
1	40	**12932_161_47**	56.1	100	65	16	7.42	***	>Chr01	17587862						
1	41	**7378_185_59**	57.2	105	76	0	4.65	**								
1	42	**11260_146_50_R**	58.9	104	77	0	4.03	**								
1	43	**8694_153_166_R**	58.9	104	77	0	4.03	**					>scaffold_9	14749119		
1	44	**10467_153_167_R**	58.9	103	77	1	3.76	*					>scaffold_9	14892380		
1	45	**10081_176_87**	61.7	102	77	2	3.49	*	>Chr01	19438598			>scaffold_9	15388184		
1	46	**6328_172_198**	62.3	102	78	1	3.2	*	>Chr01	19491352						
1	47	11431_152_152	62.3	101	79	1	2.69	-	>Chr01	19601264			>scaffold_9	15511346		
1	48	**13476_155_217**	62.3	102	79	0	2.92	*								
1	49	6030_139_59	62.9	101	79	1	2.69	-	>Chr01	11623643			>scaffold_5	11050587	>scaffold_6	15044136
1	50	**7226_159_89**	64	100	77	4	2.99	*								
1	51	782_164_191	64.6	100	81	0	1.99	-								
1	52	8528_143_112	65.2	101	80	0	2.44	-								
1	53	**W_9368_156_174_R**	66.3	98	82	1	1.42	-	>Chr04	55058050						
1	54	9003_169_247	66.9	100	81	0	1.99	-	>Chr04	66269781			>scaffold_9	16773689		
1	55	6020_166_99	67.5	101	80	0	2.44	-	>Chr02	66660246						
1	56	2189_170_139_R	67.5	101	80	0	2.44	-	>Chr01	21078680			>scaffold_3	6933857	>scaffold_9	16892636
1	57	3577_183_59	68.1	100	81	0	1.99	-								
1	58	5571_180_69	69.2	98	83	0	1.24	-	>Chr07	56386869			>scaffold_6	29260734		
1	59	**W_8488_146_32_R**	69.2	96	83	2	0.94	-								
1	60	3262_188_129	69.2	97	83	1	1.09	-								
1	61	4852_149_209_R	69.2	92	79	10	0.99	-								
1	62	13353_166_92	70.3	96	80	5	1.45	-								
1	63	**W_9340_161_200_R**	72.4	88	72	21	1.6	-								
1	64	5323_184_4	75.3	91	84	6	0.28	-					>scaffold_9	19463457		
1	65	157_170_130_R	75.3	95	82	4	0.95	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
1	66	8929_156_112	75.3	94	86	1	0.36	-								
1	67	**W_2874_181_211_R**	76.9	80	70	31	0.67	-					>scaffold_9	22053696		
1	68	1085_174_116	76.9	92	84	5	0.36	-					>scaffold_9	22053694		
1	69	7429_148_17	76.9	92	89	0	0.05	-	>Chr01	28445440			>scaffold_9	21067200		
1	70	11645_153_97	76.9	92	89	0	0.05	-								
1	71	11668_154_5_R	76.9	92	89	0	0.05	-								
1	72	**W_7371_158_206**	77.2	85	84	12	0.01	-					>scaffold_1	21056950		
1	73	12792_159_263	77.5	89	89	3	0	-	>Chr01	30470910			>scaffold_9	20456009		
1	74	4471_182_218	77.5	91	90	0	0.01	-								
1	75	4169_163_5	78.1	92	89	0	0.05	-	>Chr01	31632790			>scaffold_9	20127626		
1	76	10410_178_35	78.1	92	88	1	0.09	-								
1	77	1223_179_166	81.4	94	87	0	0.27	-	>Chr01	73447446			>scaffold_9	32782928		
1	78	63_146_119_R	83.9	75	76	30	0.01	-	>Chr01	73462248	>Chr10	5033938	>scaffold_9	32796060	>scaffold_4	3801799
1	79	1137_133_64	87.2	76	82	23	0.23	-								
1	80	2431_178_20_R	88.6	92	88	1	0.09	-					>scaffold_4	17367245		
1	81	11352_141_165	88.6	93	87	1	0.2	-								
1	82	10806_160_134_R	88.6	85	88	8	0.05	-								
1	83	6971_139_266	88.6	93	86	2	0.27	-	>super_16	2198	>super_12	3513618	>scaffold_9	21885092	>scaffold_1	38729571
1	84	3790_136_126	88.6	90	88	3	0.02	-								
1	85	2732_161_166	88.6	93	88	0	0.14	-	>Chr05	956946			>scaffold_9	31815944		
1	86	10165_172_234	90.2	94	86	1	0.36	-								
1	87	2605_160_118	90.2	88	78	15	0.6	-								
1	88	2201_154_130	90.2	88	85	8	0.05	-								
1	89	648_145_270	90.8	93	85	3	0.36	-								
1	90	1057_162_92_R	90.8	93	88	0	0.14	-								
1	91	**2662_136_35_R**	96.2	66	87	28	2.88	*								
1	92	5077_168_108_R	99.2	76	86	19	0.62	-								
1	93	10397_184_59	99.2	92	89	0	0.05	-								
1	94	188_165_23_R	99.2	92	89	0	0.05	-								
1	95	11602_156_85_R	99.2	92	89	0	0.05	-					>scaffold_9	35780817		
1	96	1420_132_22	99.5	91	87	3	0.09	-	>Chr01	49715139			>scaffold_9	36394679		
1	97	10405_154_131	99.8	89	81	11	0.38	-								
1	98	10518_162_75_R	100.9	90	88	3	0.02	-								
1	99	8189_168_130	100.9	93	88	0	0.14	-	>Chr01	49996749			>scaffold_9	36555771		
1	100	13369_159_103	100.9	93	88	0	0.14	-	>Chr01	50023272			>scaffold_9	36567954		
1	101	6168_174_106	101.5	94	87	0	0.27	-								
1	102	11460_166_254_R	101.5	94	87	0	0.27	-	>Chr01	50062677	>Chr06	53172858	>scaffold_9	36612957	>scaffold_7	26280995
1	103	1077_177_63_R	102.1	91	87	3	0.09	-								
1	104	4658_161_182_R	102.1	89	85	7	0.09	-								
1	105	13739_147_78	102.7	92	89	0	0.05	-	>Chr01	50927186			>scaffold_9	37727995		
1	106	13240_159_262_R	102.7	92	89	0	0.05	-	>Chr01	50927349			>scaffold_9	37727831		
1	107	10333_177_107_R	107.7	91	90	0	0.01	-	>Chr01	52390793			>scaffold_9	39725463		
1	108	3785_150_261_R	108.8	89	92	0	0.05	-	>Chr01	52919973			>scaffold_9	40198753		
1	109	12507_174_35_R	108.8	88	92	1	0.09	-	>Chr01	53007679			>scaffold_9	40309599		
1	110	13723_150_88_R	108.8	89	92	0	0.05	-	>Chr01	53091496			>scaffold_9	40335286		
1	111	**W_14628_136_212**	111	87	88	6	0.01	-					>scaffold_7	4791288	>scaffold_4	9040082
1	112	8468_152_11_R	111	89	92	0	0.05	-	>Chr01	53936176						
1	113	4349_158_226_R	111	89	92	0	0.05	-	>Chr01	53936471	>Chr08	10427742	>scaffold_9	41218001		
1	114	1237_183_192_R	111	88	92	1	0.09	-	>Chr06	48459281	>Chr01	54132887	>scaffold_9	41348682		
1	115	10565_180_167_R	111	89	92	0	0.05	-	>Chr01	54203044						
1	116	4644_174_214_R	111	89	92	0	0.05	-								
1	117	4121_139_74_R	111	87	91	3	0.09	-								
1	118	4756_179_87_R	111	89	92	0	0.05	-								
1	119	6127_181_95_R	111	86	91	4	0.14	-					>scaffold_7	35371672		
1	120	3655_168_140_R	111	89	92	0	0.05	-								
1	121	4502_170_73_R	111.7	84	91	6	0.28	-								
1	122	8233_143_113	113.2	84	80	17	0.1	-								
1	123	10815_174_98	114.9	88	93	0	0.14	-								
1	124	11125_172_249_R	116.6	85	96	0	0.67	-								
1	125	4063_171_26_R	117.7	86	94	1	0.36	-					>scaffold_9	42713853	>scaffold_2	45336279
1	126	9494_167_107_R	120.5	85	88	8	0.05	-								
1	127	11758_146_168	123.7	83	92	6	0.46	-	>Chr01	56869184			>scaffold_9	43868927		
1	128	8281_151_154_R	125	84	97	0	0.93	-	>Chr01	56669519			>scaffold_9	43668899		
1	129	8493_180_66_R	126.1	82	99	0	1.6	-								
1	130	217_175_216	126.1	82	99	0	1.6	-								
1	131	10372_157_83	126.1	82	99	0	1.6	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
1	132	9449_169_224_R	130.5	85	92	4	0.28	-	>Chr01	58013573			>scaffold_9	45720058		
1	133	7832_161_142_R	131.6	86	89	6	0.05	-								
1	134	1691_182_179_R	132.2	89	84	8	0.14	-								
1	135	**W_2601_164_170_R**	132.2	89	91	1	0.02	-								
1	136	4589_162_87_R	132.8	86	91	4	0.14	-	>Chr01	59059349						
1	137	11627_153_91	132.8	88	92	1	0.09	-	>Chr01	59984470			>scaffold_9	47320114		
1	138	8635_133_130_R	132.8	88	91	2	0.05	-								
1	139	9645_150_244	132.8	86	92	3	0.2	-	>Chr01	60070245	>Chr02	76745836	>scaffold_9	47410238	>scaffold_2	48602645
1	140	12093_162_19_R	133.1	86	91	4	0.14	-								
1	141	1069_169_242	133.4	87	89	5	0.02	-								
1	142	12476_151_164	134.5	86	92	3	0.2	-	>Chr06	55700983						
1	143	13434_165_92	135.6	84	96	1	0.8	-					>scaffold_9	47962871		
1	144	12147_181_102	136.2	83	96	2	0.94	-								
1	145	**W_6557_137_110**	137.3	45	89	47	14.45	******								
1	146	**W_10540_175_201**	138.4	84	97	0	0.93	-								
1	147	4430_175_46	138.4	83	95	3	0.81	-								
1	148	3100_160_131	138.4	84	97	0	0.93	-	>Chr01	60971766			>scaffold_9	48415416		
1	149	4593_178_140	142.3	82	91	8	0.47	-	>Chr01	62249633			>scaffold_9	49519056		
1	150	**W_1959_163_111**	142.9	83	97	1	1.09	-								
1	151	9980_153_45	143.5	83	98	0	1.24	-								
1	152	1088_178_156	145.7	83	97	1	1.09	-								
1	153	5863_171_114	149	81	91	9	0.58	-					>scaffold_9	51104164		
1	154	**13249_151_115**	151.2	79	102	0	2.92	*								
1	155	**4271_132_97**	151.2	79	102	0	2.92	*								
1	156	12949_138_144	151.8	79	100	2	2.46	-	>Chr01	65271236			>scaffold_9	52173626		
1	157	4728_143_51	153.8	80	99	2	2.02	-								
1	158	**W_9111_175_6**	155.1	79	101	1	2.69	-					>scaffold_9	52904473		
1	159	**13852_169_236**	155.7	79	102	0	2.92	*	>Chr01	66379913			>scaffold_9	53165654		
1	160	1250_169_200	157.9	81	100	0	1.99	-	>Chr01	66637332			>scaffold_9	53451261		
1	161	7264_171_243	157.9	81	100	0	1.99	-	>Chr01	66651747						
1	162	11490_145_4	157.9	81	100	0	1.99	-	>Chr06	27683551						
1	163	12948_172_5	157.9	81	100	0	1.99	-								
1	164	13564_168_120	157.9	79	98	4	2.04	-								
1	165	5555_181_37	157.9	81	100	0	1.99	-								
1	166	8177_157_263	159	83	98	0	1.24	-	>Chr01	66831228			>scaffold_9	53647701		
1	167	16090_159_26	159.6	84	97	0	0.93	-								
1	168	3891_151_219	159.6	84	97	0	0.93	-	>Chr01	67265255			>scaffold_9	54049379		
1	169	7446_177_67	161.8	86	76	19	0.62	-	>Chr10	11388207			>scaffold_4	11987098		
1	170	4922_164_52	164.6	86	88	7	0.02	-								
1	171	1139_168_152	166.8	85	85	11	0	-	>Chr01	69531667			>scaffold_9	55742959		
1	172	9369_138_32	166.8	89	92	0	0.05	-	>Chr01	69532194			>scaffold_9	55743509		
1	173	1447_165_90	166.8	89	92	0	0.05	-	>Chr01	69532757			>scaffold_9	55744273		
1	174	8718_181_121	166.8	89	91	1	0.02	-					>scaffold_9	55751395		
1	175	10085_163_84	167.4	90	91	0	0.01	-	>Chr06	57178992						
1	176	1949_155_259	167.4	90	90	1	0	-	>Chr01	69877613	>Chr08	1477994	>scaffold_9	56044328		
1	177	12861_141_232	167.4	89	90	2	0.01	-	>Chr01	69879730			>scaffold_9	56046768		
1	178	2137_154_47	167.4	89	90	2	0.01	-								
1	179	10794_162_40	167.4	90	91	0	0.01	-	>Chr01	69883462			>scaffold_9	56049743		
1	180	1319_166_108	168.5	87	89	5	0.02	-	>Chr01	70713644			>scaffold_9	56674812		
1	181	3472_147_220	168.5	90	91	0	0.01	-	>Chr01	70831029			>scaffold_9	56725342		
1	182	959_182_119	168.5	90	89	2	0.01	-								
1	183	10326_180_208	168.5	90	91	0	0.01	-								
1	184	11501_148_154	168.5	90	91	0	0.01	-	>Chr01	70868633			>scaffold_9	56750501		
1	185	6655_178_21	168.5	90	91	0	0.01	-								
1	186	337_138_148	168.5	90	91	0	0.01	-								
1	187	7083_151_104	169.1	91	90	0	0.01	-	>Chr01	70922136			>scaffold_9	56801617		
1	188	6968_167_6	169.7	89	88	4	0.01	-								
1	189	9749_174_12	169.7	85	86	10	0.01	-								
1	190	7605_165_82	169.7	90	91	0	0.01	-								
1	191	13593_181_204	170.3	89	92	0	0.05	-								
1	192	221_172_181	173.1	77	74	30	0.06	-								
1	193	6740_169_177	173.1	89	91	1	0.02	-								
1	194	5808_153_128	173.1	90	91	0	0.01	-	>Chr01	72120823			>scaffold_9	57774456		
1	195	12996_159_54	173.7	91	90	0	0.01	-	>Chr01	72199368			>scaffold_9	57843041		
1	196	5431_177_142	176.5	83	85	13	0.02	-	>Chr01	72965786			>scaffold_9	58455324		
1	197	7786_169_7	177.1	91	90	0	0.01	-	>Chr01	73121022			>scaffold_9	58503999		

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
2	1	9874_172_241	0	83	98	0	1.24	-	>Chr02	77531364	>Chr01	21894809	>scaffold_2	49076437		
2	2	1059_137_175	0.6	82	99	0	1.6	-	>Chr02	76980090			>scaffold_2	48768862		
2	3	236_152_38_R	1.7	82	99	0	1.6	-	>Chr02	76686398	>Chr01	59975771	>scaffold_2	48521068	>scaffold_9	47313370
2	4	6046_150_114	1.7	82	99	0	1.6	-								
2	5	13595_166_204	2.8	79	99	3	2.25	-					>scaffold_5	42637022	>scaffold_2	47721814
2	6	10272_158_212	3.9	80	101	0	2.44	-	>Chr02	76051898			>scaffold_2	47984500		
2	7	**12456_142_265**	4.5	79	102	0	2.92	*								
2	8	**13204_159_38_R**	5.6	79	102	0	2.92	*								
2	9	**13510_146_110**	6.2	78	103	0	3.45	*								
2	10	**11302_166_118**	6.2	77	103	1	3.76	*	>Chr02	75781161			>scaffold_2	47779107		
2	11	**4187_152_145**	6.2	78	103	0	3.45	*	>Chr02	75289607	>Chr01	58404104				
2	12	**6359_176_187**	7.9	75	106	0	5.31	**	>Chr02	75122910			>scaffold_2	47213252		
2	13	**4972_173_146**	7.9	75	106	0	5.31	**								
2	14	**10951_135_147**	9.6	74	107	0	6.02	**	>Chr02	74653407			>scaffold_2	46876667		
2	15	**12152_168_236_R**	9.6	74	106	1	5.69	**								
2	16	**9577_169_129**	9.6	74	107	0	6.02	**	>Chr02	74309285						
2	17	**3528_168_92**	11	70	97	14	4.37	**								
2	18	8650_158_81	12.4	73	93	15	2.41	-								
2	19	**12468_173_227_R**	13.5	77	104	0	4.03	**	>Chr02	73349708			>scaffold_2	45787439		
2	20	**11351_171_266_R**	15.2	76	104	1	4.36	**	>Chr02	72674677			>scaffold_2	45136088		
2	21	**5130_136_247**	15.2	76	105	0	4.65	**	>Chr02	72629490			>scaffold_2	45114155		
2	22	**5784_160_179**	15.2	66	88	27	3.14	*	>Chr02	72592253			>scaffold_2	45075939		
2	23	**12088_164_11_R**	15.8	77	101	3	3.24	*	>Chr02	72572178			>scaffold_2	45043866		
2	24	**13431_177_230_R**	16.4	76	105	0	4.65	**	>Chr02	72435336						
2	25	**8412_187_54_R**	16.4	76	103	2	4.07	**	>Chr02	72342752			>scaffold_2	44857189		
2	26	**12659_147_180_R**	16.4	76	103	2	4.07	**	>Chr02	72342424			>scaffold_2	44856856		
2	27	**10443_154_233_R**	17	77	103	1	3.76	*								
2	28	**14907_135_235_R**	18.1	77	104	0	4.03	**	>Chr02	71978895			>scaffold_2	44545961		
2	29	**7690_159_156_R**	18.1	77	104	0	4.03	**	>Chr02	71916458			>scaffold_2	44497803		
2	30	6075_180_214_R	19.2	78	98	5	2.27	-	>Chr02	71430744	>Chr01	65829629	>scaffold_2	44025658	>scaffold_9	52732324
2	31	7989_150_263_R	20.9	80	101	0	2.44	-	>Chr02	70750032			>scaffold_2	43397554		
2	32	12856_159_87	20.9	80	100	1	2.22	-								
2	33	5307_151_150_R	22.6	79	100	2	2.46	-	>Chr02	70468677						
2	34	**8655_134_58_R**	24.3	64	96	21	6.4	**								
2	35	**128_144_184_R**	26	76	104	1	4.36	**	>Chr02	69891682			>scaffold_2	42579699		
2	36	**788_176_221_R**	27.1	77	103	1	3.76	*								
2	37	**2221_167_112**	29.9	75	101	5	3.84	*								
2	38	**6449_171_257**	30.5	77	104	0	4.03	**								
2	39	**7769_142_101**	33.3	78	103	0	3.45	*								
2	40	**1181_138_218**	33.3	78	103	0	3.45	*								
2	41	**9833_136_182**	33.3	78	103	0	3.45	*	>Chr02	68731747			>scaffold_2	41440766		
2	42	**11582_170_175**	33.3	78	103	0	3.45	*	>Chr02	68613621			>scaffold_2	41330859		
2	43	**9998_185_96**	33.3	77	102	2	3.49	*	>Chr02	68353875			>scaffold_2	41071728		
2	44	**153_135_23**	33.3	78	103	0	3.45	*	>Chr06	59473077	>Chr02	68318543	>scaffold_2	41031612		
2	45	**12569_148_191_R**	33.3	78	103	0	3.45	*	>Chr02	68024030			>scaffold_2	40713424		
2	46	**14350_145_16**	35.5	78	103	0	3.45	*	>Chr02	66946882			>scaffold_2	39499483		
2	47	**8013_168_256_R**	35.5	78	101	2	2.96	*								
2	48	**13445_175_77_R**	36.1	74	101	6	4.17	**								
2	49	**1226_161_115**	37.2	77	104	0	4.03	**								
2	50	**8285_159_11**	38.9	78	103	0	3.45	*	>Chr02	65668326			>scaffold_2	38147996		
2	51	**1252_173_107**	38.9	78	103	0	3.45	*					>scaffold_2	37925776		
2	52	**9034_170_191_R**	40	77	103	1	3.76	*								
2	53	**1041_178_122_R**	40	76	101	4	3.53	*	>Chr02	64870733			>scaffold_2	37365559		
2	54	**5142_183_239_R**	40	78	103	0	3.45	*	>Chr02	64866923						
2	55	4610_175_205	40	66	81	34	1.53	-								
2	56	**1734_151_83**	41.2	74	96	11	2.85	*								
2	57	**5458_167_97**	44.4	74	107	0	6.02	**								
2	58	**4178_161_89**	45	70	101	10	5.62	**	>Chr02	62761126			>scaffold_2	34713045		
2	59	**6189_149_8**	48.3	71	110	0	8.4	****								
2	60	**9298_139_38**	48.9	60	87	34	4.96	**								
2	61	**12393_157_75**	49.5	71	110	0	8.4	****								
2	62	**8360_162_121**	52.8	71	110	0	8.4	****								
2	63	**4724_164_78**	55.6	71	100	10	4.92	**								
2	64	**13316_163_67**	57.3	71	110	0	8.4	****	>Chr02	60055842			>scaffold_2	31408758		
2	65	**13314_132_69**	57.3	71	110	0	8.4	****	>Chr02	60055475			>scaffold_2	31408394		

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
2	66	**9526_177_84**	57.3	71	109	1	8.02	****								
2	67	**4119_173_53**	57.3	71	110	0	8.4	****								
2	68	**2383_182_100**	57.9	70	108	3	8.11	****								
2	69	5569_140_119	59.6	67	83	31	1.71	-	>Chr01	7169253			>scaffold_2	32261828		
2	70	**6411_161_140**	60.7	71	110	0	8.4	****	>Chr02	59050506			>scaffold_2	30489499		
2	71	3529_165_189	67.9	74	106	1	5.69	**								
2	72	**W_2599_150_172**	68.5	73	106	2	6.08	**	>Chr02	57665806			>scaffold_2	29081357		
2	73	**1417_157_129**	69.6	75	104	2	4.7	**	>Chr02	57154179			>scaffold_2	28771591		
2	74	**3722_174_171**	74	77	104	0	4.03	**								
2	75	**W_15187_140_131**	75.7	68	89	24	2.81	*								
2	76	**921_179_76_R**	77.9	72	109	0	7.56	***	>Chr02	53895312			>scaffold_2	26616080		
2	77	**5652_161_265_R**	77.9	72	109	0	7.56	***	>Chr02	53876372			>scaffold_2	26629412		
2	78	**5422_136_59_R**	77.9	72	109	0	7.56	***								
2	79	**9274_173_82**	78.7	72	98	11	3.98	**								
2	80	**778_146_185**	79.5	73	108	0	6.77	***								
2	81	**9923_178_167_R**	79.5	73	108	0	6.77	***								
2	82	**W_1206_168_25**	81.4	62	109	10	12.92	******								
2	83	**11312_179_50_R**	84.5	74	103	4	4.75	**								
2	84	**6632_164_208_R**	85.6	73	107	1	6.42	**								
2	85	**8854_183_148_R**	85.6	74	107	0	6.02	**								
2	86	**11412_156_193_R**	85.6	74	106	1	5.69	**								
2	87	**11307_160_194_R**	85.6	74	107	0	6.02	**								
2	88	**4929_178_73_R**	86.4	72	101	8	4.86	**								
2	89	**5671_145_45**	87.2	75	105	1	5	**								
2	90	**W_12574_144_206**	87.9	44	106	31	25.63	*******								
2	91	**288_133_229**	90	76	105	0	4.65	**	>Chr01	5426724	>Chr02	19818696	>scaffold_2	15436351	>scaffold_5	11452256
2	92	**13470_160_201_R**	91.9	74	106	1	5.69	**								
2	93	**5557_180_206_R**	95	75	106	0	5.31	**								
2	94	**13365_151_210_R**	95	75	106	0	5.31	**	>Chr02	13730762						
2	95	**7745_184_9_R**	95	74	104	3	5.06	**								
2	96	**7808_180_83_R**	95	75	105	1	5	**								
2	97	7779_178_92	105.7	81	100	0	1.99	-								
2	98	11658_160_17	107.4	82	99	0	1.6	-					>scaffold_8	1804096	>scaffold_2	3182152
2	99	9514_171_205	107.4	82	99	0	1.6	-								
2	100	13742_146_9	110.1	76	92	13	1.52	-					>scaffold_2	9176726		
2	101	372_175_67	112.2	79	96	6	1.65	-	>Chr02	3325317			>scaffold_2	2686141		
2	102	9850_181_135	121.9	80	101	0	2.44	-	>Chr02	2808861			>scaffold_2	2353470		
2	103	**9798_158_124**	121.9	78	101	2	2.96	*	>Chr02	2729995			>scaffold_2	2301619		
2	104	**9205_181_102**	123.6	77	101	3	3.24	*								
2	105	7787_167_74	132	79	100	2	2.46	-								
2	106	4308_159_210	133.1	82	99	0	1.6	-								
2	107	6073_182_129	134.8	85	96	0	0.67	-								
2	108	9673_185_99	137.6	90	91	0	0.01	-								
2	109	10784_164_6	138.7	90	91	0	0.01	-								
2	110	1279_151_187	138.7	90	91	0	0.01	-	>Chr02	1091193			>scaffold_2	366134		
2	111	4981_181_82	143.1	88	93	0	0.14	-	>Chr02	811303			>scaffold_2	180653		
2	112	9087_136_103	144.4	83	74	24	0.52	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
3	1	**W_10683_150_125**	0	81	70	30	0.8	-								
3	2	13273_165_208_R	0.7	98	83	0	1.24	-	>Chr03	73907548			>scaffold_7	22966715		
3	3	233_134_66	1.3	97	84	0	0.93	-								
3	4	2870_133_16	3	94	87	0	0.27	-								
3	5	9476_172_30_R	5.2	92	89	0	0.05	-	>Chr03	73228812			>scaffold_5	46468427		
3	6	13045_181_168_R	5.2	92	89	0	0.05	-	>Chr03	73132392	>Chr01	58359278	>scaffold_5	46367173	>scaffold_9	45964235
3	7	10835_174_146_R	5.8	91	89	1	0.02	-	>Chr03	73081877			>scaffold_1	7438842		
3	8	4372_156_160_R	5.8	91	90	0	0.01	-								
3	9	1376_156_96_R	8.6	74	85	22	0.76	-	>Chr09	46176354	>Chr03	10684083	>scaffold_5	45824341	>scaffold_3	23641587
3	10	4222_161_172_R	8.6	90	91	0	0.01	-	>Chr03	72409006			>scaffold_5	45786885		
3	11	11094_168_148_R	11.9	90	91	0	0.01	-								
3	12	7726_161_199_R	14.1	84	92	5	0.36	-								
3	13	13166_171_142_R	16.9	89	92	0	0.05	-					>scaffold_5	45148426		
3	14	10070_172_115_R	18	86	91	4	0.14	-								
3	15	1694_159_138_R	18	86	89	6	0.05	-					>scaffold_5	44997180		
3	16	4550_136_142_R	19.1	87	94	0	0.27	-	>Chr03	70155809	>Chr01	31552941	>scaffold_5	43822653		
3	17	8868_158_136_R	21.3	89	92	0	0.05	-	>Chr03	69423202	>Chr09	49936213	>scaffold_5	43244031	>scaffold_3	20053480
3	18	1668_174_7_R	23	90	90	1	0	-								
3	19	12255_171_93_R	23	90	91	0	0.01	-	>Chr03	69140410	>Chr09	50170883	>scaffold_5	42965851		
3	20	5358_161_96_R	23	75	85	21	0.62	-	>Chr03	69099402						
3	21	13091_156_258_R	23	90	91	0	0.01	-	>Chr03	69064258			>scaffold_5	42879535		
3	22	11836_138_56_R	24.1	90	91	0	0.01	-								
3	23	11296_152_183_R	24.1	79	83	19	0.1	-								
3	24	10643_145_43_R	24.1	68	83	30	1.49	-								
3	25	7546_177_113_R	25.7	79	89	13	0.6	-								
3	26	804_148_30_R	27.4	90	91	0	0.01	-								
3	27	10135_175_79_R	28.2	89	89	3	0	-	>Chr03	68014568			>scaffold_5	41954951		
3	28	10265_172_66_R	29	85	86	10	0.01	-								
3	29	2174_169_57_R	30.1	93	88	0	0.14	-	>Chr03	67975365			>scaffold_5	41914433		
3	30	1883_158_113_R	30.1	92	87	2	0.14	-	>Chr03	67964158			>scaffold_5	41903694		
3	31	5867_166_95_R	31.4	78	83	20	0.16	-								
3	32	88_163_54	34.6	83	81	17	0.02	-								
3	33	7620_171_240	35.2	90	89	2	0.01	-	>Chr08	45308850						
3	34	6707_133_129_R	35.2	92	87	2	0.14	-	>Chr05	48459979			>scaffold_8	26843855		
3	35	**W_1098_133_150**	36.9	92	77	12	1.33	-								
3	36	4812_162_151	36.9	85	87	9	0.02	-								
3	37	9347_177_127	38	93	87	1	0.2	-	>Chr03	66129543						
3	38	3007_168_250	38.6	94	87	0	0.27	-	>Chr03	65897845			>scaffold_5	39889567		
3	39	12975_177_99	41.4	95	86	0	0.45	-								
3	40	10937_152_194	43.5	93	81	7	0.83	-								
3	41	1836_170_98	44.2	96	79	6	1.65	-	>Chr03	73958227	>Chr09	5878439	>scaffold_5	46958729		
3	42	12929_170_231	44.5	98	82	1	1.42	-					>scaffold_1	41993102		
3	43	7985_149_132	44.8	99	82	0	1.6	-								
3	44	**W_695_147_39**	45.9	99	81	1	1.8	-								
3	45	12405_167_21_R	46.5	98	81	2	1.61	-	>Chr03	63383448			>scaffold_5	37505550		
3	46	5372_184_200	46.5	97	82	2	1.26	-								
3	47	10191_178_186	46.5	92	77	12	1.33	-	>Chr03	63242573			>scaffold_5	37353609		
3	48	13737_136_200_R	46.5	98	83	0	1.24	-								
3	49	**896_142_182_R**	48.7	102	79	0	2.92	*	>Chr03	62983598						
3	50	**8719_158_39**	49.3	103	78	0	3.45	*	>Chr03	62942483			>scaffold_5	36969418		
3	51	**204_167_12**	49.9	102	79	0	2.92	*								
3	52	**7123_152_90**	49.9	102	78	1	3.2	*								
3	53	**12678_166_189_R**	49.9	102	79	0	2.92	*	>Chr03	62340715	>Chr07	943655	>scaffold_5	36348988		
3	54	**1249_175_101**	49.9	100	77	4	2.99	*	>Chr01	5086406			>scaffold_9	3800220		
3	55	**5267_177_96**	49.9	102	79	0	2.92	*	>Chr03	62256454			>scaffold_5	36265412		
3	56	152_183_115	50.5	101	80	0	2.44	-								
3	57	3899_181_130_R	50.5	100	79	2	2.46	-								
3	58	**W_8814_155_240**	50.5	101	77	3	3.24	*								
3	59	**W_7999_171_253_R**	50.5	98	79	4	2.04	-								
3	60	13899_142_26_R	50.5	100	80	1	2.22	-	>Chr03	61825203			>scaffold_5	35878898		
3	61	1393_174_8_R	50.5	101	80	0	2.44	-								
3	62	**386_178_233_R**	51.1	102	79	0	2.92	*								
3	63	**9683_170_35_R**	52.2	102	79	0	2.92	*	>Chr03	61053333			>scaffold_5	34847761		
3	64	**4947_144_17**	52.2	102	79	0	2.92	*								
3	65	**10659_167_86_R**	54.4	104	77	0	4.03	**								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
3	66	**11297_177_187_R**	55.5	106	74	1	5.69	**	>Chr03	60151722	>Chr10	992153	>scaffold_7	19105550	>scaffold_5	33935715
3	67	**9017_162_262_R**	55.5	106	75	0	5.31	**	>Chr03	60150976			>scaffold_5	33934970	>scaffold_7	19104801
3	68	**9253_164_159_R**	55.5	106	75	0	5.31	**	>Chr03	60142594			>scaffold_7	19095466	>scaffold_5	33927859
3	69	**5477_168_189_R**	56.1	89	67	25	3.1	*	>Chr03	60140774			>scaffold_5	33925526	>scaffold_7	19093500
3	70	**9149_168_240_R**	56.7	105	75	1	5	**								
3	71	**8946_147_53_R**	56.7	106	75	0	5.31	**								
3	72	**4591_166_46_R**	58.4	108	72	1	7.2	***	>Chr03	59273201	>Chr09	57068323	>scaffold_5	32937826	>scaffold_3	10713641
3	73	**7167_172_164_R**	59	102	67	12	7.25	***								
3	74	**8087_146_108_R**	60.1	106	73	2	6.08	**								
3	75	**W_955_135_131_R**	65.7	101	67	13	6.88	***								
3	76	10138_163_52_R	71.1	91	76	14	1.35	-								
3	77	**5337_171_18_R**	73.5	105	75	1	5	**								
3	78	**11000_156_170_R**	73.5	106	74	1	5.69	**	>Chr03	55752412			>scaffold_5	28947903		
3	79	**W_8683_148_115_R**	73.5	105	75	1	5	**	>Chr03	55503216			>scaffold_5	28532226		
3	80	**14163_159_5_R**	73.5	106	75	0	5.31	**	>Chr03	55488048			>scaffold_5	28504373		
3	81	**7866_172_15_R**	77.4	103	78	0	3.45	*								
3	82	4315_135_184_R	81.3	100	81	0	1.99	-								
3	83	206_144_196_R	81.3	100	81	0	1.99	-								
3	84	10305_165_176_R	82.4	98	82	1	1.42	-								
3	85	9536_172_216_R	87.9	86	80	15	0.22	-								
3	86	10370_181_75_R	88.5	97	84	0	0.93	-								
3	87	5548_170_260_R	88.5	97	84	0	0.93	-								
3	88	3065_184_169_R	88.5	97	84	0	0.93	-								
3	89	4462_184_66_R	88.5	95	81	5	1.11	-								
3	90	6462_147_78	89.6	95	81	5	1.11	-								
3	91	1064_164_136	89.6	88	74	19	1.21	-								
3	92	596_167_122	91.9	96	81	4	1.27	-	>Chr03	16190455			>scaffold_5	14075470		
3	93	**W_4217_152_159_R**	110.9	98	83	0	1.24	-					>scaffold_7	34990037		
3	94	8781_139_50_R	110.9	96	83	2	0.94	-								
3	95	9957_183_196_R	111.5	99	82	0	1.6	-								
3	96	4153_138_219_R	111.5	99	82	0	1.6	-								
3	97	4172_158_44_R	112.1	98	83	0	1.24	-	>Chr03	10831560			>scaffold_5	4863285		
3	98	8610_180_40_R	113.8	84	81	16	0.05	-								
3	99	6222_176_17_R	116	99	82	0	1.6	-								
3	100	508_167_122_R	117.7	100	81	0	1.99	-					>scaffold_5	7160527	>scaffold_6	288282
3	101	12440_151_143_R	119.4	99	82	0	1.6	-								
3	102	4152_159_109_R	121.6	96	84	1	0.8	-	>Chr03	7090456			>scaffold_5	7890630		
3	103	5272_164_123_R	121.6	97	84	0	0.93	-	>Chr03	7016980			>scaffold_5	7932230		
3	104	3495_185_115_R	122.7	97	84	0	0.93	-	>Chr03	6936952			>scaffold_5	7996796		
3	105	3729_179_52_R	122.7	95	81	5	1.11	-	>Chr03	6833662						
3	106	678_136_210_R	123.8	97	84	0	0.93	-								
3	107	6849_173_110_R	123.8	92	83	6	0.46	-	>Chr03	6414761						
3	108	3726_149_94_R	125.4	86	84	11	0.02	-								
3	109	10938_142_134_R	127.1	97	84	0	0.93	-								
3	110	**W_4873_132_194_R**	128.2	99	82	0	1.6	-								
3	111	2646_176_171_R	131	96	85	0	0.67	-	>Chr03	4243766			>scaffold_5	10505058		
3	112	246_151_50	132.7	94	80	7	1.13	-	>Chr02	19820528	>Chr01	5428332	>scaffold_5	11450567	>scaffold_2	15438146
3	113	3949_168_18	133.3	98	83	0	1.24	-	>Chr03	3392454	>Chr09	8006628	>scaffold_5	11547324	>scaffold_3	7265214
3	114	4686_140_177_R	137.2	101	80	0	2.44	-								
3	115	3132_180_156	137.2	100	80	1	2.22	-								
3	116	1775_173_24	139	85	73	23	0.91	-								
3	117	15626_176_199_R	143.3	92	89	0	0.05	-	>Chr03	2492278			>scaffold_5	12466270		
3	118	10941_168_149_R	145	93	88	0	0.14	-								
3	119	10263_163_126_R	145.6	94	87	0	0.27	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
4	1	3333_179_140_R	0	84	97	0	0.93	-	>Chr01	2186054						
4	2	11207_150_97_R	0	84	97	0	0.93	-								
4	3	408_154_100_R	2.8	83	98	0	1.24	-								
4	4	9926_158_221_R	2.8	83	97	1	1.09	-	>Chr08	1241762			>scaffold_1	10257095		
4	5	10866_166_260_R	2.8	83	98	0	1.24	-	>Chr04	861095						
4	6	9967_179_200_R	2.8	83	98	0	1.24	-								
4	7	12638_166_97_R	5	85	96	0	0.67	-								
4	8	3674_184_252_R	5.6	84	97	0	0.93	-								
4	9	6938_147_54_R	6.2	83	93	5	0.57	-								
4	10	9631_172_119_R	6.2	85	96	0	0.67	-								
4	11	13741_145_126_R	8.7	84	96	1	0.8	-					>scaffold_1	8901998		
4	12	13289_152_167	10.6	83	94	4	0.68	-								
4	13	4003_134_47	12.8	83	91	7	0.37	-								
4	14	12394_147_159	13.4	84	97	0	0.93	-	>Chr04	2785313			>scaffold_1	8285845		
4	15	1291_176_134	13.4	84	97	0	0.93	-					>scaffold_1	8284531		
4	16	1158_132_64	13.4	84	97	0	0.93	-	>Chr04	2831579			>scaffold_1	8237131		
4	17	7755_177_216	14	83	98	0	1.24	-	>Chr04	2946816			>scaffold_1	8159326		
4	18	3848_168_34	14	83	98	0	1.24	-								
4	19	3735_143_102	16.7	73	91	17	1.98	-								
4	20	**2883_169_148**	17.4	79	102	0	2.92	*	>Chr04	3281639						
4	21	**3507_178_97_R**	17.4	79	102	0	2.92	*								
4	22	**8287_149_80**	17.4	79	102	0	2.92	*	>Chr04	3709939			>scaffold_24	44271	>scaffold_1	7583106
4	23	**11375_177_128**	17.4	79	102	0	2.92	*								
4	24	425_153_3_R	20.2	80	101	0	2.44	-								
4	25	3547_181_41	20.2	80	96	5	1.45	-								
4	26	**12931_174_22**	20.2	78	101	2	2.96	*	>Chr04	4847517	>Chr10	55882814	>scaffold_1	6315234		
4	27	4785_163_115	20.8	81	100	0	1.99	-								
4	28	9300_135_43_R	26.9	86	95	0	0.45	-	>Chr04	5316023			>scaffold_1	5917433		
4	29	15431_162_213	30.8	85	96	0	0.67	-	>Chr04	5724905			>scaffold_1	5529930		
4	30	13958_142_40	33.5	85	92	4	0.28	-								
4	31	677_180_98	34.2	85	96	0	0.67	-	>Chr04	6504658			>scaffold_1	4879838		
4	32	5207_145_195	34.8	83	97	1	1.09	-	>Chr04	6686697			>scaffold_1	4655250		
4	33	13462_147_219	34.8	84	97	0	0.93	-								
4	34	8230_166_219	35.4	85	96	0	0.67	-	>Chr04	6855097			>scaffold_1	4532739		
4	35	11376_135_37	37.1	85	95	1	0.56	-								
4	36	4921_135_256_R	37.7	82	94	5	0.82	-								
4	37	3020_180_56_R	39.9	85	96	0	0.67	-	>Chr10	8999734						
4	38	548_170_114_R	40.5	84	95	2	0.68	-								
4	39	10720_146_143	44.4	85	95	1	0.56	-	>Chr02	14974744	>Chr09	3087815	>scaffold_2	11734006	>scaffold_3	3392291
4	40	8513_164_12	44.4	85	96	0	0.67	-	>Chr04	10238423			>scaffold_1	1781396		
4	41	**12059_168_21_R**	47.7	56	80	45	4.24	**	>Chr09	54254862			>scaffold_1	1251307	>scaffold_3	14035955
4	42	10741_144_49_R	49.4	78	91	12	1	-								
4	43	4567_148_130_R	51.1	76	90	15	1.18	-	>Chr04	12227698						
4	44	14713_147_31_R	52.2	82	98	1	1.42	-								
4	45	10396_163_227_R	52.8	84	97	0	0.93	-								
4	46	3619_132_163_R	52.8	84	86	11	0.02	-								
4	47	**W_2249_168_140_R**	53.1	82	95	4	0.95	-								
4	48	9140_159_116_R	53.4	83	97	1	1.09	-								
4	49	4145_185_6_R	54.5	80	98	3	1.82	-								
4	50	3315_162_61_R	55.1	81	91	9	0.58	-								
4	51	10605_169_130	55.7	83	98	0	1.24	-	>Chr04	16044695			>scaffold_1	11207069		
4	52	13215_179_217	55.7	83	98	0	1.24	-								
4	53	11425_169_229	56.3	84	97	0	0.93	-	>Chr08	9642636	>Chr04	60752987	>scaffold_7	32537895	>scaffold_5	16240334
4	54	1806_132_105	57.4	86	95	0	0.45	-					>scaffold_4	20626432		
4	55	475_143_131	58	85	96	0	0.67	-								
4	56	10128_137_34_R	58	81	94	6	0.97	-								
4	57	1548_184_140	58	82	89	10	0.29	-	>Chr04	19075067			>scaffold_1	12846711		
4	58	8008_137_200_R	58	85	95	1	0.56	-								
4	59	5028_167_267_R	58	85	96	0	0.67	-								
4	60	8417_160_122_R	58	85	95	1	0.56	-								
4	61	7671_147_182_R	58	85	96	0	0.67	-								
4	62	9360_155_138_R	58.6	86	94	1	0.36	-	>Chr04	47178731			>scaffold_1	23769730		
4	63	6639_171_36	58.6	86	95	0	0.45	-								
4	64	2021_182_213_R	60.8	86	95	0	0.45	-								
4	65	5347_149_131	65.8	87	92	2	0.14	-	>Chr04	50631464			>scaffold_1	25809789		

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
4	66	8014_154_111	65.8	87	94	0	0.27	-	>Chr04	50731835			>scaffold_1	25933304		
4	67	13083_163_240_R	65.8	87	94	0	0.27	-					>scaffold_1	26540635		
4	68	6881_153_187_R	65.8	71	82	28	0.79	-								
4	69	7924_161_63	68.6	88	92	1	0.09	-								
4	70	12086_158_246_R	69.7	88	93	0	0.14	-								
4	71	11686_147_254	70.3	89	91	1	0.02	-								
4	72	4143_185_76	70.3	89	92	0	0.05	-	>Chr04	52122500			>scaffold_1	27440253		
4	73	11229_175_210_R	71.4	87	94	0	0.27	-	>Chr04	52337532			>scaffold_1	27669650		
4	74	10517_169_238_R	72	86	95	0	0.45	-	>Chr04	52499058	>Chr06	46976093	>scaffold_1	27709098	>scaffold_7	20966991
4	75	7379_141_169	72	86	94	1	0.36	-								
4	76	5767_168_195_R	72	86	95	0	0.45	-	>Chr04	52815488			>scaffold_1	27894736		
4	77	7668_163_51	75.9	85	93	3	0.36	-					>scaffold_1	28261569	>scaffold_7	21437833
4	78	14305_136_198	76.5	86	95	0	0.45	-	>Chr04	53158172	>Chr06	47653065	>scaffold_1	28261767		
4	79	5666_167_127	79.3	85	96	0	0.67	-	>Chr04	53653885			>scaffold_1	28831103		
4	80	287_168_11	79.9	83	90	8	0.28	-								
4	81	3092_170_70_R	81	84	97	0	0.93	-	>Chr04	53839416			>scaffold_1	29017403		
4	82	6894_155_64	81.6	83	86	12	0.05	-	>Chr04	54160416	>Chr06	49289770	>scaffold_1	29341616		
4	83	4228_172_55	81.6	85	96	0	0.67	-	>Chr04	54338829						
4	84	5709_156_121_R	82.7	74	87	20	1.05	-								
4	85	10587_137_81_R	84.4	88	93	0	0.14	-	>Chr04	55056846			>scaffold_1	30193263		
4	86	6001_175_127_R	85.5	86	95	0	0.45	-								
4	87	732_171_110_R	86.1	84	94	3	0.56	-								
4	88	11090_178_181_R	86.7	86	95	0	0.45	-	>Chr04	55145257			>scaffold_1	30272700		
4	89	12674_162_87_R	86.7	86	95	0	0.45	-	>Chr04	55446627			>scaffold_1	30601947		
4	90	6963_155_112_R	86.7	86	95	0	0.45	-	>Chr04	55446810			>scaffold_1	30589724		
4	91	542_154_83_R	86.7	86	95	0	0.45	-	>Chr04	56052414			>scaffold_1	31552854		
4	92	8020_150_95_R	86.7	86	95	0	0.45	-								
4	93	**W_8603_170_251_R**	86.7	85	95	1	0.56	-								
4	94	3348_167_3_R	86.7	86	95	0	0.45	-								
4	95	14001_135_170_R	91.1	90	91	0	0.01	-	>Chr04	56312574			>scaffold_1	31744131		
4	96	5098_133_105_R	93.3	88	91	2	0.05	-								
4	97	11121_182_136_R	97.7	88	91	2	0.05	-								
4	98	13953_137_17_R	100.5	89	92	0	0.05	-								
4	99	9268_135_79_R	100.5	89	92	0	0.05	-								
4	100	607_169_134_R	102.7	93	88	0	0.14	-								
4	101	5176_171_8_R	102.7	93	88	0	0.14	-	>Chr04	62155477			>scaffold_1	34122528		
4	102	12271_142_133_R	103.3	90	89	2	0.01	-								
4	103	15273_158_257_R	104.4	92	89	0	0.05	-								
4	104	8665_166_56_R	105.1	80	79	22	0.01	-								
4	105	10339_177_15	107.8	90	90	1	0	-	>Chr04	63827295						
4	106	822_169_39	111.7	93	88	0	0.14	-								
4	107	4322_133_46	113.4	96	85	0	0.67	-								
4	108	9018_178_5	113.4	96	85	0	0.67	-								
4	109	11128_169_21	119.5	94	86	1	0.36	-								
4	110	11556_158_71	120.6	96	83	2	0.94	-								
4	111	564_148_85	122.3	96	85	0	0.67	-					>scaffold_9	8679868		
4	112	13725_147_199	126.5	86	80	15	0.22	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
5	1	7602_164_242	0	90	91	0	0.01	-								
5	2	12953_174_93	2.2	88	93	0	0.14	-								
5	3	1628_143_159	5.3	90	77	14	1.01	-					>scaffold_1	6848574		
5	4	**W_10132_152_89**	7.8	80	76	25	0.1	-								
5	5	11269_182_87	8.9	93	76	12	1.71	-								
5	6	3116_133_61	12	89	87	5	0.02	-	>Chr05	60615102			>scaffold_8	4572966		
5	7	7306_139_152	13.9	92	86	3	0.2	-								
5	8	3141_137_117	14.7	89	77	15	0.87	-					>scaffold_9	47467352		
5	9	7398_153_163	15.5	96	85	0	0.67	-								
5	10	1609_162_163_R	15.5	78	83	20	0.16	-								
5	11	85_157_178	15.5	96	84	1	0.8	-								
5	12	4671_133_140	15.5	96	85	0	0.67	-								
5	13	4730_183_107_R	15.5	96	85	0	0.67	-								
5	14	13008_137_12	16.1	95	83	3	0.81	-								
5	15	6330_159_131_R	17.8	96	84	1	0.8	-								
5	16	163_135_132_R	17.8	95	85	1	0.56	-								
5	17	793_142_86	19.5	95	86	0	0.45	-	>Chr05	60099193			>scaffold_8	38132472		
5	18	1643_164_138_R	20.1	94	87	0	0.27	-	>Chr05	59954683			>scaffold_8	37859049	>scaffold_7	21176266
5	19	986_159_26_R	20.7	93	86	2	0.27	-								
5	20	9912_172_112	30.1	92	83	6	0.46	-								
5	21	10507_175_229	30.8	93	87	1	0.2	-								
5	22	**W_3924_177_148**	32	88	87	6	0.01	-								
5	23	9059_169_124_R	37.4	88	90	3	0.02	-								
5	24	12448_154_53	43	93	86	2	0.27	-								
5	25	**W_7454_165_205**	43.6	93	66	22	4.58	**								
5	26	752_174_105	46.8	96	85	0	0.67	-	>Chr05	55069368			>scaffold_8	31074256		
5	27	2323_163_92_R	46.8	96	85	0	0.67	-								
5	28	**W_2041_169_41_R**	49	95	85	1	0.56	-								
5	29	179_156_172_R	50.1	96	85	0	0.67	-								
5	30	**W_10582_148_89_R**	51.2	88	80	13	0.38	-	>Chr03	36128173						
5	31	11486_140_130_R	51.2	94	84	3	0.56	-								
5	32	8539_151_121_R	51.8	84	88	9	0.09	-	>Chr03	65416900			>scaffold_5	39437047		
5	33	**W_6329_174_219_R**	53.5	93	87	1	0.2	-								
5	34	2671_157_107	64	94	73	14	2.64	-								
5	35	11140_151_5	70.1	91	73	17	1.98	-								
5	36	8773_170_18	72.6	92	89	0	0.05	-								
5	37	9994_155_130_R	75.9	91	88	2	0.05	-								
5	38	600_186_228_R	77	90	91	0	0.01	-								
5	39	9531_165_163	80.9	93	88	0	0.14	-								
5	40	10355_184_24_R	80.9	92	88	1	0.09	-								
5	41	5840_152_249	81.5	94	87	0	0.27	-								
5	42	391_145_191	81.5	94	87	0	0.27	-								
5	43	**6342_145_94**	84.3	94	56	31	9.63	****								
5	44	**3099_154_59**	88.6	92	69	20	3.29	*								
5	45	11174_168_197	90.2	85	82	14	0.05	-								
5	46	**4179_166_134**	93.7	100	68	13	6.1	**								
5	47	8371_153_106_R	98	79	81	21	0.02	-								
5	48	5681_159_221	98.7	93	87	1	0.2	-								
5	49	9802_177_150_R	98.7	90	86	5	0.09	-								
5	50	2444_140_100	98.7	93	88	0	0.14	-								
5	51	3650_177_63_R	98.7	93	88	0	0.14	-	>Chr07	21772109						
5	52	6724_151_115	98.7	93	88	0	0.14	-	>Chr05	41066185						
5	53	2893_176_150	98.7	93	88	0	0.14	-					>scaffold_8	21605669		
5	54	5954_181_44	98.7	93	88	0	0.14	-	>Chr09	25632083			>scaffold_8	16050307		
5	55	79_169_187	98.7	93	87	1	0.2	-								
5	56	2689_153_65	98.7	93	88	0	0.14	-								
5	57	6022_183_156	98.7	93	88	0	0.14	-								
5	58	480_135_132	99.3	94	87	0	0.27	-								
5	59	1896_136_104	101.5	81	69	31	0.96	-								
5	60	5524_175_194	103.2	95	86	0	0.45	-								
5	61	**W_1907_142_65**	104.3	95	85	1	0.56	-	>Chr05	14664827			>scaffold_8	10760587		
5	62	**W_10702_180_141**	104.3	94	83	4	0.68	-								
5	63	**W_5251_157_71_R**	105.6	87	79	15	0.39	-								
5	64	5052_162_98_R	110.4	94	87	0	0.27	-								
5	65	8697_144_76_R	112.6	78	77	26	0.01	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
5	66	158_162_106	115.9	90	88	3	0.02	-								
5	67	534_160_81_R	116.6	73	62	46	0.9	-								
5	68	**W_11206_172_157_R**	118.7	69	85	27	1.66	-								
5	69	**W_264_139_247_R**	120.4	86	88	7	0.02	-					>scaffold_8	34289088	>scaffold_3	16151187
5	70	144_163_158_R	121	90	90	1	0	-	>Chr06	58413111			>scaffold_7	31064060		
5	71	10554_149_125	121.6	92	89	0	0.05	-	>Chr05	8951020			>scaffold_8	6701905		
5	72	8063_174_43_R	122.2	93	88	0	0.14	-								
5	73	4700_175_218	123.9	94	87	0	0.27	-								
5	74	2948_145_17	123.9	94	87	0	0.27	-	>Chr05	8666303			>scaffold_8	6363560		
5	75	4023_184_213	124.5	92	86	3	0.2	-								
5	76	5770_178_121_R	128.9	90	89	2	0.01	-								
5	77	6825_167_153	128.9	91	87	3	0.09	-								
5	78	11117_151_267	128.9	91	90	0	0.01	-								
5	79	**W_15804_133_174_R**	129.5	87	89	5	0.02	-								
5	80	10348_180_30	130.1	92	87	2	0.14	-	>Chr03	3752679			>scaffold_5	11164924		
5	81	836_168_32	131.8	82	81	18	0.01	-								
5	82	10133_168_131	131.8	92	89	0	0.05	-								
5	83	4532_133_165_R	133.5	93	87	1	0.2	-	>Chr05	4479541			>scaffold_8	3358507		
5	84	4235_171_32	135.2	92	89	0	0.05	-					>scaffold_8	3035611		
5	85	4125_147_85_R	136.9	83	90	8	0.28	-					>scaffold_8	3035302		
5	86	4008_165_29	140.8	89	91	1	0.02	-								
5	87	7251_187_88	141.9	90	88	3	0.02	-								
5	88	7170_179_97	145.8	89	92	0	0.05	-								
5	89	7907_176_161_R	148	86	91	4	0.14	-								
5	90	4202_171_173	149.1	89	92	0	0.05	-	>Chr05	2574395			>scaffold_8	1574518	>scaffold_1	28511090
5	91	2037_167_34	150.2	78	79	24	0.01	-								
5	92	6097_173_216_R	151.9	90	90	1	0	-								
5	93	4428_171_108	153.6	89	92	0	0.05	-								
5	94	6571_158_38_R	154.2	88	93	0	0.14	-								
5	95	10940_156_25	156.4	90	89	2	0.01	-								
5	96	347_163_108	158.1	91	90	0	0.01	-								
5	97	10539_144_122_R	163.1	87	92	2	0.14	-								
5	98	8080_181_45	165.9	79	89	13	0.6	-					>scaffold_8	102851	>scaffold_7	35878537
5	99	12598_155_5_R	165.9	87	93	1	0.2	-	>Chr05	294154	>Chr08	334096				
5	100	2779_178_210	165.9	87	94	0	0.27	-	>Chr08	123769	>Chr05	93294	>scaffold_6	33385073		
5	101	3802_167_202	165.9	87	94	0	0.27	-	>Chr08	43408	>Chr05	51006	>scaffold_7	35900960	>scaffold_8	76214
5	102	**W_6050_167_244**	168.1	88	91	2	0.05	-								
5	103	3465_139_196	170.9	90	90	1	0	-								
5	104	**9422_175_93**	178.7	100	56	25	12.41	******								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
6	1	**10915_149_204**	0	112	69	0	10.22	****								
6	2	**6152_169_229**	0.6	113	68	0	11.19	*****	>Chr06	57885496			>scaffold_7	30579364		
6	3	**5951_163_147**	6.5	96	57	28	9.94	****								
6	4	**3395_164_109**	8.4	109	72	0	7.56	***	>Chr06	57018754						
6	5	**4651_165_205**	9	108	73	0	6.77	***	>Chr06	56942643			>scaffold_7	29612953		
6	6	**7354_178_60**	12.9	109	72	0	7.56	***					>scaffold_9	14046376		
6	7	**4691_157_132**	16	95	66	20	5.22	**								
6	8	**2277_151_99**	16.8	104	74	3	5.06	**								
6	9	**6762_177_126**	16.8	106	73	2	6.08	**								
6	10	**13938_149_205**	18.5	103	78	0	3.45	*								
6	11	**15221_145_4**	18.5	103	78	0	3.45	*	>Chr03	5246411			>scaffold_7	26409813		
6	12	8583_187_34	19.1	100	79	2	2.46	-	>Chr06	53278277	>Chr01	10866010	>scaffold_2	22828164	>scaffold_7	23670529
6	13	**1035_137_68**	19.9	84	61	36	3.65	*								
6	14	**7412_135_72**	20.9	94	59	28	8.01	****								
6	15	**2984_173_159**	25.2	107	74	0	6.02	**	>Chr06	52214956			>scaffold_7	25397836		
6	16	**12009_173_199**	25.8	102	70	9	5.95	**	>Chr06	52040687			>scaffold_7	25187081		
6	17	**W_12595_133_1**	26.9	104	71	6	6.22	**	>Chr06	59928102			>scaffold_7	25152799		
6	18	**8124_143_145_R**	29.7	103	78	0	3.45	*								
6	19	4477_177_223_R	29.7	98	78	5	2.27	-								
6	20	**4167_168_41_R**	29.7	103	78	0	3.45	*	>Chr06	51176892			>scaffold_7	24443262		
6	21	**5752_183_76_R**	31.4	102	79	0	2.92	*								
6	22	**872_156_152_R**	31.4	102	77	2	3.49	*								
6	23	8778_178_5	32	99	79	3	2.25	-	>Chr06	49650104			>scaffold_7	22920738		
6	24	2956_154_26	32	101	80	0	2.44	-								
6	25	**W_9036_147_145**	34.2	95	77	9	1.88	-								
6	26	945_164_178	35.3	97	83	1	1.09	-	>Chr06	48747363			>scaffold_7	22126628		
6	27	9779_167_83	36.4	97	84	0	0.93	-								
6	28	10950_156_78	37	96	85	0	0.67	-	>Chr06	47674520			>scaffold_7	21448156		
6	29	**W_9504_172_97**	38.3	94	78	9	1.49	-								
6	30	8482_182_131	41.5	96	85	0	0.67	-								
6	31	**W_4049_161_182**	43.2	96	84	1	0.8	-								
6	32	9948_162_153	43.8	86	77	18	0.5	-								
6	33	9363_159_122	44.4	97	83	1	1.09	-	>Chr06	45792322			>scaffold_7	20130339		
6	34	8078_136_243	44.4	97	84	0	0.93	-	>Chr06	45227845			>scaffold_7	19927425		
6	35	9177_175_58	44.4	95	82	4	0.95	-								
6	36	7072_161_192	44.4	97	84	0	0.93	-								
6	37	9038_144_17	45.1	92	75	14	1.73	-	>super_13	2583426	>Chr03	33940074				
6	38	3879_168_124	49.4	95	84	2	0.68	-	>Chr06	44591842			>scaffold_7	19664195		
6	39	5032_164_71_R	56.6	86	80	15	0.22	-								
6	40	7534_156_239_R	57.7	95	85	1	0.56	-								
6	41	10575_180_128_R	58.3	96	85	0	0.67	-								
6	42	8205_162_131_R	58.3	95	85	1	0.56	-	>Chr04	56761444						
6	43	4648_178_184_R	58.3	96	85	0	0.67	-								
6	44	7868_155_144_R	58.3	95	85	1	0.56	-								
6	45	**W_10590_135_265_R**	61	83	76	22	0.31	-								
6	46	4853_166_107_R	61	94	86	1	0.36	-								
6	47	8667_167_162_R	61	95	86	0	0.45	-	>Chr06	53653615			>scaffold_2	5062408		
6	48	10589_181_123_R	61.6	94	87	0	0.27	-								
6	49	7724_171_196_R	62.7	94	87	0	0.27	-								
6	50	9929_134_66_R	62.7	94	87	0	0.27	-								
6	51	3323_173_244_R	63.3	93	88	0	0.14	-	>Chr06	37988490			>scaffold_7	17742497		
6	52	13069_182_99_R	64.4	91	90	0	0.01	-	>Chr06	37670143						
6	53	6245_155_25	66.6	82	80	19	0.02	-								
6	54	11513_173_153_R	68.5	87	84	10	0.05	-	>Chr04	4709242			>scaffold_1	6435285		
6	55	13611_136_81_R	70.7	71	82	28	0.79	-					>scaffold_7	22869472		
6	56	13957_173_237_R	73.9	69	88	24	2.3	-								
6	57	7203_138_154_R	76	92	89	0	0.05	-								
6	58	8601_160_194_R	76	92	89	0	0.05	-	>Chr06	32826493			>scaffold_7	17312798		
6	59	**W_5602_160_127**	77.7	74	85	22	0.76	-								
6	60	1796_170_169	78.8	82	82	17	0	-	>Chr06	26342472	>Chr05	50006957	>scaffold_7	16623925		
6	61	**W_10968_162_230_R**	79.4	92	89	0	0.05	-								
6	62	13042_186_85_R	80	92	88	1	0.09	-	>Chr06	10567957						
6	63	6291_163_249_R	80	91	88	2	0.05	-								
6	64	**W_6481_169_146_R**	80	92	86	3	0.2	-								
6	65	6351_151_94_R	81.1	80	79	22	0.01	-	>Chr06	6076586			>scaffold_7	9453044		

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
6	66	12011_156_159	82.7	76	80	25	0.1	-								
6	67	897_135_130	82.9	89	86	6	0.05	-								
6	68	796_162_248_R	84.4	91	90	0	0.01	-								
6	69	5620_176_245	85	90	91	0	0.01	-								
6	70	7931_168_131	85.6	89	92	0	0.05	-	>Chr06	4217790			>scaffold_7	11880449		
6	71	2440_161_9_R	87.8	85	91	5	0.2	-								
6	72	4488_156_36_R	90.6	93	86	2	0.27	-	>Chr01	71467637			>scaffold_3	20331079		
6	73	9153_172_230	92.3	91	87	3	0.09	-	>Chr04	3694908			>scaffold_1	7588454	>scaffold_24	2311
6	74	8005_183_138	97.9	89	91	1	0.02	-								
6	75	2296_177_202	99.6	89	74	18	1.38	-								
6	76	12958_155_140	101.8	94	87	0	0.27	-								
6	77	1595_142_99	101.8	94	87	0	0.27	-								
6	78	10509_170_172	101.8	94	85	2	0.45	-								
6	79	3271_158_186_R	103.7	87	73	21	1.23	-								
6	80	8377_160_150	113.2	105	62	14	11.07	*****								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
7	1	5636_167_120_R	0	77	98	6	2.52	-								
7	2	**3047_162_170_R**	2.3	78	103	0	3.45	*								
7	3	12615_182_211_R	3.4	78	98	5	2.27	-	>Chr07	1418172			>scaffold_6	7926238		
7	4	2214_162_258	3.4	80	101	0	2.44	-	>Chr07	1445709			>scaffold_6	7972123		
7	5	7767_175_201_R	4.5	80	101	0	2.44	-					>scaffold_1	196307		
7	6	1564_178_113_R	4.5	80	101	0	2.44	-	>Chr07	1738649			>scaffold_6	7540023		
7	7	4468_165_257	4.5	80	101	0	2.44	-	>Chr07	1745156			>scaffold_6	7533450		
7	8	**W_77_164_34_R**	4.5	80	100	1	2.22	-	>Chr07	1821446			>scaffold_6	7506880		
7	9	4224_167_49	4.5	70	88	23	2.05	-								
7	10	**1144_178_84_R**	6.1	77	104	0	4.03	**	>Chr07	1993989			>scaffold_6	7417243	>scaffold_1	6072292
7	11	**2972_149_131_R**	7.8	78	101	2	2.96	*								
7	12	**968_176_98_R**	7.8	78	101	2	2.96	*	>Chr07	2122792			>scaffold_6	7316487		
7	13	**4334_138_101**	7.8	78	103	0	3.45	*	>Chr07	2128417			>scaffold_6	7313092		
7	14	10188_142_26_R	8.4	79	101	1	2.69	-	>Chr03	65949041			>scaffold_1	37643032	>scaffold_5	39908708
7	15	**231_162_216**	8.4	79	102	0	2.92	*								
7	16	682_183_115_R	10.1	80	101	0	2.44	-					>scaffold_6	33606952		
7	17	5286_179_157	11.2	82	97	2	1.26	-	>Chr07	2764511			>scaffold_6	6561843		
7	18	7133_149_239	12.9	83	98	0	1.24	-								
7	19	10102_170_234	12.9	83	98	0	1.24	-	>Chr07	2879821			>scaffold_6	6238249		
7	20	7300_140_109	12.9	83	98	0	1.24	-								
7	21	9722_168_75_R	13.5	84	97	0	0.93	-								
7	22	6191_154_189	14.1	83	98	0	1.24	-								
7	23	8153_135_106_R	15.8	80	101	0	2.44	-	>Chr07	3481163			>scaffold_6	5597368		
7	24	887_178_53_R	18	82	99	0	1.6	-								
7	25	11135_161_109_R	18.6	83	98	0	1.24	-	>Chr07	4223553			>scaffold_6	4860188		
7	26	8007_145_146_R	23	77	94	10	1.69	-								
7	27	9423_173_161_R	25.8	78	86	17	0.39	-	>Chr07	5267937			>scaffold_6	3982283		
7	28	7090_155_84_R	25.8	84	97	0	0.93	-	>Chr07	5358092			>scaffold_6	3939293		
7	29	8800_170_114_R	25.8	79	94	8	1.3	-								
7	30	12700_162_159_R	30.8	87	93	1	0.2	-								
7	31	12837_159_114	34.1	91	90	0	0.01	-								
7	32	11240_160_236_R	34.1	91	89	1	0.02	-								
7	33	3098_165_161_R	34.1	79	86	16	0.3	-								
7	34	9354_160_93	39.1	93	86	2	0.27	-								
7	35	6617_162_158	40.7	85	83	13	0.02	-								
7	36	12469_165_83	40.7	88	87	6	0.01	-								
7	37	9066_149_35	40.7	86	82	13	0.1	-								
7	38	13960_178_8	40.7	91	90	0	0.01	-								
7	39	214_180_100	40.7	89	87	5	0.02	-								
7	40	2549_148_210	42.9	95	86	0	0.45	-								
7	41	1457_152_63	42.9	95	85	1	0.56	-								
7	42	13164_142_8	43.5	94	87	0	0.27	-								
7	43	10534_168_137	44.1	95	86	0	0.45	-	>Chr07	49891928			>scaffold_6	23669773		
7	44	11430_162_271	44.1	95	86	0	0.45	-								
7	45	10077_154_47	44.1	93	84	4	0.46	-								
7	46	8817_184_84	44.1	95	85	1	0.56	-								
7	47	7841_166_75	44.1	95	86	0	0.45	-	>Chr07	50428697	>Chr02	56115471				
7	48	10545_161_153	44.1	95	86	0	0.45	-								
7	49	3176_169_22	44.9	83	75	23	0.41	-								
7	50	1795_167_4	47.4	93	87	1	0.2	-	>Chr07	52566908						
7	51	11241_145_261	48.5	93	88	0	0.14	-	>Chr07	53450009	>Chr10	9352353	>scaffold_6	27033675		
7	52	15050_155_128	50.3	87	79	15	0.39	-								
7	53	**3400_139_128**	54.5	93	65	23	4.96	**								
7	54	3804_174_127	55.1	95	86	0	0.45	-								
7	55	11373_177_141	55.7	94	87	0	0.27	-								
7	56	3993_141_33	57.4	93	88	0	0.14	-								
7	57	3229_178_189	58.5	92	88	1	0.09	-								
7	58	12610_158_216	58.5	93	88	0	0.14	-	>Chr07	54361896						
7	59	13145_177_73	58.5	93	88	0	0.14	-								
7	60	7663_159_119	59.7	88	81	12	0.29	-					>scaffold_6	27480306		
7	61	1857_164_100	71.2	85	82	14	0.05	-								
7	62	9853_175_155	71.8	81	81	19	0	-								
7	63	13971_153_58	74.6	90	89	2	0.01	-								
7	64	4474_155_103	83.2	87	94	0	0.27	-								
7	65	239_178_192	83.2	85	88	8	0.05	-	>Chr07	62495181			>scaffold_6	32630588		

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
7	66	9057_182_111	83.8	88	93	0	0.14	-								
7	67	9901_175_131	84.9	88	93	0	0.14	-								
7	68	11057_174_177	87.1	86	95	0	0.45	-								
7	69	7388_182_253_R	91	85	96	0	0.67	-								
7	70	363_168_59	93.2	87	94	0	0.27	-								
7	71	6624_175_12_R	93.8	79	90	12	0.72	-								
7	72	2175_164_201_R	95.5	85	96	0	0.67	-								
7	73	5880_178_113	97.7	85	92	4	0.28	-	>Chr07	59471253						
7	74	4583_186_73	98.3	88	93	0	0.14	-	>Chr07	59119620						
7	75	10516_147_207_R	99.4	90	91	0	0.01	-	>Chr07	59031047			>scaffold_6	35653821	>scaffold_4	38494273

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
8	1	**W_13286_180_56_R**	0	89	88	4	0.01	-								
8	2	9751_157_51_R	0	91	90	0	0.01	-	>Chr08	55256970			>scaffold_3	50625389		
8	3	5129_135_102_R	5.5	91	90	0	0.01	-								
8	4	8434_172_24_R	5.5	91	90	0	0.01	-								
8	5	6606_182_122_R	8.8	87	90	4	0.05	-								
8	6	6035_173_15_R	8.8	91	90	0	0.01	-					>scaffold_9	26879072	>scaffold_3	49168963
8	7	13661_144_254_R	11	88	92	1	0.09	-	>Chr02	57725535			>scaffold_2	29130185		
8	8	1477_171_144_R	11	79	85	17	0.22	-	>Chr08	52853513	>Chr01	12722416	>scaffold_3	49644776	>scaffold_9	10519473
8	9	4438_170_13_R	15.4	88	92	1	0.09	-					>scaffold_3	48223403		
8	10	12389_168_97	19.3	94	86	1	0.36	-	>Chr08	51019265			>scaffold_3	47753715		
8	11	**205_153_47**	21.4	88	57	36	6.63	**								
8	12	12690_169_215	24.3	86	91	4	0.14	-								
8	13	506_179_128_R	24.9	86	93	2	0.27	-	>Chr08	51405442			>scaffold_3	48108241		
8	14	10318_170_194_R	26	87	93	1	0.2	-								
8	15	**W_3686_132_141_R**	26.6	88	91	2	0.05	-								
8	16	8675_164_27_R	27.2	88	93	0	0.14	-	>Chr08	50805099			>scaffold_3	47639799		
8	17	6781_183_254	27.8	87	94	0	0.27	-								
8	18	13578_178_38_R	27.8	87	94	0	0.27	-	>Chr08	52504371			>scaffold_3	47634943		
8	19	**W_13495_163_97_R**	27.8	83	92	6	0.46	-					>scaffold_3	47633356		
8	20	2761_137_27_R	28.4	86	95	0	0.45	-								
8	21	6053_180_17_R	29.5	86	95	0	0.45	-								
8	22	9518_175_167_R	29.5	75	91	15	1.54	-	>Chr08	50598364			>scaffold_3	47388344		
8	23	4472_155_89_R	30.6	75	92	14	1.73	-								
8	24	9411_185_217_R	31.7	85	95	1	0.56	-								
8	25	9445_149_140_R	35	86	95	0	0.45	-								
8	26	14775_141_130_R	37.8	85	92	4	0.28	-	>Chr08	48700984						
8	27	4520_165_120_R	38.9	79	96	6	1.65	-	>Chr08	48449309						
8	28	4177_146_218_R	39.5	83	97	1	1.09	-								
8	29	8098_142_145	40.1	85	95	1	0.56	-								
8	30	**W_3894_181_48**	42.3	85	94	2	0.45	-								
8	31	**W_6499_182_42_R**	42.3	82	96	3	1.1	-								
8	32	6393_178_55_R	42.3	85	96	0	0.67	-	>Chr08	47707945						
8	33	6391_180_217_R	45.1	84	96	1	0.8	-	>Chr08	47165539	>Chr05	59756367				
8	34	11479_156_85_R	45.7	82	98	1	1.42	-								
8	35	2007_168_187	47.9	85	96	0	0.67	-	>Chr08	45292270			>scaffold_3	44399067		
8	36	8720_180_52	49	85	96	0	0.67	-	>Chr08	45159035			>scaffold_3	44498755		
8	37	13918_162_42	51.8	88	93	0	0.14	-								
8	38	3692_173_18	51.8	88	93	0	0.14	-								
8	39	10_176_215	53.5	86	91	4	0.14	-								
8	40	2768_132_96_R	54.6	68	79	34	0.82	-	>Chr03	47909046			>scaffold_3	46089653		
8	41	11482_136_260	55.2	88	93	0	0.14	-	>Chr08	40107145			>scaffold_3	46450691		
8	42	4432_156_188_R	55.2	69	84	28	1.47	-	>Chr05	58963112	>Chr02	5182588				
8	43	2935_170_232	55.2	86	92	3	0.2	-								
8	44	5238_174_143_R	55.8	87	94	0	0.27	-								
8	45	4704_176_92_R	57.5	86	95	0	0.45	-								
8	46	1134_168_109	57.5	86	95	0	0.45	-	>Chr08	9220986			>scaffold_7	32602144	>scaffold_8	9296186
8	47	8104_151_156_R	58.1	87	94	0	0.27	-	>Chr08	7081794			>scaffold_7	32749719		
8	48	1193_171_132_R	58.1	70	82	29	0.95	-								
8	49	7756_148_194	58.1	86	94	1	0.36	-								
8	50	1591_169_147	58.1	76	81	24	0.16	-	>Chr06	46226037			>scaffold_7	20451082		
8	51	11303_156_130	60.9	86	95	0	0.45	-	>Chr10	9695360	>Chr08	17891314	>scaffold_3	5584915		
8	52	13135_177_110	62	83	92	6	0.46	-								
8	53	11520_139_226_R	62.6	85	96	0	0.67	-								
8	54	12491_157_221	63.2	86	95	0	0.45	-								
8	55	7996_173_177	63.8	71	84	26	1.09	-					>scaffold_6	6851578		
8	56	544_185_103_R	65.5	88	93	0	0.14	-	>Chr08	5378900			>scaffold_7	33281807		
8	57	629_154_84_R	67.7	88	93	0	0.14	-								
8	58	10362_133_186_R	70.5	90	90	1	0	-								
8	59	10975_163_115_R	70.5	91	90	0	0.01	-								
8	60	7684_186_130_R	79.4	92	85	4	0.28	-								
8	61	11605_168_112_R	79.4	93	88	0	0.14	-	>Chr08	2874256	>Chr04	3479821	>scaffold_7	35543903	>scaffold_8	854146
8	62	5081_164_257_R	81.6	91	90	0	0.01	-	>Chr05	2930916			>scaffold_5	38418982		
8	63	1485_182_8_R	87.7	92	87	2	0.14	-								
8	64	11661_165_16_R	87.7	92	87	2	0.14	-								
8	65	3898_177_133_R	90.5	93	88	0	0.14	-								
8	66	6475_140_130_R	91.6	91	89	1	0.02	-								
8	67	8843_161_45_R	92.7	89	91	1	0.02	-								
8	68	11361_143_250_R	96.7	71	80	30	0.54	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
9	1	11526_180_182_R	0	91	81	9	0.58	-								
9	2	2030_170_211_R	0	95	86	0	0.45	-					>scaffold_7	22216432	>scaffold_4	8030828
9	3	13791_152_180	3.2	97	83	1	1.09	-								
9	4	10032_164_123	3.8	96	82	3	1.1	-								
9	5	7794_175_54	4.6	98	79	4	2.04	-								
9	6	6045_188_111_R	5.4	98	82	1	1.42	-	>Chr09	57921457			>scaffold_3	9450022		
9	7	9420_165_154_R	7.1	98	83	0	1.24	-								
9	8	10527_161_223_R	7.1	98	83	0	1.24	-	>Chr09	57807290	>Chr03	57828197	>scaffold_3	9556574	>scaffold_5	30735861
9	9	11797_164_186	8.2	82	73	26	0.52	-	>Chr09	57687274	>Chr03	58094896				
9	10	12163_160_154	9.3	96	85	0	0.67	-					>scaffold_3	9799581		
9	11	4479_176_91	9.9	95	85	1	0.56	-								
9	12	8982_174_85	12.7	92	89	0	0.05	-	>Chr09	56939237			>scaffold_3	10847227		
9	13	11687_170_206	13.3	91	90	0	0.01	-	>Chr09	56771880	>Chr03	59687740	>scaffold_5	33369969	>scaffold_3	11090505
9	14	11040_182_54_R	14.4	85	88	8	0.05	-								
9	15	1434_179_83_R	15	90	91	0	0.01	-								
9	16	5498_172_148_R	15	78	78	25	0	-								
9	17	13265_171_115_R	16.1	86	89	6	0.05	-								
9	18	7165_156_132	17.2	90	91	0	0.01	-	>Chr06	54609214						
9	19	12026_153_139_R	20.5	90	91	0	0.01	-								
9	20	11380_166_94_R	20.5	90	90	1	0	-	>Chr09	55820890			>scaffold_3	12094566		
9	21	5567_165_126_R	21.1	89	92	0	0.05	-	>Chr09	55472010			>scaffold_9	35007628	>scaffold_3	12577659
9	22	11988_168_261_R	21.1	88	92	1	0.09	-								
9	23	9931_178_145_R	21.1	81	84	16	0.05	-								
9	24	865_141_269_R	23.3	89	92	0	0.05	-	>Chr09	55255819			>scaffold_3	12886313		
9	25	9560_168_113	25	92	89	0	0.05	-					>scaffold_3	14852837		
9	26	235_169_173	25.6	91	90	0	0.01	-								
9	27	971_152_108	25.6	91	90	0	0.01	-								
9	28	1516_168_263	26.2	92	89	0	0.05	-								
9	29	255_182_24	26.2	90	89	2	0.01	-					>scaffold_1	2540819		
9	30	8278_178_81	26.2	89	86	6	0.05	-								
9	31	9703_140_66	26.2	90	89	2	0.01	-								
9	32	8160_184_10	26.2	92	89	0	0.05	-								
9	33	10143_143_234	29.5	94	87	0	0.27	-	>Chr09	52085868			>scaffold_3	17548953		
9	34	3798_152_39	29.8	81	70	30	0.8	-	>Chr09	52073700	>Chr01	70350851	>scaffold_3	17569973		
9	35	10788_172_144	30.1	94	83	4	0.68	-								
9	36	12459_157_93	30.7	94	87	0	0.27	-					>scaffold_3	17621091		
9	37	9451_182_80	30.7	94	86	1	0.36	-					>scaffold_3	17681929		
9	38	328_176_61	30.7	92	85	4	0.28	-	>Chr09	51863757			>scaffold_3	17745265		
9	39	11053_186_9	30.7	94	87	0	0.27	-								
9	40	9051_164_149	30.7	94	87	0	0.27	-								
9	41	6072_163_153	31.3	93	88	0	0.14	-	>Chr09	51764631						
9	42	9392_164_193	36.8	87	76	18	0.74	-								
9	43	2608_182_59	38	92	84	5	0.36	-	>Chr09	**58704170**						
9	44	1051_180_121	38.6	94	87	0	0.27	-	>Chr09	**50599108**			>scaffold_3	19180611		
9	45	6000_169_247	38.6	93	86	2	0.27	-								
9	46	11858_166_78	43	91	89	1	0.02	-	>Chr09	**57908807**	>Chr02	8677834				
9	47	125_149_99	43	92	89	0	0.05	-	>Chr09	50119196			>scaffold_3	19678111		
9	48	10179_178_231	43	92	89	0	0.05	-	>Chr09	49969323						
9	49	12475_155_231	45.2	88	93	0	0.14	-	>Chr09	49740626			>scaffold_3	20275737		
9	50	4314_136_110	48.7	82	74	25	0.41	-	>Chr09	49485223			>scaffold_3	20594557		
9	51	5537_171_135	50.8	84	97	0	0.93	-	>Chr09	49020508			>scaffold_3	21084727		
9	52	6284_182_153	52.5	83	97	1	1.09	-								
9	53	4676_147_132	52.5	83	94	4	0.68	-	>Chr02	54961703			>scaffold_3	9228037	>scaffold_2	32314702
9	54	545_132_215	53.6	82	98	1	1.42	-								
9	55	**8852_151_231**	56.9	79	102	0	2.92	*	>Chr09	46385241	>Chr03	72549449	>scaffold_3	23257034		
9	56	**9797_179_190**	56.9	79	102	0	2.92	*					>scaffold_3	23695899		
9	57	3357_177_168	56.9	78	99	4	2.49	-								
9	58	**9521_168_73**	56.9	79	102	0	2.92	*								
9	59	**9795_163_121**	56.9	79	102	0	2.92	*								
9	60	3397_172_88	58.6	79	99	3	2.25	-								
9	61	2425_163_112	61.8	78	91	12	1	-								
9	62	13859_161_191	62.4	79	101	1	2.69	-								
9	63	**11725_134_31**	62.5	79	102	0	2.92	*								
9	64	**3306_180_239**	62.5	79	102	0	2.92	*								
9	65	**4621_171_81**	62.5	79	102	0	2.92	*	>Chr02	64837457	>Chr01	18428458	>scaffold_2	37223044		

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
9	66	4363_152_185	62.5	79	101	1	2.69	-								
9	67	7257_172_75	62.5	76	93	12	1.71	-								
9	68	**8736_158_112**	62.5	79	102	0	2.92	*								
9	69	**3945_160_192**	62.5	79	102	0	2.92	*	>Chr10	4508966						
9	70	**6830_168_153**	63.1	78	103	0	3.45	*	>Chr09	10709336						
9	71	**1995_175_36**	63.1	73	95	13	2.88	*								
9	72	**24_155_48**	63.7	79	102	0	2.92	*								
9	73	**6332_178_64**	64.3	78	101	2	2.96	*								
9	74	**4521_171_73**	64.3	78	103	0	3.45	*	>Chr09	9809027			>scaffold_3	4615680		
9	75	1947_154_196	75.6	78	66	37	1	-								
9	76	2306_152_121	89.1	79	76	26	0.06	-								
9	77	10087_163_95	98.8	79	101	1	2.69	-								
9	78	**7336_167_80**	102.7	76	102	3	3.8	*								
9	79	**5179_166_54**	102.7	76	105	0	4.65	**	>Chr09	8825360			>scaffold_3	7477331		
9	80	**3544_160_103**	104.9	72	107	2	6.84	***								
9	81	**5865_152_144**	105.5	71	110	0	8.4	****					>scaffold_3	6812095	>scaffold_5	10621298
9	82	**W_7091_164_92**	106.1	69	111	1	9.8	****					>scaffold_4	33135373		
9	83	**7215_170_143**	107.2	70	111	0	9.29	****								
9	84	**7472_173_146**	110	67	114	0	12.2	******	>Chr09	5591426			>scaffold_7	32236918		
9	85	**10254_176_140**	113.3	62	118	1	17.42	*******								
9	86	**W_483_151_42**	113.9	60	114	7	16.76	*******								
9	87	**3269_172_155**	115	60	110	11	14.71	******								
9	88	**2968_151_30**	115	64	117	0	15.52	*******								
9	89	**9459_169_36**	115.6	64	114	3	14.04	******	>Chr09	5099758			>scaffold_7	31975006		
9	90	**13257_176_68**	116.2	64	117	0	15.52	*******					>scaffold_6	19394028	>scaffold_5	32239517
9	91	**6977_172_183**	116.8	65	116	0	14.37	******								
9	92	**12137_167_80**	120.1	63	116	2	15.69	*******								
9	93	**3111_151_140**	124.3	53	102	26	15.49	*******								
9	94	**W_10814_161_168**	127.9	64	107	10	10.81	****	>Chr09	3591277			>scaffold_3	3640230		
9	95	**W_6448_169_210**	130.7	72	108	1	7.2	***								
9	96	**13294_138_248**	132.9	76	104	1	4.36	**	>Chr09	3110978			>scaffold_3	3405968		
9	97	**7704_160_104**	133.5	77	104	0	4.03	**								
9	98	**12662_147_241**	133.5	77	104	0	4.03	**								
9	99	**9439_149_172**	134.6	77	104	0	4.03	**								
9	100	**8255_175_77**	135.2	74	101	6	4.17	**	>Chr09	2964857						

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
10	1	1079_180_208	0	92	87	2	0.14	-								
10	2	9579_178_58	0	91	84	6	0.28	-	>Chr10	59998095			>scaffold_4	39552138		
10	3	11277_182_2	0	93	88	0	0.14	-								
10	4	8427_155_155	0.6	92	88	1	0.09	-					>scaffold_4	38715547	>scaffold_1	8086430
10	5	**W_5974_165_110**	1.2	93	88	0	0.14	-								
10	6	10495_134_99	2.3	85	70	26	1.45	-					>scaffold_4	36457944	>scaffold_8	13307316
10	7	588_176_69	3.3	92	86	3	0.2	-								
10	8	4663_167_60	3.3	93	88	0	0.14	-								
10	9	10837_157_158	3.3	93	88	0	0.14	-								
10	10	9565_159_110	3.3	93	88	0	0.14	-	>Chr10	58750480			>scaffold_4	36692342		
10	11	4739_183_53	3.9	92	89	0	0.05	-								
10	12	12403_137_116	5.3	85	76	20	0.5	-								
10	13	**W_10379_134_58**	6.7	93	88	0	0.14	-	>Chr06	60191183			>scaffold_3	1714691		
10	14	14058_135_120	6.7	93	88	0	0.14	-	>Chr10	57794333			>scaffold_4	37476691		
10	15	4234_179_232	6.7	93	88	0	0.14	-					>scaffold_4	37675160		
10	16	15298_134_84	6.7	93	88	0	0.14	-								
10	17	4199_173_189	6.7	86	86	9	0	-								
10	18	6659_143_210_R	13.9	90	90	1	0	-								
10	19	11699_171_63	16.1	88	82	11	0.21	-					>scaffold_4	31502900		
10	20	7097_145_259_R	16.7	93	87	1	0.2	-								
10	21	**W_9812_167_55_R**	17.3	92	86	3	0.2	-								
10	22	13625_147_189_R	18.4	92	89	0	0.05	-					>scaffold_4	31830051		
10	23	13184_157_120	19	91	88	2	0.05	-								
10	24	2865_176_160_R	20.6	78	83	20	0.16	-								
10	25	4547_178_215_R	20.6	90	91	0	0.01	-								
10	26	**W_6735_137_46_R**	23.3	88	48	45	11.76	*****								
10	27	13205_142_44_R	24	90	91	0	0.01	-	>Chr10	53630422			>scaffold_4	32948772		
10	28	559_148_166	24	90	85	6	0.14	-								
10	29	15672_169_61_R	24	90	91	0	0.01	-								
10	30	11983_157_207	24	90	91	0	0.01	-								
10	31	13592_159_223	24	90	91	0	0.01	-								
10	32	12629_155_62_R	24.6	89	92	0	0.05	-								
10	33	12509_151_9	27.9	91	82	8	0.47	-								
10	34	8361_174_60_R	30.7	87	87	7	0	-								
10	35	5891_133_196	31.3	89	92	0	0.05	-								
10	36	13891_149_114	31.3	89	91	1	0.02	-								
10	37	**W_8207_135_151_R**	34.1	71	84	26	1.09	-								
10	38	888_153_216	35.8	89	92	0	0.05	-								
10	39	3335_175_145	36.4	90	91	0	0.01	-								
10	40	10161_183_131_R	36.4	90	91	0	0.01	-	>Chr10	50506269						
10	41	1309_143_114_R	38.1	91	90	0	0.01	-	>super_59	515019	>Chr05	6840046	>scaffold_14	121931	>scaffold_12	306331
10	42	11433_170_44_R	38.1	91	90	0	0.01	-					>scaffold_1	39385026	>scaffold_7	18587880
10	43	**W_9508_167_106_R**	39.2	91	90	0	0.01	-								
10	44	5443_185_60_R	39.8	92	89	0	0.05	-	>Chr10	49607125			>scaffold_4	36371187		
10	45	10328_160_119	47	96	83	2	0.94	-								
10	46	3212_171_51	47.3	84	73	24	0.77	-								
10	47	3208_142_55	47.6	94	84	3	0.56	-								
10	48	3382_163_156_R	48.2	97	84	0	0.93	-	>Chr10	47859714			>scaffold_4	28678631		
10	49	11220_169_12_R	48.2	94	83	4	0.68	-	>Chr10	47793398			>scaffold_4	28499242		
10	50	**1196_180_117**	49.3	98	75	8	3.06	*					>scaffold_2	30664587		
10	51	11852_166_11	49.9	98	83	0	1.24	-	>Chr10	47137378			>scaffold_4	27998007	>scaffold_1	1781370
10	52	9610_175_12_R	50.5	99	82	0	1.6	-	>Chr04	65847655	>Chr09	4136052	>scaffold_1	40569339		
10	53	7440_171_143	50.5	99	78	4	2.49	-								
10	54	11653_175_59	50.5	99	79	3	2.25	-								
10	55	7744_166_136	50.5	99	82	0	1.6	-								
10	56	2169_158_200_R	52.1	80	74	27	0.23	-								
10	57	12200_177_237	52.1	96	85	0	0.67	-								
10	58	9229_174_51_R	52.1	96	84	1	0.8	-								
10	59	3963_154_30	52.1	96	85	0	0.67	-								
10	60	14450_163_202_R	52.1	95	84	2	0.68	-								
10	61	13245_144_37_R	52.1	96	85	0	0.67	-								
10	62	956_156_23	52.1	96	85	0	0.67	-	>Chr01	69657108	>Chr10	43526818	>scaffold_4	26045863	>scaffold_1	14266216
10	63	10678_165_173	52.1	90	82	9	0.37	-								
10	64	13925_173_160_R	52.1	96	82	3	1.1	-								
10	65	6148_162_169	52.1	95	85	1	0.56	-								

Supplementary Table 3.3: Maternal and paternal maps with comparative markers

LG	Marker No.	Locus Name	cM (Kos)	a	h	-	X2	Signif.	1° hit sorghum	loc (bp)	2° hit sorghum	loc (bp)	1° hit Setaria	loc (bp)	2° hit Setaria	loc (bp)
10	66	900_147_232	52.1	96	85	0	0.67	-								
10	67	13888_145_160	52.1	96	85	0	0.67	-								
10	68	7642_170_68	52.7	90	77	14	1.01	-	>Chr07	61585987			>scaffold_4	23576178	>scaffold_1	13622696
10	69	808_141_162_R	53.8	97	84	0	0.93	-								
10	70	7694_181_66_R	53.8	95	79	7	1.47	-					>scaffold_4	15528619		
10	71	**7977_157_110**	57.4	95	67	19	4.84	**								
10	72	10973_171_124	61.3	94	76	11	1.91	-	>Chr01	2190084						
10	73	10287_182_247	62.7	99	82	0	1.6	-								
10	74	9085_149_221_R	62.7	99	82	0	1.6	-	>Chr10	17509943			>scaffold_4	15291848		
10	75	1071_167_192_R	62.7	99	82	0	1.6	-								
10	76	349_137_183	62.7	99	82	0	1.6	-								
10	77	11469_169_257	63.3	100	81	0	1.99	-								
10	78	3025_177_107	63.3	100	81	0	1.99	-	>Chr10	14415635						
10	79	6296_145_127	63.9	86	66	29	2.63	-	>Chr10	14353614			>scaffold_4	13562600		
10	80	13224_174_150_R	65.6	98	83	0	1.24	-								
10	81	**4105_168_162_R**	67.8	102	79	0	2.92	*								
10	82	285_141_209_R	69.5	101	80	0	2.44	-	>Chr10	11485212						
10	83	13879_165_150	69.5	101	80	0	2.44	-	>Chr10	11413724	>Chr04	66364344	>scaffold_4	12028180	>scaffold_1	40977071
10	84	4134_170_86	69.5	101	80	0	2.44	-	>Chr10	11308269						
10	85	883_174_184_R	69.5	92	76	13	1.52	-	>Chr10	11307930			>scaffold_4	11929132		
10	86	6419_168_130	69.5	94	80	7	1.13	-								
10	87	12943_164_188_R	69.5	100	80	1	2.22	-					>scaffold_4	9774468		
10	88	11477_152_139	70.6	101	79	1	2.69	-					>scaffold_8	33611344		
10	89	11323_171_27	70.6	101	80	0	2.44	-								
10	90	5237_152_59_R	74.5	79	68	34	0.82	-	>Chr10	9002153	>Chr06	59961775	>scaffold_4	9674916	>scaffold_6	31127358
10	91	**13450_153_3_R**	75.6	102	79	0	2.92	*	>Chr10	8991359			>scaffold_4	9666232		
10	92	10497_178_219_R	76.7	100	80	1	2.22	-								
10	93	11166_174_52_R	76.7	100	81	0	1.99	-								
10	94	10426_161_17_R	79.9	83	72	26	0.78	-								
10	95	13662_139_27_R	80.5	97	84	0	0.93	-	>Chr10	7202206			>scaffold_4	6023318		
10	96	7801_143_129_R	82	98	82	1	1.42	-								
10	97	8029_181_98	82.7	97	84	0	0.93	-	>Chr10	6457798			>scaffold_4	6920025		
10	98	11940_160_128	84.7	98	80	3	1.82	-								
10	99	12478_132_169_R	86	101	80	0	2.44	-								
10	100	10119_175_25	88.8	98	82	1	1.42	-								
10	101	5791_170_28	89.4	97	84	0	0.93	-	>Chr10	5498862			>scaffold_4	3259244		
10	102	6996_182_206_R	90.5	99	82	0	1.6	-								
10	103	10198_171_85_R	91.1	89	82	10	0.29	-								
10	104	721_169_161	92.8	93	77	11	1.51	-	>Chr10	4881923			>scaffold_4	3942123		
10	105	27_160_66_R	92.8	99	82	0	1.6	-					>scaffold_4	4052116		
10	106	**W_6281_135_81_R**	92.8	93	80	8	0.98	-	>Chr02	75680292			>scaffold_6	19068993		
10	107	2235_171_180_R	94.4	95	80	6	1.29	-								
10	108	1482_156_127_R	96.1	97	82	2	1.26	-								
10	109	3953_183_151	96.1	99	82	0	1.6	-	>Chr10	3669912						
10	110	9852_176_50_R	96.1	99	82	0	1.6	-	>Chr10	3582104			>scaffold_4	5135144		
10	111	**8544_175_96**	96.7	98	76	7	2.78	*	>Chr10	3401287			>scaffold_4	5328704		
10	112	622_181_119	100	98	83	0	1.24	-					>scaffold_9	49710612		
10	113	9981_165_93	101.3	92	76	13	1.52	-	>Chr10	2780933			>scaffold_4	2172292		
10	114	**W_1091_134_54_R**	114.2	66	87	28	2.88	*								
10	115	3137_149_161_R	114.2	93	88	0	0.14	-	>Chr10	1247413			>scaffold_4	999819		
10	116	7142_146_54_R	114.2	72	79	30	0.32	-	>Chr10	1100208			>scaffold_4	767200		
10	117	8170_154_59_R	114.2	93	88	0	0.14	-	>Chr10	1048278	>Chr04	55350778	>scaffold_4	730731		
10	118	4078_173_5_R	114.2	93	88	0	0.14	-	>Chr02	45402217			>scaffold_2	24138152		
10	119	12977_174_128_R	115.9	94	87	0	0.27	-					>scaffold_4	346245		
10	120	10488_171_59_R	115.9	87	79	15	0.39	-								
10	121	1997_176_171_R	117	92	89	0	0.05	-								
10	122	6297_132_90_R	117	92	89	0	0.05	-								
10	123	7296_169_212_R	117.6	93	88	0	0.14	-								
10	124	2965_182_201_R	117.6	93	88	0	0.14	-					>scaffold_4	202302		
10	125	10710_184_225_R	117.6	90	84	7	0.21	-	>Chr10	238104						

CHAPTER 4

IDENTIFYING QUANTITATIVE TRAIT LOCI (QTL) FOR SALT TOLERANCE TRAITS IN AN F1 MAPPING POPULATION DERIVED FROM PARENTAL LINES DIFFERING IN SALT TOLERANCE[3]

[3] Douglas M Eudy, Paul Raymer, and Katrien M. Devos. To be submitted to *PLOS ONE*

Abstract

Seashore paspalum is a highly salt tolerant grass species which utilizes a sodium exclusion mechanism in order to survive salt exposure. An F1 mapping population was generated from two paspalum genotypes previously identified to differ in salt tolerance level and screened under high salt conditions for organ biomass fractions as well as sodium accumulation, potassium accumulation, and K^+/Na^+ ratio in green leaf fractions. A QTL analysis for these traits resulted in the identification of a QTL on linkage group (LG) 9 for sodium accumulation explaining ~12% of the phenotypic variation as well as co-localizing QTL on linkage group 4 for potassium accumulation and K^+/Na^+ ratio, which explained ~20% and ~16% of the variation, respectively. Both the LG 4 and LG 9 QTL were contributed by the paternal genotype, which had previously been identified as the parent with superior salt tolerance. A candidate gene approach conducted in the orthologous regions of the *Sorghum bicolor* genome identified three candidates for the sodium accumulation trait and four candidates for potassium accumulation and ion ratio traits including a high-affinity potassium transporter (HKT6-like).

Introduction

The lack of salinity tolerance in most crop species will become an increasingly pressing food security issue as more and more arable land becomes affected by salt. Understanding the components which comprise individual salt tolerance mechanisms as well as the genes which contribute to them will aid in improving salt tolerance in crops and ensuring food security. One approach that could lead to salt tolerant varieties of crops is the introgression of genes which condition salt-tolerance from crop wild-relatives and/or unadapted germplasm. Improving crops in this way, through traditional breeding, requires inter-fertile germplasm containing the trait of

interest. Alternatively, genes conferring salt tolerance traits could be moved from one species to another using a transgenics approach or, potentially, native DNA sequences could be modified using gene editing; to date neither of these approaches have led to salt tolerant cultivars that have been commercialized, but both methods have promise, since they have the potential to leverage genes or elite alleles that are not naturally found in the primary germplasm pools of crops. Regardless of the approach, quantitative trait loci (QTL) and potential candidate genes must first be identified.

Since early in the era of the study of plant quantitative genetics with mapping populations, salt tolerance has been a phenomenon of interest. Foolad and Jones (1993) followed soon after by Bretó et al. (1994), both working in tomato, published the first papers to identify QTL for salt tolerance. Since that time, salt tolerance genetic mapping research has expanded to a number of other systems. Flowers and Flowers (2005) provide a summary of salt tolerance mapping studies and communicate the importance of considering potential tolerance mechanisms as well as plant developmental stages when studying salt tolerance. Another important consideration is that among the systems most commonly studied for salt tolerance, the majority of those represent agricultural crops which generally have low levels of salinity tolerance. As such, the QTL identified in most studies condition a relatively minor level of salinity tolerance increase when compared to the levels exhibited by the so-called halophytes. Species such as seashore paspalum (*Paspalum vaginatum* Sw.) are therefore potentially very attractive systems with which to study salinity tolerance because they naturally exhibit such a high level of tolerance. In fact, as evidenced by the areas which it commonly inhabits, seashore paspalum contains loci that enable it to survive in exceptionally high levels of salt, including at or above that of seawater. However, to date, only two studies have sought to identify the specific genes

associated with salt tolerance in seashore paspalum, and neither of these used a genetic mapping approach, rather they evaluated gene expression data (Endo et al. 2005; Chen et al. 2016). With map location information, in combination with comparative genomics, it could be possible to identify candidate genes for salt tolerance, even in the absence of a *Paspalum vaginatum* reference genome. This could have important implications for salt tolerance research and breeding as paspalum QTL and/or candidate genes could potentially confer impressive levels of salt tolerance.

Materials and Methods

Population Generation and Maintenance

In order to identify an appropriate pair of genotypes with which to develop a mapping population, we relied on two selection criteria: maximizing genetic diversity between the individuals while simultaneously selecting lines which exhibited performance differences when exposed to increasing salt levels from freshwater up to nearly ocean strength. Performance under salt exposure was assessed by growing 89 accessions of seashore paspalum at four salt levels. The salinity levels are represented by the conductivity of each sea-salt-based solution: 0 dS/m, 15 dS/m, 30 dS/m, and 45 dS/m. The performance values recorded in this experiment included quantitative values of biomass taken repeatedly, as well as qualitative ratings of leaf damage. These data were generated by Dr. Paul Raymer, the seashore paspalum breeder at UGA Griffin. We also referenced earlier salt screen results from work performed at UGA in the late 1990s (Lee 2000). The individuals selected as parents, HI33 and 509022, were assayed in both sets of experiments and were identified in both cases as varying in salt tolerance level. The genetic diversity data were obtained by screening the accessions with 43 microsatellite markers (Chapter 2).

To generate the mapping population, three clones of each parent were grown in six inch pots and placed together under a pollen screen for isolation. Because seashore paspalum is largely self-incompatible, no effort was made to prevent self-pollination. The parental lines were grown in 2013 under natural day length from late Spring to early Fall in greenhouses at Griffin, GA and pollinations were made by allowing random intercrossing between the parental clones. Seed was collected by harvesting mature seedheads from each of the six parental plants with the genotype and clone identity being recorded as 22A, 22B, 22C, 33 orange, 33 yellow, and 33 green. Seeds from all six sources were stored and germinated in the winter of 2013/2014. To eliminate the potential for differing maternal effects, all progeny used in our experiments were from flowers of line 22A. A total of 226 progeny were planted in 4 inch square plastic pots containing the UGA Botany Greenhouses' pine bark soil mix (125 gallons pine bark, 8 cups limestone, 2 cups superphosphate, 1 cup each gypsum, calcium nitrate, potassium nitrate and Micromax[a source of micronutrients produced by Everris]). The plants were maintained as sources for stolon material multiplied used in a mapping experiment. These "clonal mothers" were fertilized with a liquid concentrate that was applied at the discretion of the greenhouse staff. As a part of maintaining these lines as sources of propagules, the top growth was periodically trimmed with shears. In order to clone the population to provide replicates for the salt tolerance QTL study, the stolons were left to grow for several weeks in order to have at least 12 nodes available for the propagation procedure.

Cone Prep/Mapping Grow Out

In order to produce multiple clones of lines from the mapping population, stolon nodes were propagated in river sand placed in UV stabilized (yellow) SC-10 (164mL) Ray Leach Cone-tainers™ (Stuewe and Sons Inc, Tangent, Oregon) in June 2014. Approximately 25 mL of

landscape pea gravel was placed in the bottom of each Cone-tainer to provide drainage. Stolons were prepared so that each propagule had only a single node. The nodes selected for propagation each had a single small shoot emerging and the shoots selected were at or between the 3 and 5 leaf stage (meaning the shoots were between 1 ½ and 5 centimeters tall at the time of propagation). Each single stolon piece was pressed into the wetted sand of an individual cone, so that the node (source of roots) was below the soil line and a majority of the shoot (stem and leaves) was above. Eight clones per genotype were required for the experiment, but up to sixteen clones per genotype were propagated to insure enough material was available in the case that there was a low propagation success rate. Propagules were placed in 98-cell racks under a mister (operating every 15 minutes) in a greenhouse under natural light. The propagules were fertilized every third day by saturating the sand with a solution of 2 grams of Excel Plug and Bedding Special (13-2-12) liquid feed fertilizer (E99120 Everris NA Inc, Dublin, OH) and 0.6 grams magnesium sulfate each, per gallon. During the establishment of these clones, a single leaf was excised from all clones of a genotype and pooled into a single 2mL microcentrifuge tube for DNA extraction and the subsequent GBS-marker development and genetic analysis (Chapter 3). After allowing the propagules to establish roots for about 5 weeks, the salt screen was conducted. For each progeny, the eight plantlets with the greatest consistency in size were selected and each plantlet was randomly assigned to one of eight blocks. Progeny were randomized within each of the eight blocks, and two blocks were set up within a single ebb and flow bin. Each population member was present once in each block, thus twice per bin; each parental genotype was present six times per block, thus twelve times per bin. There were eight blocks split between two treatment levels, 15 dS/m and 45 dS/m. An ebb and flow bench setup was used to control the watering of the plants. Our total screening capacity was 1764 cones at a time.

Ebb and Flow Irrigation System

The ebb and flow irrigation system consisted of a flood table (which is iteratively fed a nutrient solution containing 2 grams of Excel Plug and Bedding Special liquid feed fertilizer (E99120 Everris NA Inc, Dublin, OH) and 0.6 grams magnesium sulfate each, per gallon, from a below bench reservoir using a pump system activated by a timer. The reservoirs (Tuff Stuff Brand) had a 110 gallon capacity and had a brass plug in the side allowing for solutions to be drained and replaced weekly. At predetermined times throughout the day, the pump (a fountain pump, 500GPH capacity) was switched on and pumped the nutrient/salt solution from the reservoir into the flood table. An overflow drain feeding back to the reservoir allowed for longer pump times. When the timer shut off power to the pump, gravity returned the solution to the reservoir by flowing back through the pump. During the salt grow out experiment, the timers were set to run for the first ten minutes of each hour during daytime as well as three additional times throughout the night. This regular cycling kept the sand moist during the experiment.

Salt Tolerance Screen

The first two weeks after being placed in the ebb and flow bins, plants were irrigated with nutrient solution lacking salt. On August 10th 2014, the reservoir salt levels of both the control and salt treatments were raised at the same rate until all reached a conductivity of ~15 dS/m. Then every third or fourth day the salt levels of the two salt treatment reservoirs were further elevated ~5 dS/m until salt levels reached 45dS/m. The nutrient/salt solutions were refreshed weekly until the conclusion of the experiment. Samples from three blocks were harvested and subsequently analyzed, rep. 1 (labeled 1_1) was harvested during the period 11/17/14-11/21/14 and samples from reps. 2 and 3 (labeled 1_2 and 4_1) were harvested between 12/15/14-12/21/14; these samples comprise only plants grown at the high salt level.

At the conclusion of the salt screen, the aerial portions of the plants were separated from the roots at the crown. The roots were washed free of river sand by rinsing while gently rubbing them in tap water. Cleaned roots were removed from the crown. Leaf blades were excised from the rest of the aerial plant fraction by cutting at the ligule. Leaves were separated into healthy and injured/dead fractions by visual inspection. Entirely green leaves were put into the healthy fraction. Entirely yellow or brown leaves were put into the injured/dead fraction. Leaves that showed transitions from green to yellow were cut at the area of transition and each part placed into the appropriate fraction (healthy or injured dead). Stolons and stoloniferous parts of shoots were separated into a stolon fraction and the remaining stem material retained in a stem fraction. Individual fractions (dead/injured leaf parts, green leaf parts, stems, stolons, and roots) were placed into paper sacks dried at 65°C. Samples were stored at 65 ^{0}C until dry weight (DW) measurements were taken.

Trait Data Collected

Biomass

Dry weight of each organ fraction was recorded for the parents and all progeny using a Mettler digital balance linked via serial port to a laptop PC. The software WinWedge was used to automate data entry.

Chemical Analysis

Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) was used to measure analyte abundances in the healthy leaf fraction of the salt grown plant's biomass. The analysis was completed at the Center for Applied Isotope Studies at the University of Georgia. The green leaf fractions which were composed of large amounts of leaf material were ball milled with a

SPEX mill, samples containing too little leaf material to grind and have sufficient material transferred for chemical analysis were not mill homogenized. After grinding, the powdered leaf samples (and those samples that remained unground) were stored in sealed glass scintillation vials. Prior to weighing, the vials with loosened caps were placed in a forced air oven set to 60°C for a minimum of 12 hours. Samples were removed immediately prior to weighing and their caps sealed, samples were then allowed to cool to room temperature (with sealed caps). About 20mg of each sample was moved to a ceramic crucible and the sample's mass recorded to one decimal place; in cases of unground sample, all of the leaf material was used. Samples were converted to ash in a muffle furnace at 500°C overnight. Samples were acid extracted with Aqua Regia at a rate of 500µL acid for every 10mg of pre-ashed sample. After digestion, ~1 mL of acid extract was moved to a polymer 20mL scintillation vial and diluted with 10mL of 10Mohm water. These diluted samples were used for sodium and potassium analysis using ICP-OES at the Center for Applied Isotope Studies at UGA. The results were presented as the parts per million (ppm) of each analyte in the plant samples. In addition to individual sodium and potassium abundances, K^+/Na^+ ratios were calculated.

QTL Detection

Biomass data from organ specific fractions, and sodium ion abundance, potassium ion abundance, and K^+/Na^+ ratio from green leaf blades were used in combination with the parental genetic maps (Chapter 3) to detect QTL for salt tolerance using the composite interval mapping algorithm (CIM) in the program WinQTLCart (Wang et al. 2012). The default likelihood ratio (LR) of 11.5, which translates into a logarithm of odds (LOD) threshold of 2.5, was used to identify QTL. The data collected for each trait were analyzed independently for each repetition because each was exposed to the experimental conditions for different lengths of time. The

biomass and ion datasets were filtered to remove values from samples where the root, leaf, or stem fraction measured less than 0.1 gram. A second filtering approach was applied independently: the ion abundance values were ranked from low to high for each block and then compared across blocks. When genotypes displayed rank values that were more than thirty positions away from the ranks attained for that genotype in the other blocks, the trait value associated with the non-similar rank was removed from the dataset. In addition, outliers below predetermined thresholds were removed from the ion abundance datasets prior to issuing ranks. The lower limit for sodium was 5000 ppm and 20000 ppm for potassium.

Candidate Gene Identification

Paspalum markers flanking each QTL were used to delineate the orthologous regions in the *Sorghum bicolor* genome. A list of genes present in these sorghum intervals and their functional descriptions were downloaded from Phytozome (phytozome.jgi.doe.gov) using Biomart. The resulting genes were manually scanned to identify previously annotated genes with putative functions relevant to salt tolerance and the specific trait used to detect that QTL. The protein sequences from the sorghum genes identified as candidates were then cross-referenced in SwissProt and UniProt to collect more comprehensive information about each candidate including specific functions described in the literature as a supplement to the more generic descriptions from Phytozome.

Results

Trait Data

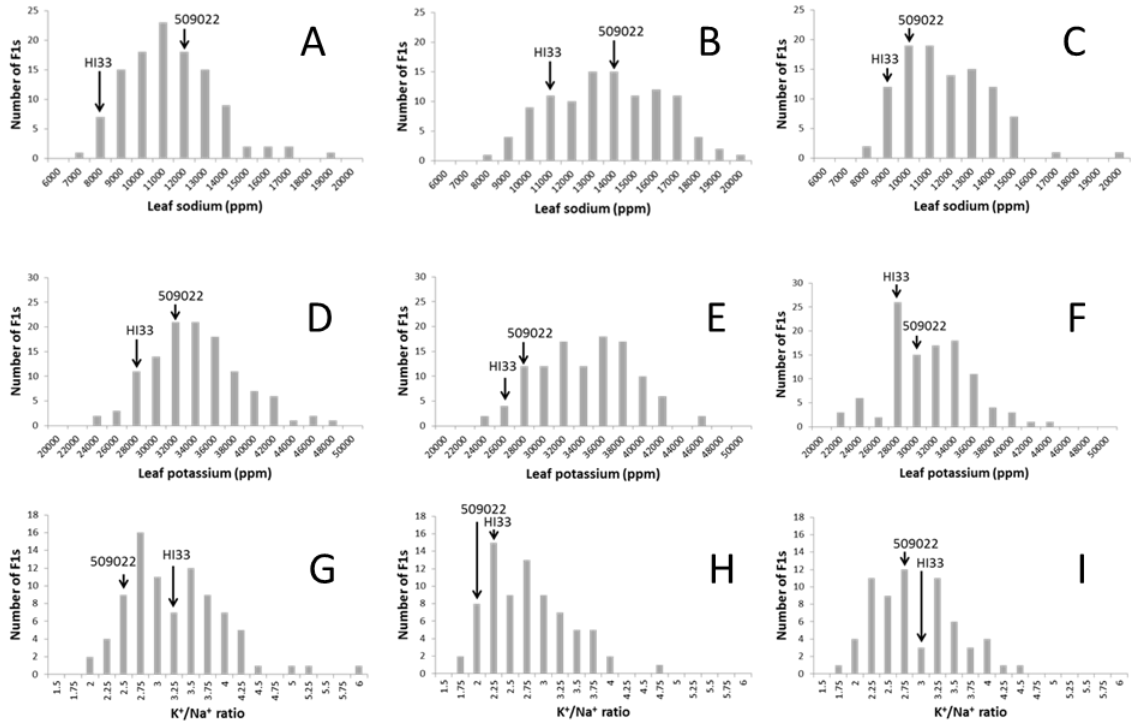

Figure 4.1: Parent and progeny trait value distributions

Parental means (n=6) are indicated on the histograms. First column (A,D,G) shows values from Rep 1, second column (B,E,H) for Rep 2 and the third column (C,F,I) for Rep 3

Values collected for trait data include: individual organ biomass, sodium and potassium abundances, and K^+/Na^+ and are tabulated in a supplementary table: The mean parental values (n=6) and the range of progeny values for ion related traits are displayed (Figure 1). The Shapiro-Wilk test was used to determine normality; with that approach it was determined that none of the sodium datasets were normally distributed, each of the potassium datasets were normally distributed, and that K^+/Na^+ ratios were normally distributed only in the third set.

Summary of QTL Detected

Each parental map was used individually to detect QTL for the traits of interest. We only considered QTL if they were present above the LOD threshold in all three replicates. In the maternal parent, no QTL were detected for salt tolerance traits. In the paternal parent, no QTL were consistently detected for biomass of individual organ fractions or total biomass, but QTL were consistently detected for sodium and potassium abundances as well as K^+/Na^+ ratio. The QTL associated with sodium abundance was detected on linkage group (LG) 9, but only in the dataset filtered for a genotype's consistency of rank (Figure 2). The QTL associated with potassium abundance was detected on LG 4 and was detected in datasets prepared by both filtering approaches (Figure2). Additionally, a QTL for K^+/Na^+ localized to the same interval on LG 4 as potassium abundance; this QTL was also detected in datasets prepared by both filtering approaches (Figure 3). For potassium abundance and K^+/Na^+, the QTL peaks from the datasets filtered for consistency of rank had higher LOD scores than those filtered to remove values from plants with very low biomass (Table 1 and Figures 3 and 4).

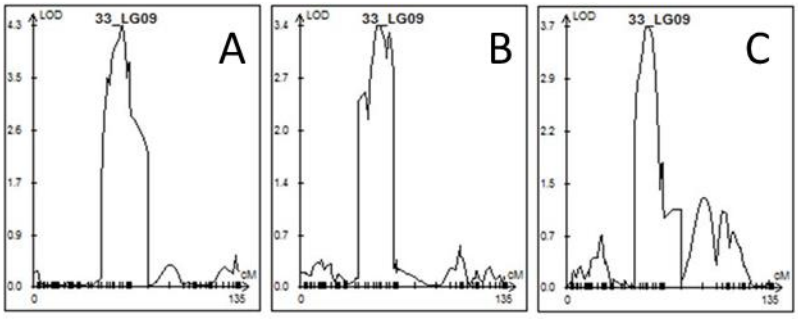

Figure 4.2: QTL peaks for Na+ abundance in HI33
QTL peaks on LG09 in HI33 for Na+ abundance in samples filtered for rank;
A: Rep 1; B: Rep 2; C: Rep 3

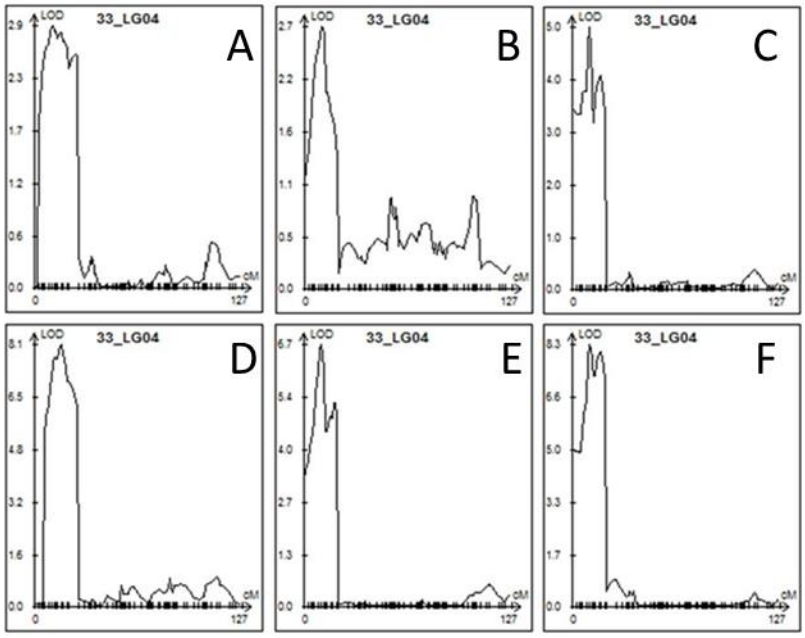

Figure 4.3: QTL peaks for K^+ abundance in HI33
QTL peaks on LG04 in HI33 for K+ abundance in samples filtered for biomass (A-C) and filtered for rank (D-F); A and D: Rep1; B and E: Rep 2; C and F: Rep 3

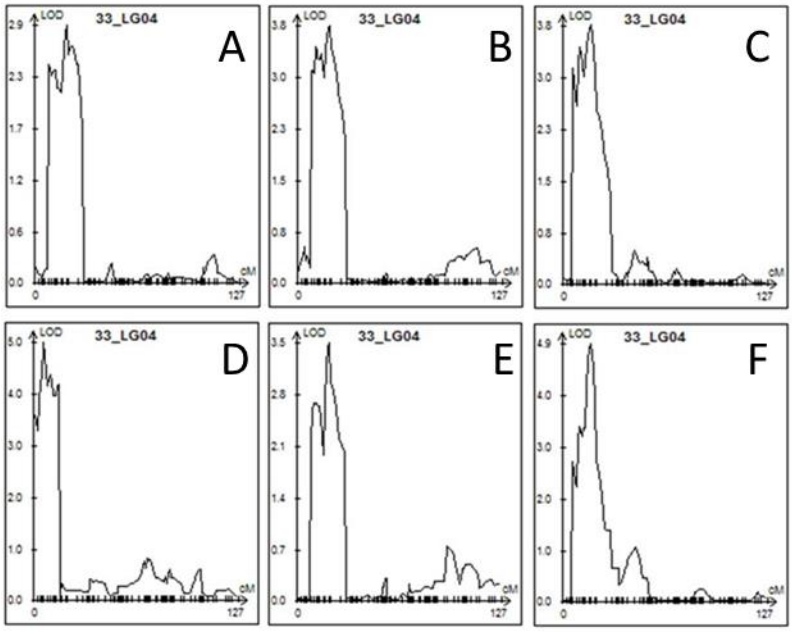

Figure 4.4: QTL peaks for ion ratio in HI33
QTL peaks on LG04 in HI33 for K^+/Na^+ in samples filtered for biomass (A-C) and filtered for rank (D-F); A and D: Rep1; B and E: Rep 2; C and F: Rep 3

Table 4.1: Detected QTL Summary

QTL ID	Linkage group (LG)	Marker Interval (Position range)	LOD value	Range of % phenotypic variance explained (R2)	Broad Sense Heritablilty (H2)
Na_1_rank	9	m50-m73 (48.7-64.3 cM)	4.3	9.8-13.4%	0.22
Na_2_rank	9	m50-m60 (48.7-58.6 cM)	3.4	9.4-10.6%	0.49
Na_3_rank	9	m50-m59 (48.7-56.9 cM)	3.7	10.2-12.0%	0.01
K_1_rank	4	m10-m27 (6.2-20.8 cM)	8.0	13.8-20.1%	0.61
K_2_rank	4	m2-m26 (0-20.2 cM)	6.7	9.2-17.9%	0.70
K_3_rank	4	m2-m26 (0-20.2 cM)	8.3	14.4-23.0%	-0.61
K/Na_1_rank	4	m2-m18 (0-14 cM)	5.0	13.0-19.0%	0.57
K/Na_2_rank	4	m11-m26 (8.7-20.2 cM)	3.4	7.0-11.7%	0.20
K/Na_3_rank	4	m12-m26 (10.6-20.2 cM)	4.9	12.1-18.0%	-0.08
K_1_biomass	4	m10-m27 (6.2-20.8 cM)	2.9	6.4-7.2%	0.74
K_2_biomass	4	m11-m27 (8.7-20.8 cM)	2.7	6.2-8.7%	0.88
K_3_biomass	4	m2-m26 (0-20.2 cM)	4.9	8.6-13.0%	-0.87
K/Na_1_biomass	4	m26* (20.2 cM)	2.9	6.3%	0.65
K/Na_2_biomass	4	m11-m27 (8.7-20.8 cM)	3.7	6.2-8.7%	0.29
K/Na_3_biomass	4	m10-m26 (6.2-20.2 cM)	3.8	6.9-9.8%	0.42

* adjacent markers all had LRs below the 11.5 threshold

Table 4.2: Candidate genes underlying the QTL for sodium abundance in the orthologous region of *S. bicolor*

Candidate Genes Underlying the QTL for Sodium Abundance on Paspalum Linkage Group 9 in *Sorghum bicolor*				
Gene Name	Description (from Phytozome)	Gene name from Swiss-Prot	Gene Start (bp)	Gene End (bp)
Sobic.009G089000	Similar to putative sodium/hydrogen exchanger	CHX19 or CHX18 or CHX17	15398378	15401199
Sobic.009G105900	Similar to cation chloride cotransporter	CCC1	42888312	42899575
Sobic.009G146800	(M=4) KOG0500 - Cyclic nucleotide-gated cation channel CNGA1-3 and related proteins	AKT2/3	50370739	50377457
Sobic.009G147000	(M=12) PTHR10217:SF6 - EAG-RELATED VOLTAGE-GATED POTASSIUM CHANNEL	AKT2/3	50387111	50389536
Sobic.009G147200	Similar to potassium channel protein ZMK2	AKT2/3	50416397	50419635
Sobic.009G147500	Similar to potassium channel protein ZMK2	AKT2/3	50441882	50451626

Table 4.3: Candidate genes underlying the QTL for potassium abundance and K^+/Na^+ ratio in the orthologous region of *S. bicolor*

Candidate Genes Underlying the QTL for Potassium Abundance and K+/Na+ Detected on Paspalum Linkage Group 4 in *Sorghum bicolor*				
Gene Name	Description (from Phytozome)	Gene name from Swiss-Prot	Gene Start (bp)	Gene End (bp)
Sobic.004G036400	Similar to putative calcium exchanger	VCX1 or CAX4 or CAX5	2924622	2927441
Sobic.004G057400	Similar to cation-transporting ATPase	RAN1	4590758	4595694
Sobic.004G059800	Similar to probable cation transporter HKT6	HKT6 (HKT1;3)	4795018	4797561
Sobic.004G060100	Similar to vacuolar ATP synthase catalytic subunit A	VHA-a	4812180	4818074

Candidate Genes Underlying QTL

In the sorghum genome, the region orthologous to the QTL detected for leaf sodium abundance spans ~41 Mb on chromosome 9 (9,809,027-50,599,108 Mb) and contains 737 predicted genes. From among those, seven genes belonging to three gene families were identified as having putative functions potentially related to ion transport and could be associated with sodium accumulation (Table 4.2). For the QTL detected for leaf potassium abundance and K^+/Na^+ ratio, the orthologous interval in sorghum spans ~4.5 Mb on chromosome 4 (861,095-5,316,023 Mb) and contains 571 predicted genes. From among those, four candidates with putative functions that relate to leaf potassium abundance and K^+/Na^+ ratio were identified (Table 4.3).

Discussion

Salt Tolerance in Seashore Paspalum

Seashore paspalum is widely recognized as an exceptionally salt tolerant species with the capability to survive in environments with salinity levels at or even greater than the ocean (Duncan and Carrow 2000; Endo et al. 2005). As such, it is clear that seashore paspalum must be able to leverage mechanisms that help it deal with the osmotic and ionic components of salt stress that would be extreme under such conditions (Munns and Tester 2008b). Glycinebetaine has been identified as the primary osmolyte used by seashore paspalum to ameliorate the osmotic effects of salt stress (Lee et al. 2008). However, exactly how it maintains its survival in the face of potential ionic effects is not clear. It has been shown using comparisons of ion accumulation in shoots that seashore paspalum utilizes a sodium exclusion mechanism, but the genes involved have not been identified (Marcum and Murdoch 1994). Our QTL analysis identified two

regions of the genome which appear to be contributing to ion control, one strongly associated with sodium abundance in leaves and another strongly associated with both potassium abundance in leaves as well as the maintenance of a higher K^+/Na^+ ratio in leaves. However it is important to remember that with the pseudo testcross approach, detectable QTL are limited to those that are conditioned by a locus which is heterozygous in one of the parents and homozygous in the other (Grattapaglia and Sederoff 1994b). In this way, if a QTL is detected, we are directly detecting the variation at that locus in one parent, while indirectly confirming the lack of phenotypic variation at that locus in the other parent. With this in mind, we can understand both of these salt tolerance traits as segregating in the genomic contribution of the father (HI33) to its progeny. However, because the sodium accumulation QTL explained only ~12% of the variation and the potassium accumulation QTL ~20% of the variation, additional QTL, presumably of small effects and possibly segregating in both parents, must be segregating in the population. Nevertheless, because HI33 has a lower mean sodium accumulation than 509022 (Figure 1), we can speculate that HI33 carries a superior allele for sodium exclusion on LG 9. Because HI33 also has lower mean potassium accumulation than 509022, which is typically associated with higher salt sensitivity, we hypothesize that salt tolerance in HI33 could be further enhanced by introducing an allele for high K accumulation at the QTL locus on LG 4.

Candidate Genes for Sodium Abundance

With regard to the candidates for the sodium abundance QTL, there is a no clear best candidate. The gene Sobic.009G089000, annotated as a putative sodium/hydrogen exchanger, was included as a candidate because it could be involved in sodium movement; however, it lies outside of the QTL interval detected in two of the three reps. When the corresponding protein was BLASTed

against the Swiss-Prot database, several members of the CHX protein family were among the best hits. Members of the CHX family have been implicated in cation homeostasis, with CHX21 and CHX23 being implicated in K^+ relationships in pollen development (Evans et al. 2012). Another member, CHX17, which was among the best hits returned from Swiss-Prot for our candidate gene, has been shown to be expressed in roots exposed to salt but not freshwater controls; however it is thought to be involved primarily with potassium uptake not sodium (Cellier et al. 2004). The gene Sobic.009G105900 was annotated as being similar to a cation chloride cotransporter (CCC1), so it was included as a candidate gene. However, this gene has been implicated specifically in potassium/chloride cotransport and has been documented to not have a role in sodium homeostasis (Kong et al. 2011).

The features identified as Sobic.009G146800, Sobic.009G147000, Sobic.009G147200, and Sobic.009G147500 were all annotated as having relation to a voltage and ligand gated potassium channel (AKT2/3). This gene has been implicated to be involved in sugar loading in phloem vessels and has no recognized putative involvement in salt tolerance in general, or specifically with sodium ion movement (Deeken et al. 2002).

Candidate Genes for Potassium Abundance and K^+/Na^+ ratio.

The gene Sobic.004G036400 closely matches sequence of CAX4 and CAX5 which are members of the cation exchanger family of proteins. This group of proteins was initially identified as being involved in Ca^{+2} transport into the vacuole, but it is now known that some members have affinities for other metal ions including sodium and potassium (Mäser et al. 2001). These authors advise that substrate specificity, general regulation as well as membrane localization of

these antiporters cannot be predicted with certainty from phylogenetic relationships, and as such, individual functional characterization is required to identify the specific roles of each.

The gene SobicG057400 was listed in phytozome as a cation transporting ATPase, however it has strong homology to RAN1, a copper-transporting P-type ATPase which has linked copper to ethylene response (Binder et al. 2010). As such, this gene is no longer a strong candidate for salt tolerance in paspalum and has no particular connection to potassium transport.

The gene Sobic.004G059800 was described in Phytozome as having similarity to a probable cation transporter, HKT6. Swiss-Prot confirmed that a number of HKT members have strong homology to this locus. The high-affinity potassium transporters (HKTs), in particular the HKT1 class of which HKT6 is a member (HKT6 alias is HKT1;3), are acknowledged to play a key role in salinity tolerance by enabling potassium transport while limiting sodium movement (Hamamoto et al. 2015; Rosas-Santiago et al. 2015). Rosas-Santiago et al. (2015) indicate that *Os*HKT1;3 functions as a sodium-selective transporter/channel and not as an Na+/K+ symporter. Mickelbart et al. (2015) review similar functions for *Tm*HKT1;4-A and *Tm*HKT1;5-A implicating then in sodium compartmentalization at the leaf sheath and the root, respectively. Other groups have confirmed this group of genes' role as maintaining potassium transport while limiting sodium uptake (Huang, Spielmeyer et al. 2006, Byrt, Platten et al. 2007, James, Blake et al. 2011). As such, this is a very strong candidate gene for this QTL.

The gene Sobic.004G060100 was identified as having similarity to a vacuolar H+-ATP synthase sub-unit. The functional enzyme of which this gene is a subunit is involved in pumping H+ into the vacuole; this contributes to building a H+ gradient across this membrane which could be leveraged by cation exchanger proteins to push cations back across the vacuolar membrane for compartmentalization (Zimniak et al. 1988). As such, this enzyme could be

tangentially related to sodium exclusion, by supporting a potentially increased capacity for sodium sequestration/vacuolar compartmentalization.

Filtering the Datasets for Biomass and Consistency of Rank:

Filtering out data from samples with very low biomass was done because exceptionally small plants were expected to exhibit effects from non-salt related issues. In general, these plants' reduced performance could have been an artifact of a plant simply not cloning well or they might have been more affected by crowding during the experiment than most of the lines in general. Ion concentrations in these plants typically were at the extreme ends of the ion concentration range measured in the mapping progeny, perhaps indicating that these plants were not sufficiently healthy to keep Na+ from accumulating in the leaves or that smaller tissue quantities are more likely to propagate errors. Clearly, consistency of rank was also a reasonable criterion with which to filter as this process, in general, returned peaks with higher LOD scores at the same locations than filtering on biomass alone (Table 4.1). This also may explain why the dataset filtered with consistency of rank returned a significant QTL for sodium abundance, but when the dataset was filtered only for biomass no QTL were detected. How consistency of rank might improve the dataset set is clear: genotypes that perform well for genetic reasons should consistently perform well across replications while genotypes that perform poorly for genetic reasons should consistently perform poorly. Why the values between replications were not consistent in some cases is less obvious. One possibility is that these samples were generated from green leaves as well as the green parts of leaves; it may be that some samples were composed of greater proportions of green leaf pieces coming from injured leaves than others and

as a result, those samples had higher sodium abundances than other samples representing the same genotype.

Conclusions

QTL for salt tolerance traits (sodium accumulation in leaves, potassium accumulation in leaves, and K^+/Na^+ ratio in leaves) are segregating in a F1 mapping population derived from two seashore paspalum lines previously identified as differing in salt tolerance. Though the parents differ in salt tolerance, with this mapping approach we are not mapping the differences between the parents *per se*, rather differences present within each parent. Referencing the orthologous regions associated with these QTL in the *Sorghum bicolor* genome has led to the identification of several candidate genes for these salt tolerance traits. A transcriptome analysis will be performed on parental materials exposed to freshwater and saline conditions to determine if any of these candidates are differentially regulated between the parents, show salt-specific responses, or organ-specific expression patterns.

In terms of breeding in seashore paspalum, we have provided a potentially valuable example that demonstrates the utility of this approach for identifying genomic regions controlling traits of interest. In the case of salt tolerance, by having molecular markers associated with desirable alleles, it is now possible to track those alleles without having to conduct additional salt tolerance screens. This has the potential to accelerate the breeding cycle while reducing costs. By forwarding recombinants identified as having the preferred allelic combination for salt tolerance, turf quality traits or other traits of interest could remain the focus of selection, while ensuring that salt tolerance level will not suffer. This could lead to the development of an elite cultivar that would be more difficult or costly to develop otherwise.

Additionally, this process could be extended to other traits of interest including dollar-spot resistance, shade tolerance, or any number of other things. Developing a suite of traits that could be pursued in this manner would accelerate breeding by enabling molecular marker based pre-screens for demonstrated traits. By halting the advancement of inferior lines before they are installed in selection blocks, time and energy spent would be focused on more elite materials. This opens the door to the molecular breeding era in seashore paspalum.

References

Binder, B. M., F. I. Rodriguez and A. B. Bleecker (2010). "The copper transporter RAN1 is essential for biogenesis of ethylene receptors in *Arabidopsis*." J Biol Chem 285(48): 37263-37270.

Bretó, M. P., M. J. Aśins and E. A. Carbonell "Salt tolerance in *Lycopersicon* species. III. Detection of quantitative trait loci by means of molecular markers." Theoretical and Applied Genetics 88(3): 395-401.

Cellier, F., G. Conejero, L. Ricaud, D. T. Luu, M. Lepetit, F. Gosti and F. Casse (2004). "Characterization of AtCHX17, a member of the cation/H+ exchangers, CHX family, from *Arabidopsis thaliana* suggests a role in K+ homeostasis." Plant J 39(6): 834-846.

Chen, Y., C. Chen, Z. Tan, J. Liu, L. Zhuang, Z. Yang and B. Huang (2016). "Functional identification and characterization of genes cloned from halophyte seashore paspalum conferring salinity and cadmium tolerance." Frontiers in Plant Science 7: 102.

Deeken, R., D. Geiger, J. Fromm, O. Koroleva, P. Ache, R. Langenfeld-Heyser, N. Sauer, S. T. May and R. Hedrich (2002). "Loss of the AKT2/3 potassium channel affects sugar loading into the phloem of *Arabidopsis*." Planta 216(2): 334-344.

Duncan, R. and R. Carrow (2000). Seashore Paspalum: the Environmental Turfgrass, Sleeping Bear Press.

Endo, N., K. Yoshida, M. Akiyoshi, Y. Yoshida and N. Hayashi (2005). "Putative UDP-galactose epimerase and metallothioneine of *Paspalum vaginalum* enhanced the salt tolerance of rice, *Oryza sativa* L. from transplanting to harvest stages." Breeding Science 55(2): 163-173.

Evans, A. R., D. Hall, J. Pritchard and H. J. Newbury (2012). "The roles of the cation transporters CHX21 and CHX23 in the development of *Arabidopsis thaliana*." J Exp Bot 63(1): 59-67.

Flowers, T. J. and S. A. Flowers (2005). "Why does salinity pose such a difficult problem for plant breeders?" Agricultural Water Management 78(1-2): 15-24.

Hamamoto, S., T. Horie, F. Hauser, U. Deinlein, J. I. Schroeder and N. Uozumi (2015). "HKT transporters mediate salt stress resistance in plants: from structure and function to the field." Current Opinion in Biotechnology 32: 113-120.

Kong, X. Q., X. H. Gao, W. Sun, J. An, Y. X. Zhao and H. Zhang (2011). "Cloning and functional characterization of a cation-chloride cotransporter gene OsCCC1." Plant Mol Biol 75(6): 567-578.

Lee, G. (2000). Comparative Salinity Tolerance and Salt Tolerance Mechanisms of Seashore Paspalum Ecotypes. Athens, GA, University of Georgia.

Lee, G., R. Carrow, R. Duncan, M. Eiteman and M. Rieger (2008). "Synthesis of organic osmolytes and salt tolerance mechanisms in *Paspalum vaginatum*." Environ Exp Bot 63: 19 - 27.

Marcum, K. and C. Murdoch (1994). "Salinity tolerance mechanisms of six C4 turfgrasses." J Am Soc Hort Sci 119: 779 - 784.

Mäser, P., S. Thomine, J. I. Schroeder, J. M. Ward, K. Hirschi, H. Sze, I. N. Talke, A. Amtmann, F. J. M. Maathuis, D. Sanders, J. F. Harper, J. Tchieu, M. Gribskov, M. W. Persans, D. E. Salt, S. A. Kim and M. L. Guerinot (2001). "Phylogenetic relationships within cation transporter families of *Arabidopsis*." Plant Physiology 126(4): 1646-1667.

Munns, R. and M. Tester (2008). "Mechanisms of salinity tolerance." Annu Rev Plant Biol 59: 651-681.

Rosas-Santiago, P., D. Lagunas-Gómez, B. J. Barkla, R. Vera-Estrella, S. Lalonde, A. Jones, W. B. Frommer, O. Zimmermannova, H. Sychrová and O. Pantoja (2015). "Identification of rice cornichon as a possible cargo receptor for the golgi-localized sodium transporter OsHKT1;3." Journal of Experimental Botany 66(9): 2733-2748.

Wang, S., C. J. Basten and Z.-B. Zeng (2012). Windows QTL Cartographer 2.5. Raleigh, NC, North Carolina State University.

Zimniak, L., P. Dittrich, J. P. Gogarten, H. Kibak and L. Taiz (1988). "The cDNA sequence of the 69-kDa subunit of the carrot vacuolar H+-ATPase. Homology to the beta-chain of F0F1-ATPases." J Biol Chem 263(19): 9102-9112.

CHAPTER 5

A PRELIMINARY EVALUATION OF SALT EXPOSURE RESPONSES IN ROOTS AND LEAVES OF TWO *PASPALUM VAGINATUM* ACCESSIONS THAT DIFFER IN SODIUM AND POTASSIUM ACCUMULATION BY RNA-SEQ[4]

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Abstract

Salt-tolerance, specifically tolerance to sodium, is an ability lacking in many crop plants. Identifying genes which may be involved in conferring the exceptional sodium tolerance of the grass seashore paspalum (*Paspalum vaginatum* Sw.) may provide insights that could be applied to other plant systems. Here we conducted a gene expression study utilizing an RNA-Seq approach on samples of roots and leaves from two genotypes identified as differing in salt tolerance level through screens of biomass and ion accumulation. Organ specific expression patterns were observed as well as salt-specific responses shared between the parents. In leaves, 11 transcripts were identified as upregulated (4-fold or greater) in response to salt, including a gene involved in proline accumulation. In roots, 114 transcripts were up regulated (4-fold or greater) in response to salt. Of those, 54 (47%) appeared to be associated with ribosomal sequence from bacteria, though many genes with stress-related annotations were identified as well. Additionally, one of the most highly expressed genes in both root and leaf transcriptomes was a gene encoding a metallothionein protein. This gene appears to be more highly expressed in the tolerant parent HI33 than in 509022. Metallothionein is a protein with metal-chelating abilities that is known to be involved in heavy metal-tolerance and has been previously discussed in the context of salt tolerance and seashore paspalum. Interestingly, in the sorghum genome, a sequence fragment with homology to metallothionein exists in a region orthologous to an interval adjacent to the QTL for sodium accumulation detected on HI33 linkage group 9. This implicates this gene, specifically its level of expression, in conferring the differences in salt tolerance observed between the parental genotypes. Additionally, the expression of HKT6, a positional candidate for the potassium abundance and ion ratio QTL, was found expressed in

leaves, but not roots, however significant differences in expression level between the parents were not detected

Introduction

Seashore paspalum is an exceptionally salt tolerant grass species that primarily utilizes a sodium exclusion mechanism to control sodium accumulation in leaf blades and may leverage vacuolar compartmentalization secondarily, to reduce the potential toxicity of sodium that does accumulate there (Marcum and Murdoch 1994). These processes are generally understood to be the primary mechanisms conferring tolerance to the ionic-effects of salt exposure in plants (Munns and Tester 2008). Though essential for most of the non-osmotic related salt tolerance observed in plants, the organ-specific localization of these processes and the genes involved often vary from organism to organism (Mickelbart et al. 2015). We have previously identified QTL for several component traits which are proxies for salt tolerance (sodium and potassium abundance in leaves as well as K^+/Na^+ ion ratio) in an F1 mapping population and have a strong candidate gene (HKT6) which may control both potassium level and the ion ratio maintenance in leaf blades (Chapter 4). HKT6 (alias HKT1;3), is a member of a sub-class of high-affinity potassium transporters whose other members have been implicated in salt tolerance in grass species (Huang et al. 2006; Byrt et al. 2007; Byrt et al. 2014). Interestingly, though these genes are closely related, they function as ion control points in distinct organ contexts: in roots, leaf blades, and in leaf sheath (Huang et al. 2006; Byrt et al. 2007; James et al. 2011; Byrt et al. 2014). For this reason, understanding HKT6's expression pattern in seashore paspalum, including whether it exhibits organ-specificity and/or is upregulated under salt stress could help clarify whether it is involved in these potassium associated traits previously observed.

Next-generation sequencing (NGS) technologies allow analysis of complete transcriptomes and hence have great potential to help uncover the genes responsible for specific biological processes. Furthermore, this is possible in the absence of a reference genome because software such as Trinity can perform *de novo* transcript assemblies (Haas et al. 2013). Read alignments against the transcript assemblies can be used to estimate transcript abundances and detect genes that are differentially expressed. Assemblies of interest can be compared to reference genomes, including those from other species with functional gene annotations in order to identify putative roles for genes exhibiting interesting patterns of expression. In general, these technologies allow genomic research to be carried out in non-model systems such as seashore paspalum, potentially leading to discoveries that may have broad implications for genetics, physiology and breeding.

Studies seeking to identify salt exposure responses as well as comparisons between tolerant and sensitive genotypes have leveraged NGS to conduct transcriptome surveys to implicate genes in salt response pathways and specifically in tolerance mechanisms. A number of studies of this sort have been done in grasses such as rice (Huang et al. 2014; Zhou et al. 2016), maize (Kravchik and Bernstein 2013; Zhang et al. 2015), wheat (Eren et al. 2015) and a barley wild-relative (Bahieldin et al. 2015). To date, we are aware of only one published study which has evaluated seashore paspalum salt tolerance with next-generation transcriptome sequencing (Jia et al. 2014). Our familiarity with seashore paspalum as a system provides a context-specific knowledge that is augmented by access to genetic maps and comparative genomics information as well as physiological data and QTL information relevant to the lines we have chosen to assay with a transcriptomic approach.

Materials and Methods

Plant Material

Two paspalum genotypes from the UGA breeder's collection (HI33 and 509022) which had been selected as parents of a mapping population developed to study salt tolerance in seashore paspalum (Chapters 3 and 4) were the focus of this study. These lines were selected for salt tolerance studies from a larger panel of germplasm because preliminary salt tolerance screening had identified them as varying in their salt tolerance abilities while a diversity analysis completed with microsatellite markers (Chapter 2) indicated that they represented genetic extremes among diploid lines in a diversity panel.

The two lines were multiplied for this experiment in the summer of 2012 by stolon propagation from single plants acquired from Paul Raymer (UGA Griffin). Single stolons were removed from mother plants and propagated in six inch pots in a mixture of the UGA Plant Biology Greenhouse Complex custom pine bark-based soil mix and Turface. A total of nine clones were propagated per genotype. Clones were allowed to grow into their pots for several months, but were trimmed regularly during this time to manage growth.

Grow Out and Harvest Timepoints

Two pots of each genotype were placed into a Sterlite bin which had been filled with 8 quarts of vermiculite (Figure 5.1). A total of 4 bins were prepared (Figure 5.2). The bins were placed on two ebb and flow tables (2 bins per table) which had been set up at the UGA Plant Biology Greenhouse Complex. Greenhouse temperatures were set to 80°F/60°F (day/night) and there was no artificial light supplementation. Sterlite bins capable of holding ~30 gallons were used as reservoirs for irrigation solutions, which were kept at 78°F +/- 2°F using 100W aquarium heaters

(Tetra). Once a day, the solution was pumped from the reservoir into the ebb and flow table where it flowed into the Sterlite bins containing vermiculite and plants by way of dozens of evenly spaced holes ~1mm in size, which had been drilled into the sides and bottom of each Sterlite bin. The duration of each daily irrigation event was 5 minutes. After the cessation of pumping, gravity drained the solution from the bins and returned it to the reservoirs.

Figure 5.1: Composite photo showing a single Sterlite bin with plants

HI33 clones (left) are indicated with blue tags while 509022 clones (right) are indicated with red tags. Stolons from each plant were allowed to grow out of the pots and "creep" in the vermiculite

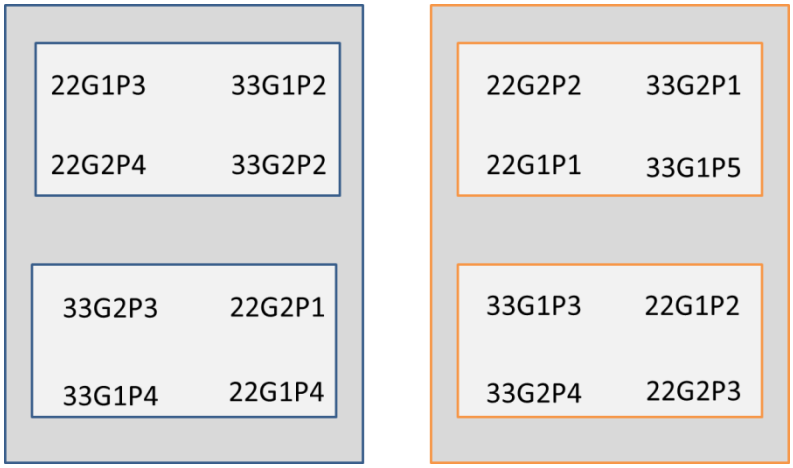

Figure 5.2: Sample layout for clones used in the transcriptome analysis.

Each bin, indicated with lighter grey, contains two clones of each genotype. The ebb and flow tables, indicated with darker grey, were filled with either a freshwater growth solution for control plants (shown on the left) or a saltwater growth solution for salt-exposed plants (shown on the right). 33 indicates genotype HI33; 22 indicates genotype 509022.

Two treatments, a freshwater control which was maintained with a solution conductivity of ~0.6 dS/m for the duration of the experiment and a saltwater treatment containing a sea salt mix (Oceanic) which was increased from ~0.6 dS/m to ~42 dS/m during the course of the experiment, were used (Figure 5.3). The salinity of the saltwater reservoir was increased by adding sea salt, ~100 grams at a time, until the conductance of the irrigation solution, as measured with an Oakton conductivity meter, was at the desired level. During the experiment, stolons were allowed to grow out of each pot and spread laterally through the vermiculite matrix. At various time points (Figure 5.3) the last ~8 inches of a single stolon was harvested (Figure 5.4) and separated into the following organ fractions: leaf, stem, root, node, internode, and stolon tip with a razor blade (GEM). Material from each of these organ fractions was placed into individually labeled microcentrifuge tubes, flash frozen in liquid nitrogen and stored at -80°C until RNA extractions were performed.

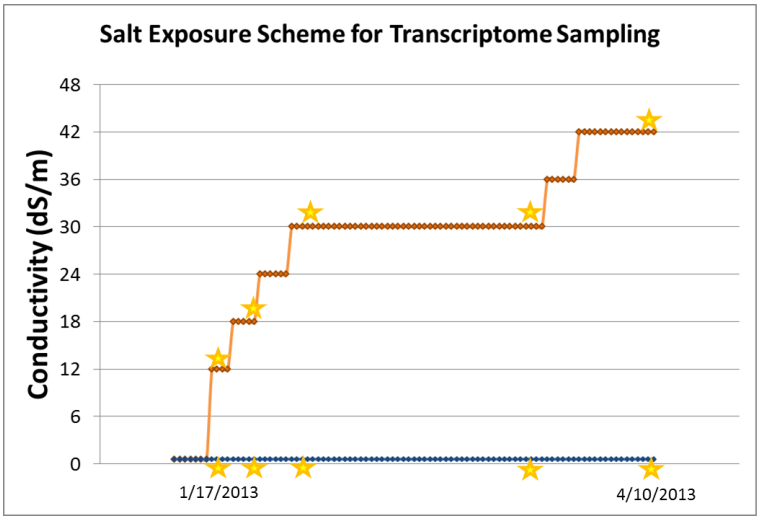

Figure 5.3 Time points for sample collection (indicated with yellow stars) and treatment salt levels.

The blue line represents the freshwater control; the orange line represents the salt treatment. Stars indicate collection time points.

Harvests were made at time points indicated in Figure 5.3 and listed below, but only time points marked by an '*' were analyzed here:

January 17, 2013; ~1hr after initial exposure to a salt solution of 12dS/m *

January 25, 2013; after salt levels had been held at ~18 dS/m for 5 days

February 3, 2013; after salt levels had first stabilized at ~30 dS/m for 5 days

March 20, 2013; after salt levels had been maintained at ~30 dS/m for 45 days

April 10, 2013; after salt levels had been held at ~42 dS/m for 14 days *

In total, four hundred and eighty samples were collected, from which forty-eight were selected for RNASeq analysis. These samples are presented in Table 5.1.

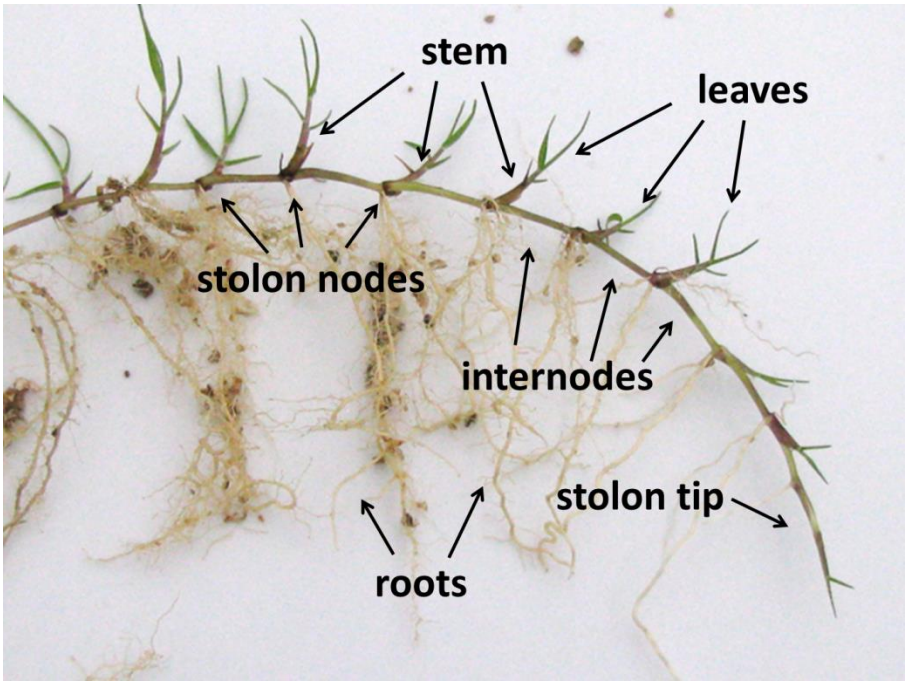

Figure 5.4: Photo example of harvested stolon section

Samples were taken from the last 8 inches of a growing stolon. Organ fractions collected were: roots, stem, leaf, stolon node, stolon internode, and stolon tip.

RNA Extraction and Library Preparation

Total RNA was extracted from ~75mg of bead ground and homogenized sample using Trizol reagent according to the manufacturer's instructions (Invitrogen). RNA quality (RNA integrity number – RIN) was assessed with the RNA 6000 Nano Kit on a BioAnalyzer 2100 machine (Agilent). Transcriptome libraries were produced at the Georgia Genomics Facility (GGF) using the Stranded RNA-Seq Kit (Kapa Biosystems). The libraries' concentrations were quantified and equimolar amounts were pooled together and sequenced for paired end (PE) reads 150 bp in length on a Nextseq using v2 300 cycle reagents (Supplementary Table 5.1).

Table 5.1: Libraries for RNA sequencing

Library #	sample name	organ	genotype	individual (Group#Plant#)	collection timepoint	Salt Treatment Level (dS/m)
1	12_RT_22G1P3	root	509022	G1P3	short term exposure	0.6 dS/m
2	12_RT_33G1P2	root	HI33	G1P2	short term exposure	0.6 dS/m
3	12_RT_22G2P2	root	509022	G2P2	short term exposure	12 dS/m
4	12_RT_33G2P1	root	HI33	G2P1	short term exposure	12 dS/m
5	12_RT_22G2P4	root	509022	G2P4	short term exposure	0.6 dS/m
6	12_RT_33G2P2	root	HI33	G2P2	short term exposure	0.6 dS/m
7	12_RT_22G1P1	root	509022	G1P1	short term exposure	12 dS/m
8	12_RT_33G1P5	root	HI33	G1P5	short term exposure	12 dS/m
9	12_RT_33G2P3	root	HI33	G2P3	short term exposure	0.6 dS/m
10	12_RT_22G2P1	root	509022	G2P1	short term exposure	0.6 dS/m
11	12_RT_33G1P3	root	HI33	G1P3	short term exposure	12 dS/m
12	12_RT_22G1P2	root	509022	G1P2	short term exposure	12 dS/m
13	12_RT_33G1P4	root	HI33	G1P4	short term exposure	0.6 dS/m
14	12_RT_22G1P4	root	509022	G1P4	short term exposure	0.6 dS/m
15	12_RT_33G2P4	root	HI33	G2P4	short term exposure	12 dS/m
16	12_RT_22G2P3	root	509022	G2P3	short term exposure	12 dS/m
17	40_RT_22G1P3	root	509022	G1P3	long term exposure	0.6 dS/m
18	40_LF_22G1P3	leaf	509022	G1P3	long term exposure	0.6 dS/m
19	40_RT_33G1P2	root	HI33	G1P2	long term exposure	0.6 dS/m
20	40_LF_33G1P2	leaf	HI33	G1P2	long term exposure	0.6 dS/m
21	40_RT_22G2P2	root	509022	G2P2	long term exposure	42 dS/m
22	40_LF_22G2P2	leaf	509022	G2P2	long term exposure	42 dS/m
23	40_RT_33G2P1	root	HI33	G2P1	long term exposure	42 dS/m
24	40_LF_33G2P1	leaf	HI33	G2P1	long term exposure	42 dS/m
25	40_RT_22G2P4	root	509022	G2P4	long term exposure	0.6 dS/m
26	40_LF_22G2P4	leaf	509022	G2P4	long term exposure	0.6 dS/m
27	40_RT_33G2P2	root	HI33	G2P2	long term exposure	0.6 dS/m
28	40_LF_33G2P2	leaf	HI33	G2P2	long term exposure	0.6 dS/m
29	40_RT_22G1P1	root	509022	G1P1	long term exposure	42 dS/m
30	40_LF_22G1P1	leaf	509022	G1P1	long term exposure	42 dS/m
31	40_RT_33G1P5	root	HI33	G1P5	long term exposure	42 dS/m
32	40_LF_33G1P5	leaf	HI33	G1P5	long term exposure	42 dS/m
33	40_RT_33G2P3	root	HI33	G2P3	long term exposure	0.6 dS/m
34	40_LF_33G2P3	leaf	HI33	G2P3	long term exposure	0.6 dS/m
35	40_RT_22G2P1	root	509022	G2P1	long term exposure	0.6 dS/m
36	40_LF_22G2P1	leaf	509022	G2P1	long term exposure	0.6 dS/m
37	40_RT_33G1P3	root	HI33	G1P3	long term exposure	42 dS/m
38	40_LF_33G1P3	leaf	HI33	G1P3	long term exposure	42 dS/m
39	40_RT_22G1P2	root	509022	G1P2	long term exposure	42 dS/m
40	40_LF_22G1P2	leaf	509022	G1P2	long term exposure	42 dS/m
41	40_RT_33G1P4	root	HI33	G1P4	long term exposure	0.6 dS/m
42	40_LF_33G1P4	leaf	HI33	G1P4	long term exposure	0.6 dS/m
43	40_RT_22G1P4	root	509022	G1P4	long term exposure	0.6 dS/m
44	40_LF_22G1P4	leaf	509022	G1P4	long term exposure	0.6 dS/m
45	40_RT_33G2P4	root	HI33	G2P4	long term exposure	42 dS/m
46	40_LF_33G2P4	leaf	HI33	G2P4	long term exposure	42 dS/m
47	40_RT_22G2P3	root	509022	G2P3	long term exposure	42 dS/m
48	40_LF_22G2P3	leaf	509022	G2P3	long term exposure	42 dS/m

Read Trimming, Trinity Assembly and Transcript Abundance Estimation

Quality trimming of the raw reads and adapter removal was done with Trimmomatic (Bolger et al. 2014); the output of trimmed forward reads became the paired or unpaired forward reads and the output of the trimmed reverse reads became the paired or unpaired reverse reads. The ILLUMINACLIP parameters were 2:30:10 and adapters identities from TruSeq3-PE-2 were used to trim adaptors. Leading and trailing quality parameters in Trimmomatic were each set to 20 and the minimum length to keep a read was set to 35.

Trinity (Haas et al. 2013) was used to assemble the paired end data from all forty eight libraries into a single assembly. Reads were *in-silico* normalized and because these were paired-end libraries developed with the dUTP method, the library-type parameter was set to 'RF.' Read abundance estimation was achieved by utilizing the default parameters in RSEM with Bowtie via a Trinity wrapper (Li and Dewey 2011). To identify transcripts with differential expression, edgeR was fed counts matrices generated with RSEM (Robinson et al. 2010). The default parameters from edgeR were used to extract transcripts with differential expression (p-value cutoff = 0.001 and fold change = 4). To identify expression clusters, the hierarchically clustered tree generated by edgeR was cut into 25 sub clusters and then each sub cluster plot manually inspected for patterns consistent with salt-response (elevated expression in salt exposed plants). These steps were completed on the root and leaf samples from the later collection time point (salt levels: 0.6 dS/m and 42 dS/m) and evaluated independently for each organ.

Results

Organ-specific expression patterns

A correlation matrix containing all 48 libraries was produced by RSEM and illustrates two distinct expression patterns consistent with organ-specificity (Figure 5.4). In general, it confirms that replicate samples were most similar to other replicates of the same genotype, organ, and treatment combination. The exceptions were the root samples from genotype 509022 sampled immediately after initial salt exposure which clustered with their freshwater controls. Library 12_RT_22G1P3_F appeared to be inexplicably distinct from all other libraries (Figure 5.5) and was removed from the subsequent analyses.

Treatment-specific expression patterns

Expression profiles in roots and leaves collected after long-term exposure to ocean strength salt were compared with those of their freshwater controls (Figure 5.6 and Figure 5.8). In each set, transcripts upregulated 4-fold in response to salt, but not necessarily differentially expressed between the parents were identified (Figure 5.7 and Figure 5.9). In leaf samples, 11 transcripts were found to be upregulated in response to salt, while in roots, 114 transcripts were determined to be upregulated in response to salt. The identity of each of these assembled transcripts was ascertained by comparing the transcript sequence to the NCBI database with BLAST. In the case of the root samples, 54 of 114 (47%) matched bacterial ribosomal sequence. In leaves, 2 of 11 transcript assemblies (~18%) were found to likely correspond to 60S ribosomal RNA. The transcripts and their putative functions are listed in Supplementary Tables 5.2 and 5.3. The majority of genes not identifies as ribosomal, were annotated for functions which indicate roles in salt exposure response.

Table 5.2: Read Content and Alignment Rate

Library #	sample name	reads (TOTAL)	reads that aligned		reads that failed to align	
			number	% of total	number	% of total
1	12_RT_22G1P3	11992985	4718553	39.34%	7274432	60.66%
2	12_RT_33G1P2	10294985	4392050	42.66%	5902935	57.34%
3	12_RT_22G2P2	8798819	3771329	42.86%	5027490	57.14%
4	12_RT_33G2P1	7956399	3554986	44.68%	4401413	55.32%
5	12_RT_22G2P4	8747866	3998647	45.71%	4749219	54.29%
6	12_RT_33G2P2	8690860	3673525	42.27%	5017335	57.73%
7	12_RT_22G1P1	8974885	3726285	41.52%	5248600	58.48%
8	12_RT_33G1P5	10444181	4525037	43.33%	5919144	56.67%
9	12_RT_33G2P3	10215890	4292205	42.01%	5923685	57.99%
10	12_RT_22G2P1	9806788	4375660	44.62%	5431128	55.38%
11	12_RT_33G1P3	10411240	4582110	44.01%	5829130	55.99%
12	12_RT_22G1P2	9431998	4030682	42.73%	5401316	57.27%
13	12_RT_33G1P4	9147255	4112542	44.96%	5034713	55.04%
14	12_RT_22G1P4	9251944	4159474	44.96%	5092470	55.04%
15	12_RT_33G2P4	8851508	4097283	46.29%	4754225	53.71%
16	12_RT_22G2P3	9955453	4211657	42.31%	5743796	57.69%
17	40_RT_22G1P3	12241749	5375041	43.91%	6866708	56.09%
18	40_LF_22G1P3	9919526	4432507	44.68%	5487019	55.32%
19	40_RT_33G1P2	10150731	4654273	45.85%	5496458	54.15%
20	40_LF_33G1P2	10201437	4484147	43.96%	5717290	56.04%
21	40_RT_22G2P2	10018939	4125053	41.17%	5893886	58.83%
22	40_LF_22G2P2	8990069	3931500	43.73%	5058569	56.27%
23	40_RT_33G2P1	8805052	3615229	41.06%	5189823	58.94%
24	40_LF_33G2P1	9654328	4085672	42.32%	5568656	57.68%
25	40_RT_22G2P4	11826828	5130297	43.38%	6696531	56.62%
26	40_LF_22G2P4	9744129	4330917	44.45%	5413212	55.55%
27	40_RT_33G2P2	10503145	4525943	43.09%	5977202	56.91%
28	40_LF_33G2P2	9676105	4278732	44.22%	5397373	55.78%
29	40_RT_22G1P1	10156112	4346499	42.80%	5809613	57.20%
30	40_LF_22G1P1	9139099	4610731	50.45%	4528368	49.55%
31	40_RT_33G1P5	9392976	3890431	41.42%	5502545	58.58%
32	40_LF_33G1P5	11213429	4754851	42.40%	6458578	57.60%
33	40_RT_33G2P3	11513357	5466879	47.48%	6046478	52.52%
34	40_LF_33G2P3	9658166	4451851	46.09%	5206315	53.91%
35	40_RT_22G2P1	9742173	4608072	47.30%	5134101	52.70%
36	40_LF_22G2P1	8757674	4019312	45.89%	4738362	54.11%
37	40_RT_33G1P3	9113849	4202886	46.12%	4910963	53.88%
38	40_LF_33G1P3	9328748	4276397	45.84%	5052351	54.16%
39	40_RT_22G1P2	8881233	4011671	45.17%	4869562	54.83%
40	40_LF_22G1P2	11486559	4862909	42.34%	6623650	57.66%
41	40_RT_33G1P4	11101569	5039003	45.39%	6062566	54.61%
42	40_LF_33G1P4	7707004	3322088	43.10%	4384916	56.90%
43	40_RT_22G1P4	8407806	3818758	45.42%	4589048	54.58%
44	40_LF_22G1P4	9744129	4330917	44.45%	5413212	55.55%
45	40_RT_33G2P4	10160319	4547057	44.75%	5613262	55.25%
46	40_LF_33G2P4	8974622	4067419	45.32%	4907203	54.68%
47	40_RT_22G2P3	8946508	4183594	46.76%	4762914	53.24%
48	40_LF_22G2P3	8310159	3673867	44.21%	4636292	55.79%

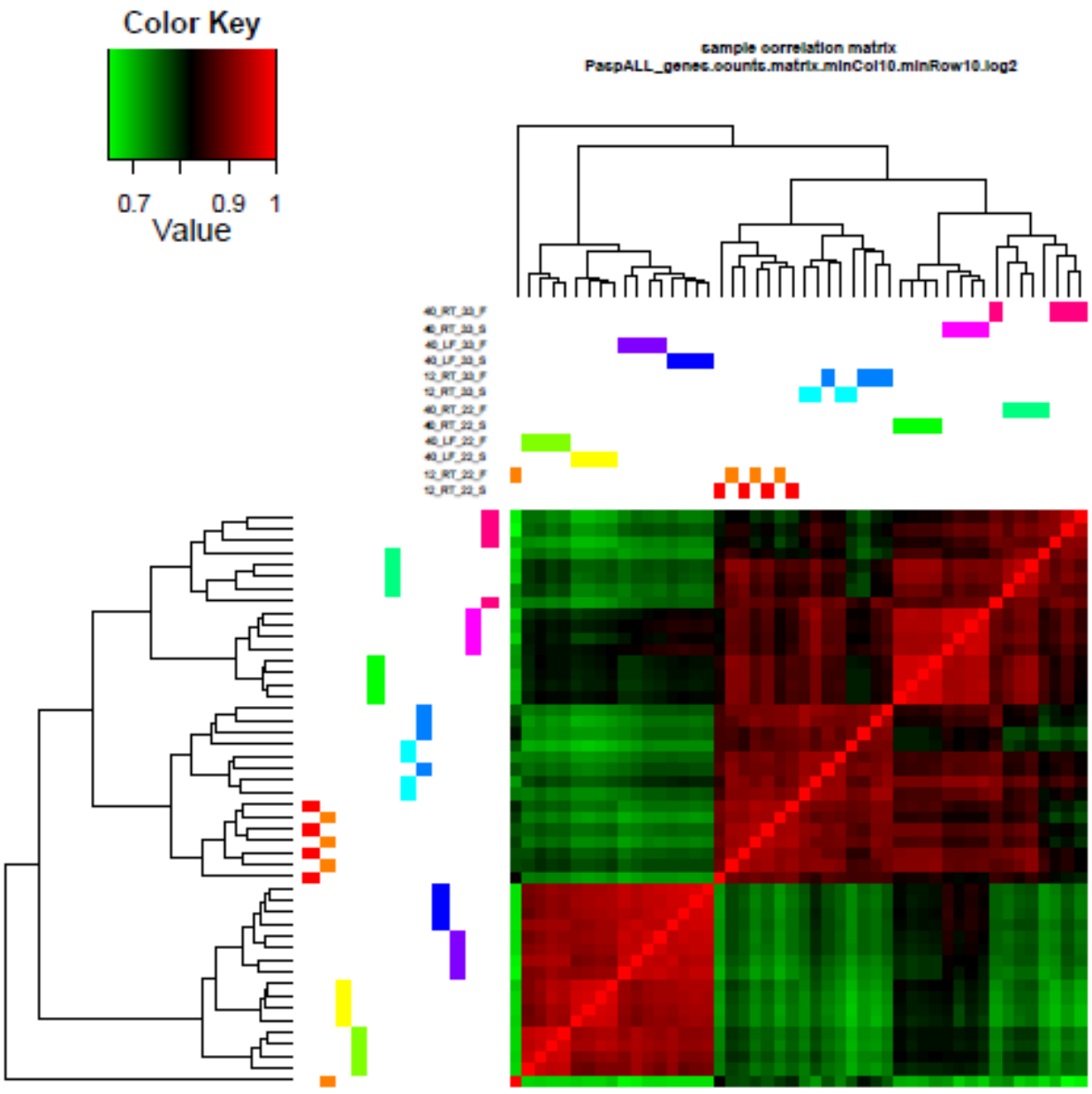

Figure 5.5: Library correlation matrix (all 48 libraries)

Library names for Figure 5.5 are listed in order in Table 5.3

Table 5.3: List of library names associated with correlation matrix (Figure 5.5)

Libraries are listed in order, as they appear from top to bottom on the y-axis of the correlation matrix illustrated in Figure 5.5. Samples IDs indicate timepoint (12 or 40); organ root (RT) or leaf (LF); individual plant; and whether the sample was grown in freshwater (F) or under salt (S).

Library Orders from Figure 5.4 (right side, top to bottom)			
1	40_RT_33G2P3_F	25	12_RT_22G1P2_S
2	40_RT_33G1P4_F	26	12_RT_22G1P4_F
3	40_RT_33G1P2_F	27	12_RT_22G2P3_S
4	40_RT_22G1P4_F	28	12_RT_22G2P1_F
5	40_RT_22G2P1_F	29	12_RT_22G1P1_S
6	40_RT_22G1P3_F	30	12_RT_22G2P4_F
7	40_RT_22G2P4_F	31	12_RT_22G2P2_S
8	40_RT_33G2P2_F	32	40_LF_33G2P1_S
9	40_RT_33G2P4_S	33	40_LF_33G1P5_S
10	40_RT_33G1P5_S	34	40_LF_33G2P4_S
11	40_RT_33G1P3_S	35	40_LF_33G1P3_S
12	40_RT_33G2P1_S	36	40_LF_33G2P2_F
13	40_RT_22G2P3_S	37	40_LF_33G1P2_F
14	40_RT_22G1P2_S	38	40_LF_33G2P3_F
15	40_RT_22G2P2_S	39	40_LF_33G1P4_F
16	40_RT_22G1P1_S	40	40_LF_22G2P3_S
17	12_RT_33G2P3_F	41	40_LF_22G1P2_S
18	12_RT_33G2P2_F	42	40_LF_22G2P2_S
19	12_RT_33G1P4_F	43	40_LF_22G1P1_S
20	12_RT_33G1P5_S	44	40_LF_22G1P4_F
21	12_RT_33G2P1_S	45	40_LF_22G1P3_F
22	12_RT_33G1P2_F	46	40_LF_22G2P4_F
23	12_RT_33G2P4_S	47	40_LF_22G2P1_F
24	12_RT_33G1P3_S	48	12_RT_22G1P3_F

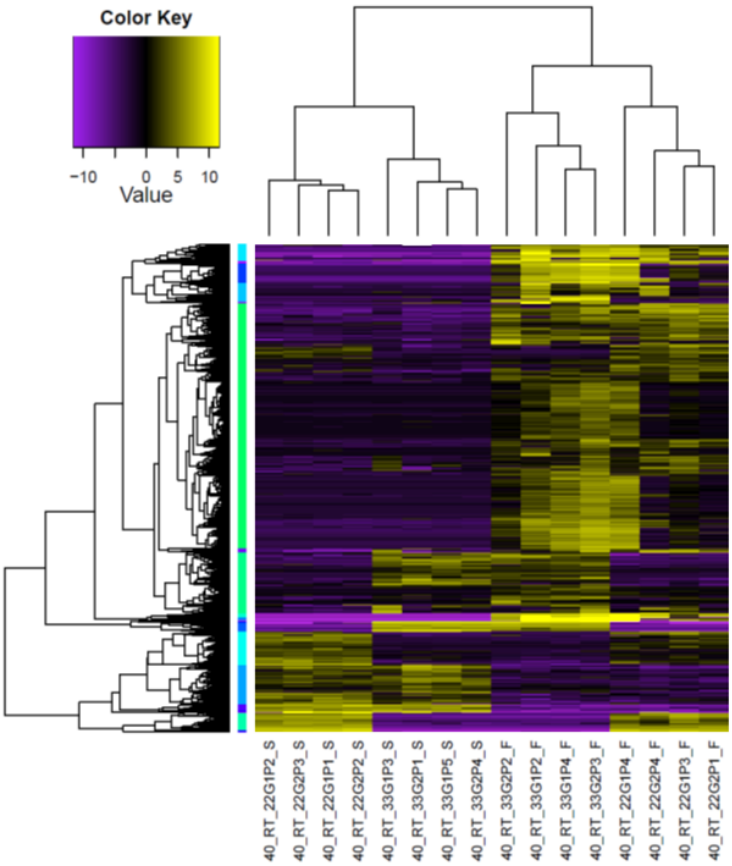

Figure 5.6: Expression clusters detected in root samples grown for long duration (k=25)

Samples include 4 biological replicates of each parental genotype grown at 0.6 dS/m (freshwater) and 42 dS/m (ocean strength salt water)

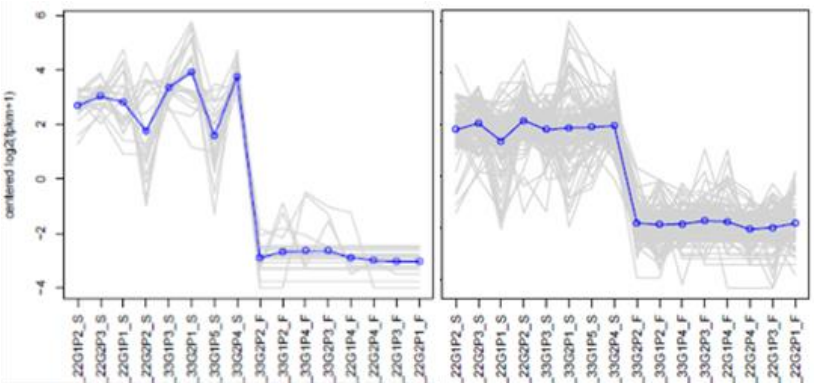

Figure 5.7: Subclusters showing transcripts with salt specific transcription level increases in root

Library identities are shown on x-axis, expression fold change is indicated on y-axis

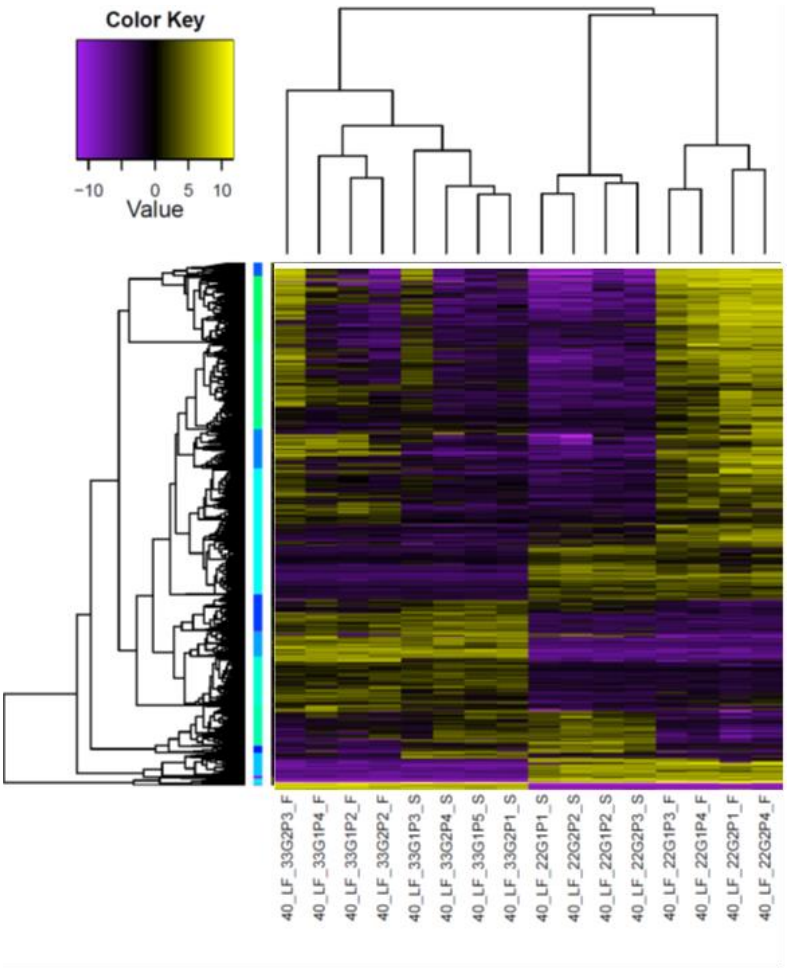

Figure 5.8: Expression clusters detected in leaf samples (k=25)
Samples include 4 biological replicates of each parental genotype grown at 0.6 dS/m (F, freshwater) and 42 dS/m (S, ocean strength salt water)

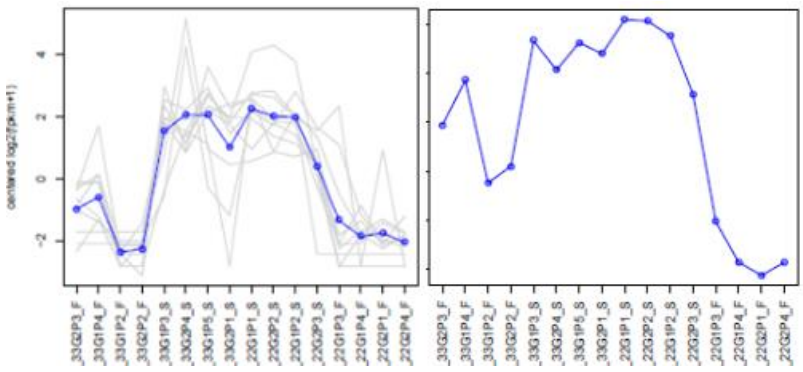

Figure 5.9: Subclusters showing transcripts with salt specific transcription level increases in leaf
Library identities are shown on x-axis, expression fold change is indicated on y-axis

Virus-like transcripts compose a large proportion of the paternal transcriptome

A single transcript family assembled in Trinity contributed to a large proportion of the reads from the paternal parent (HI33). BLAST comparisons made with the NCBI database determined that many of these sequences have homology to sequences annotated as plant virus. In the leaves of HI33 under both fresh and saltwater conditions, these virus-like sequences comprised ~15-18% of the total aligned reads on average. In general, HI33 exhibited these virus-like transcripts to a much greater extent: at all time points, under all conditions, and in both organs whereas 509022 did not (Figure 5.10).

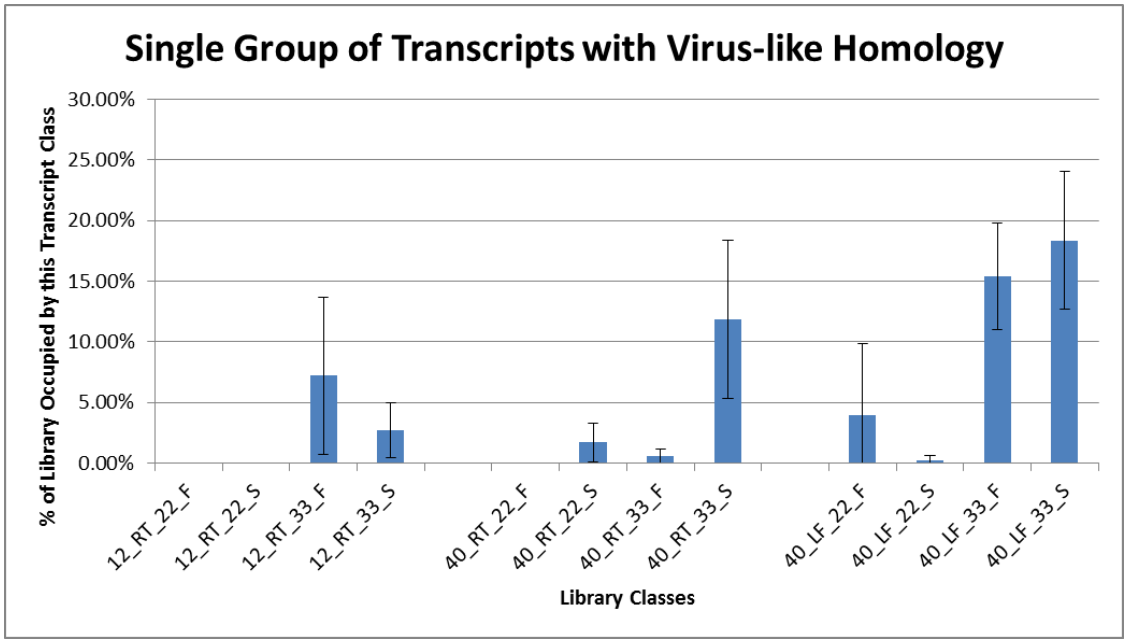

Figure 5.10: Read allocation to a single virus-like set of transcripts

Error bars indicate standard errors from four biological replicates. Values displayed are the means of the sum of the TPM values for each of four biological replicates

HKT6-like Expression illustrates Organ Specificity

An HKT6-like gene was identified as a candidate for a QTL for K^+ abundance and K^+/Na^+ ratio on paspalum chromosome 4 (Chapter 4). The sorghum HKT6 sequence was used to query the Trinity transcript set with BLAST. A single transcript was found to have homology. Evaluating its expression levels in leaf- and root-specific libraries indicated that it exhibits organ specificity, but not differential expression between the parents or expression changes in response to salinity level (Figure 5.11). A three–way ANOVA conducted between samples collected at the later time point (salt level at 42 dS/m) with genotype, organ, and salt treatment level as factors identified organ as significant ($p<0.0001$), while no significant effect was found for genotype nor salt exposure level.

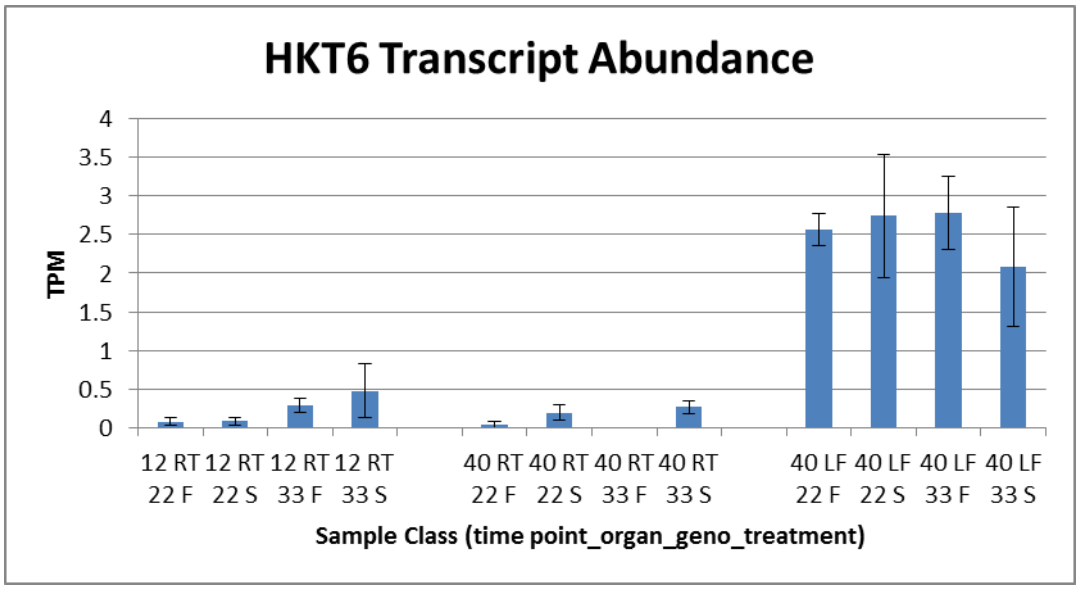

Figure 5.11: Expression levels of a HKT6-like gene in seashore paspalum

Error bars indicate standard errors from four biological replicates.

Metallothionein Is Highly Expressed in Paspalum Organs

A survey of highly expressed transcripts (>1500 transcripts per million [TPM]) identified metallothionein as consistently having high expression levels across sample types. Eleven transcripts were assembled with five (g1,g2,g5,g8, and g11) showing expression levels greater than 100 TPM, with g11 only occurring in HI33 (Figures 5.13 and 5.14); a transcript containing the 3' UTR was assembled separately with Trinity and consists of four alleles in total with two present in each parent (Figure 5.12) A two-way ANOVA on root transcript abundance data for the 3'UTR containing regions with genotype and treatment as factors found transcript abundance differences associated with treatment ($p < 0.001$) as well as genotype ($p < 0.05$), but no significant interaction between the factors. Because the biological relevance of the five expressed transcripts was unclear, no statistical comparison was made, however in roots, in general, transcript abundance consistently appears to be higher in samples from HI33 (Figure 5.13)

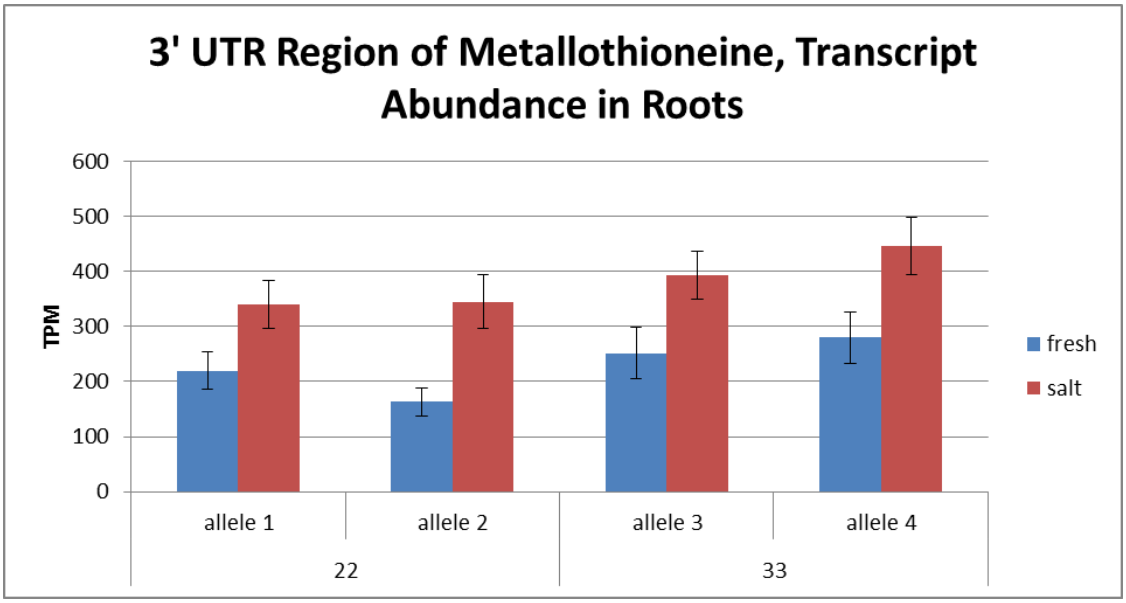

Figure 5.12: Expression levels of the 3' UTR region of the metallothionein gene
Error bars indicate standard errors from four biological replicates.

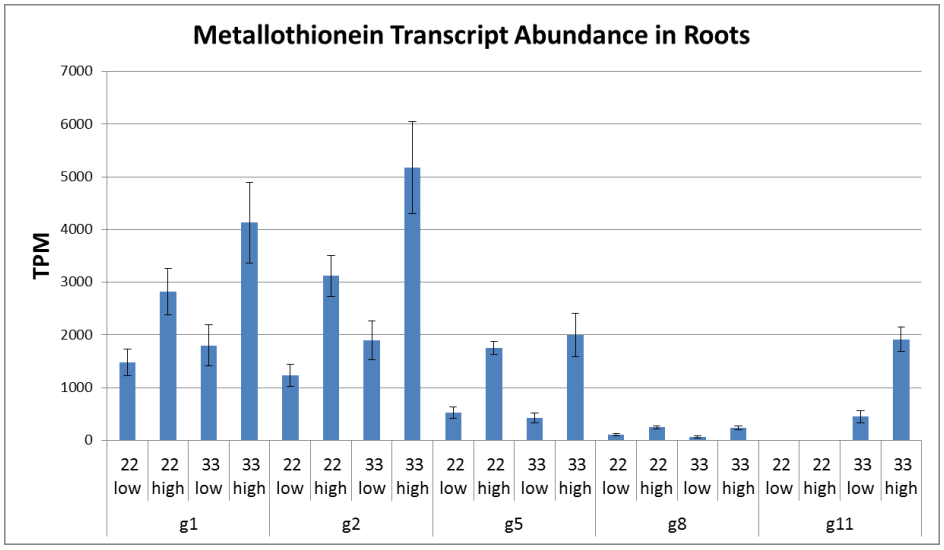

Figure 5.13: Expression levels of metallothionein in roots; g1, g2, g5, g8, and g11 indicate individually assembled trinity transcripts
Error bars indicate standard errors from four biological replicates.

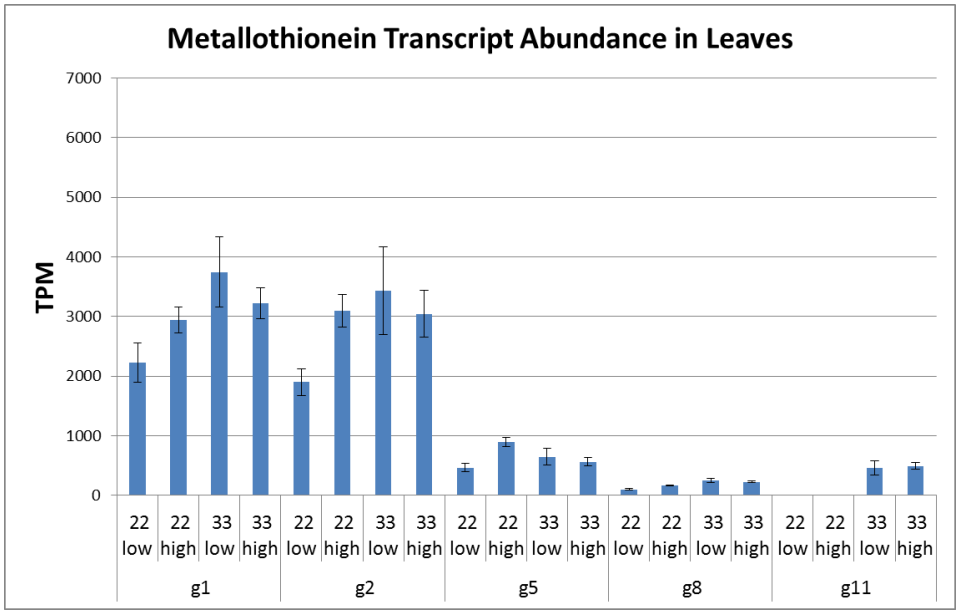

Figure 5.14: Expression levels of metallothionein in roots; g1, g2, g5, g8, and g11 indicate individually assembled trinity transcripts
Error bars indicate standard errors from four biological replicates.

Discussion

In general, most plants lack salinity tolerance mechanisms and even small amounts of salt in the soil can cause severe injury and/or death for many species. Seashore paspalum is a salt tolerant grass which has an impressive amount of salt tolerance and experiences its greatest productivity under salt exposure of ~15dS/m (Lee 2000 and P. Raymer, personal communication). In order to understand what loci are responsible for this ability we undertook studies to develop resources for studying the genetics of salt tolerance in seashore paspalum. Toward that aim, we identified QTL for salt tolerance traits in a seashore paspalum mapping population (Chapter 4). To support evaluating positional candidate genes, we conducted a transcriptome analysis of root and leaf material from the parental lines grown under different salt levels. Because the lines had previously been identified to vary in salt tolerance ability, screening for transcriptional pattern differences could elucidate biologically relevant processes.

The majority of the samples evaluated here were from plants grown at a salt level nearing that of ocean water (~42 dS/m) along with freshwater controls. We identified organ-specific expression patterns which distinguished roots from leaves and surveyed each of those organs for transcripts that were upregulated in material from plants grown under high salinity. A relatively small number of transcripts (on the order of tens) out of thousands were found to be upregulated in response to salt at a 4-fold increase. Many of these were found to match genes with functions related to different processes associated with stress response including heat-shock proteins, late embryogenesis abundant proteins, starch processing and dehydration stress .

We also specifically characterized the expression pattern of HKT6, a positional candidate from a QTL study (Chapter 4), that is a member of a family of transporters known to be involved in ion homeostasis. We observed that it was expressed consistently in leaves, but not in roots.

And though it was expressed in leaves, neither genotypic nor treatment level effects were found to be significant. As such, if HKT6 is involved in salt tolerance in paspalum, the lack of observed expression differences between the parents complicates an interpretation of how it would cause the phenotypic differences observed in the parents as well as those detected in the mapping population. Obviously it is possible that there could be relevant expression differences in organs which were not assayed (e.g. stem or leaf sheath). It is also possible that expression levels in leaves change in an age dependent-manner (the samples collected and analyzed in this experiment were relatively young). This was done specifically to prevent developmental or sample age differences from affecting inter-individual comparisons; however such a sampling regime precludes observing patterns in older leaves.

It is also possible that the abundance of virus-like sequence, which was disproportionately greater in the paternal genotype (HI33) compared to the maternal genotype (509022), particularly in leaves, may have played a role in obscuring significant differences. This is not a suggestion that the virus itself would have had direct effects on the expression levels detected, but rather because transcript abundance was presented in TPM, which is a normalized value of library content, that an indirect effect on transcript abundance estimates is possible, even likely. Since most some paternal libraries contained almost 20% of viral sequence and HKT6 expression was rather low (estimated at only ~2.5 TPM in leaves or ~0.00025% of the libraries total content) this is not an unreasonable explanation. Owing to these potential complications, more work is necessary before HKT6 is accepted or rejected as the gene responsible for the potassium and ion homeostasis phenotypes observed.

Despite the potential complications involved in detecting significant differences between libraries comprised of differing amounts of viral sequence, a metallothionein was determined to

be significantly more highly expressed in the roots of the paternal parent than in the maternal parent. The fact that the father had significantly higher expression despite the presence of virus as described above, should be taken as an indication that the actual genotypic differences are likely even greater than those observed. It is important to reiterate that the metallothionein gene was consistently among the most highly expressed protein coding genes detected. More importantly, increased expression in the parents and a likely role in metal ion (sodium) sequestration suggests that this gene could be relevant for salt tolerance. Importantly, despite the fact that this gene was not identified as a candidate gene in the QTL study (Chapter 4), it may still have relevance; a gene fragment with homology to the coding region of this gene is present on LG9 of sorghum in a genomic interval adjacent to region containing the sodium abundance QTL.

Conclusions

Salt tolerance is a complex trait that has the potential to involve large numbers of genes. In the salt tolerant grass seashore paspalum, we found significant differences in the expression patterns of more than 50 root expressed transcripts that were associated with salt response as well as 9 leaf expressed transcripts. Though these genes seem to be related to normal stress response processes, it should be noted that they were observed in plants growing in a solution with the salt strength of the ocean. As such, it is unlikely that such genes would be responsible for the high salt tolerance observed in this species without other actors present. We detected a gene that was constitutively expressed (metallothionein) and is associated with salinity tolerance *per se*, not just ameliorating the effects of salt. We are unable to confirm with certainty from this experiment that this genes confers paspalum's impressive level of salt tolerance, but taken

together with the fact its function aligns perfectly with the trait associated with the sodium QTL, it should be considered a strong candidate and investigated further.

References

Bahieldin, A., A. Atef, J. S. Sabir, N. O. Gadalla, S. Edris, A. M. Alzohairy, N. A. Radhwan, M. N. Baeshen, A. M. Ramadan, H. F. Eissa, S. M. Hassan, N. A. Baeshen, O. Abuzinadah, M. A. Al-Kordy, F. M. El-Domyati and R. K. Jansen (2015). "RNA-Seq analysis of the wild barley (*H. spontaneum*) leaf transcriptome under salt stress." C R Biol 338(5): 285-297.

Bolger, A. M., M. Lohse and B. Usadel (2014). "Trimmomatic: a flexible trimmer for Illumina sequence data." Bioinformatics 30(15): 2114-2120.

Byrt, C. S., J. D. Platten, W. Spielmeyer, R. A. James, E. S. Lagudah, E. S. Dennis, M. Tester and R. Munns (2007). "HKT1;5-like cation transporters linked to Na(+) exclusion loci in wheat, Nax2 and Kna1." Plant Physiology 143(4): 1918-1928.

Byrt, C. S., B. Xu, M. Krishnan, D. J. Lightfoot, A. Athman, A. K. Jacobs, N. S. Watson-Haigh, D. Plett, R. Munns, M. Tester and M. Gilliham (2014). "The Na(+) transporter, TaHKT1;5-D, limits shoot Na(+) accumulation in bread wheat." Plant J 80(3): 516-526.

Eren, H., M. Y. Pekmezci, S. Okay, M. Turktas, B. Inal, E. Ilhan, M. Atak, M. Erayman and T. Unver (2015). "Hexaploid wheat (*Triticum aestivum*) root miRNome analysis in response to salt stress." Annals of Applied Biology 167(2): 208-216.

Haas, B. J., A. Papanicolaou, M. Yassour, M. Grabherr, P. D. Blood, J. Bowden, M. B. Couger, D. Eccles, B. Li, M. Lieber, M. D. MacManes, M. Ott, J. Orvis, N. Pochet, F. Strozzi, N. Weeks, R. Westerman, T. William, C. N. Dewey, R. Henschel, R. D. LeDuc, N.

Friedman and A. Regev (2013). "De novo transcript sequence reconstruction from RNA-Seq: reference generation and analysis with Trinity." Nature protocols 8(8): 10.1038/nprot.2013.1084.

Huang, L., F. Zhang, W. Wang, Y. Zhou, B. Fu and Z. Li (2014). "Comparative transcriptome sequencing of tolerant rice introgression line and its parents in response to drought stress." BMC Genomics 15(1): 1-16.

Huang, S., W. Spielmeyer, E. S. Lagudah, R. A. James, J. D. Platten, E. S. Dennis and R. Munns (2006). "A sodium transporter (HKT7) is a candidate for Nax1, a gene for salt tolerance in durum wheat." Plant Physiology 142(4): 1718-1727.

James, R. A., C. Blake, C. S. Byrt and R. Munns (2011). "Major genes for Na+ exclusion, Nax1 and Nax2 (wheat HKT1;4 and HKT1;5), decrease Na+ accumulation in bread wheat leaves under saline and waterlogged conditions." Journal of Experimental Botany 62(8): 2939-2947.

Jia, X., Y. Deng, X. Sun, L. Liang and X. Ye (2014). "Characterization of the global transcriptome using Illumina sequencing and novel microsatellite marker information in seashore paspalum." Genes & Genomics 37(1): 77-86.

Kravchik, M. and N. Bernstein (2013). "Effects of salinity on the transcriptome of growing maize leaf cells point at cell-age specificity in the involvement of the antioxidative response in cell growth restriction." BMC Genomics 14(1): 1-13.

Lee, G. (2000). Comparative Salinity Tolerance and Salt Tolerance Mechanisms of Seashore Paspalum Ecotypes. Athens, GA, University of Georgia.

Li, B. and C. N. Dewey (2011). "RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome." BMC Bioinformatics 12: 323.

Marcum, K. and C. Murdoch (1994). "Salinity tolerance mechanisms of six C4 turfgrasses." J Am Soc Hort Sci 119: 779 - 784.

Mickelbart, M. V., P. M. Hasegawa and J. Bailey-Serres (2015). "Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability." Nat Rev Genet 16(4): 237-251.

Munns, R. and M. Tester (2008). "Mechanisms of salinity tolerance." Annu Rev Plant Biol 59: 651 - 681.

Robinson, M. D., D. J. McCarthy and G. K. Smyth (2010). "edgeR: a Bioconductor package for differential expression analysis of digital gene expression data." Bioinformatics 26(1): 139-140.

Zhang, M., X. Kong, X. Xu, C. Li, H. Tian and Z. Ding (2015). "Comparative transcriptome profiling of the maize primary, crown and seminal root in response to salinity stress." PLoS ONE 10(3): e0121222.

Zhou, Y., P. Yang, F. Cui, F. Zhang, X. Luo and J. Xie (2016). "Transcriptome analysis of salt stress responsiveness in the seedlings of Dongxiang wild rice (*Oryza rufipogon* Griff.)." PLoS ONE 11(1): e0146242.

Supplementary Table: 5.1

Position	Template	Sample Conc. (ng/uL)	Volume Sample for 3 ug	Volume Water for 50 uL	Well Pos	Lib Conc. (ng/ul)	20ul @ 2ng/ul	index i7	index i7 seq	index i5	index i5 seq
1	12_RT_22G1P3	100	30.00	20.00	A01	4.17	9.58	i701	ATTACTCG	i501	TATAGCCT
2	12_RT_33G1P2	267	11.24	38.76	B01	14.88	2.69	i701	ATTACTCG	i502	ATAGAGGC
3	12_RT_22G2P2	143	20.98	29.02	C01	17.92	2.23	i701	ATTACTCG	i503	CCTATCCT
4	12_RT_33G2P1	233	12.88	37.12	D01	23.71	1.69	i701	ATTACTCG	i504	GGCTCTGA
5	12_RT_22G2P4	373	8.04	41.96	E01	7.65	5.23	i701	ATTACTCG	i505	AGGCGAAG
6	12_RT_33G2P2	266	11.28	38.72	F01	6.33	6.32	i701	ATTACTCG	i506	TAATCTTA
7	12_RT_22G1P1	259	11.58	38.42	G01	4.06	9.85	i701	ATTACTCG	i507	CAGGACGT
8	12_RT_33G1P5	254	11.81	38.19	H01	11.68	3.42	i701	ATTACTCG	i508	GTACTGAC
9	12_RT_33G2P3	307	9.77	40.23	A02	3.05	13.12	i702	TCCGGAGA	i501	TATAGCCT
10	12_RT_22G2P1	571	5.25	44.75	B02	3.34	11.96	i702	TCCGGAGA	i502	ATAGAGGC
11	12_RT_33G1P3	130	23.08	26.92	C02	7.69	5.20	i702	TCCGGAGA	i503	CCTATCCT
12	12_RT_22G1P2	165	18.18	31.82	D02	16.62	2.41	i702	TCCGGAGA	i504	GGCTCTGA
13	12_RT_33G1P4	161	18.63	31.37	E02	4.90	8.17	i702	TCCGGAGA	i505	AGGCGAAG
14	12_RT_22G1P4	168	17.86	32.14	F02	5.38	7.44	i702	TCCGGAGA	i506	TAATCTTA
15	12_RT_33G2P4	207	14.49	35.51	G02	13.51	2.96	i702	TCCGGAGA	i507	CAGGACGT
16	12_RT_22G2P3	359	8.36	41.64	H02	5.90	6.78	i702	TCCGGAGA	i508	GTACTGAC
17	40_RT_22G1P3	501	5.99	44.01	A03	5.12	7.81	i703	CGCTCATT	i501	TATAGCCT
18	40_LF_22G1P3	674	4.45	45.55	B03	5.62	7.12	i703	CGCTCATT	i502	ATAGAGGC
19	40_RT_33G1P2	495	6.06	43.94	C03	13.19	3.03	i703	CGCTCATT	i503	CCTATCCT
20	40_LF_33G1P2	135	22.22	27.78	D03	13.37	2.99	i703	CGCTCATT	i504	GGCTCTGA
21	40_RT_22G2P2	146	20.55	29.45	E03	4.50	8.88	i703	CGCTCATT	i505	AGGCGAAG
22	40_LF_22G2P2	481	6.24	43.76	F03	9.75	4.10	i703	CGCTCATT	i506	TAATCTTA
23	40_RT_33G2P1	389	7.71	42.29	G03	7.23	5.53	i703	CGCTCATT	i507	CAGGACGT
24	40_LF_33G2P1	603	4.98	45.02	H03	7.33	5.46	i703	CGCTCATT	i508	GTACTGAC
25	40_RT_22G2P4	353	8.50	41.50	A04	5.22	7.67	i704	GAGATTCC	i501	TATAGCCT
26	40_LF_22G2P4	284	10.56	39.44	B04	9.76	4.10	i704	GAGATTCC	i502	ATAGAGGC
27	40_RT_33G2P2	246	12.20	37.80	C04	10.93	3.66	i704	GAGATTCC	i503	CCTATCCT
28	40_LF_33G2P2	533	5.63	44.37	D04	3.98	10.06	i704	GAGATTCC	i504	GGCTCTGA
29	40_RT_22G1P1	428	7.01	42.99	E04	4.55	8.78	i704	GAGATTCC	i505	AGGCGAAG
30	40_LF_22G1P1	881	3.41	46.59	F04	2.63	15.24	i704	GAGATTCC	i506	TAATCTTA
31	40_RT_33G1P5	578	5.19	44.81	G04	3.24	12.34	i704	GAGATTCC	i507	CAGGACGT
32	40_LF_33G1P5	182	16.48	33.52	H04	9.16	4.37	i704	GAGATTCC	i508	GTACTGAC
33	40_RT_33G2P3	487	6.16	43.84	A05	7.23	5.53	i705	ATTCAGAA	i501	TATAGCCT
34	40_LF_33G2P3	990	3.03	46.97	B05	6.73	5.95	i705	ATTCAGAA	i502	ATAGAGGC
35	40_RT_22G2P1	484	6.20	43.80	C05	13.67	2.93	i705	ATTCAGAA	i503	CCTATCCT
36	40_LF_22G2P1	431	6.96	43.04	D05	6.64	6.02	i705	ATTCAGAA	i504	GGCTCTGA
37	40_RT_33G1P3	403	7.44	42.56	E05	6.51	6.15	i705	ATTCAGAA	i505	AGGCGAAG
38	40_LF_33G1P3	303	9.90	40.10	F05	7.67	5.22	i705	ATTCAGAA	i506	TAATCTTA
39	40_RT_22G1P2	265	11.32	38.68	G05	7.68	5.21	i705	ATTCAGAA	i507	CAGGACGT
40	40_LF_22G1P2	179	16.76	33.24	H05	7.01	5.71	i705	ATTCAGAA	i508	GTACTGAC
41	40_RT_33G1P4	371	8.09	41.91	A06	7.98	5.01	i706	GAATTCGT	i501	TATAGCCT
42	40_LF_33G1P4	324	9.26	40.74	B06	6.43	6.23	i706	GAATTCGT	i502	ATAGAGGC
43	40_RT_22G1P4	333	9.01	40.99	C06	10.57	3.78	i706	GAATTCGT	i503	CCTATCCT
44	40_LF_22G1P4	528	5.68	44.32	D06	10.28	3.89	i706	GAATTCGT	i504	GGCTCTGA
45	40_RT_33G2P4	467	6.42	43.58	E06	6.83	5.86	i706	GAATTCGT	i505	AGGCGAAG
46	40_LF_33G2P4	452	6.64	43.36	F06	6.39	6.26	i706	GAATTCGT	i506	TAATCTTA
47	40_RT_22G2P3	205	14.63	35.37	G06	6.66	6.01	i706	GAATTCGT	i507	CAGGACGT
48	40_LF_22G2P3	552	5.43	44.57	H06	6.51	6.14	i706	GAATTCGT	i508	GTACTGAC

Supplementary Table 5.2

Subcluster	Trinity transcript ID	megablast; discontiguous megablast	role	connected to salt exposure
Sc 14	TR258107|c0_g1	ribosomal 60S	protein synthesis	unclear
Sc 14	TR205197|c3_g10	no significant similarity with megablast; putative disease resistance gene	disease resistance	unclear
Sc 14	TR261661|c0_g3	ribonuclease 1-like	RNA processing	unclear
Sc 14	TR279848|c0_g1	PREDICTED: Setaria italica glycine-rich cell wall structural protein-like (LOC101771250), mRNA, paspalum SSR	xylem cell wall integrity	unclear
Sc 14	TR238187|c0_g2	uncharacterized protein	unknown	unclear
Sc 14	TR264032|c1_g1	ribosomal 60S	protein synthesis	unclear
Sc 14	TR275405|c0_g1	Sorghum bicolor delta 1-pyrroline-5-carboxylate synthetase 1 (P5CS1)	proline accumulation	probable
Sc 14	TR224745|c0_g1	hypothetical protein	unknown	unclear
Sc 14	TR243250|c0_g1	holotricin-3-like	fungal resistance (in insects)	unclear
Sc 14	TR237488|c3_g4	ribonuclease 3-like	RNA processing	unclear
Sc 20	TR243250|c4_g5	holotricin-3-like	fungal resistance (in insects)	unclear

Supplementary Table: 5.3

Subcluster	Trinity transcript ID	best blast results	role/source	connected to salt exposure
Sc 13	TR210917|c3_g2	ribosome	bacterial contaminant	n/a
Sc 13	TR202962|c4_g11	ribosome	bacterial contaminant	n/a
Sc 13	TR208562|c0_g1	ribosome	bacterial contaminant	n/a
Sc 13	TR224745|c0_g1	ABA induced gene BAC 8070 sorghum	unknown role, stress-related	unclear, probable
Sc 13	TR262433|c0_g1	no significant similarity found	unknown	unclear
Sc 13	TR278719|c22_g8	ribosome	bacterial contaminant	n/a
Sc 13	TR283723|c0_g4	ribosome	bacterial contaminant	n/a
Sc 13	TR264630|c0_g1	ribosome	bacterial contaminant	n/a
Sc 13	TR14191|c0_g3	ribosome	bacterial contaminant	n/a
Sc 13	TR210751|c0_g2	ribosome	bacterial contaminant	n/a
Sc 13	TR232934|c0_g2	ribosome	bacterial contaminant	n/a
Sc 13	TR13627|c0_g2	PREDICTED: Setaria italica glycine-rich cell wall structural protein-like	xylem cell wall integrity	unclear
Sc 13	TR283726|c1_g2	ribosome	bacterial contaminant	n/a
Sc 13	TR42694|c1_g1	ribosome	bacterial contaminant	n/a
Sc 13	TR10162|c0_g1	ribosome	bacterial contaminant	n/a
Sc 13	TR280459|c1_g6	ribosome	bacterial contaminant	n/a
Sc 13	TR202936|c6_g2	ribosome	bacterial contaminant	n/a
Sc 13	TR227615|c0_g1	ribosome	bacterial contaminant	n/a
Sc 13	TR260764|c1_g3	no significant similarity found	unknown	unclear
Sc 13	TR197085|c0_g1	ribosome	bacterial contaminant	n/a
Sc 15	TR211373|c0_g3	PREDICTED: Setaria italica eukaryotic peptide chain release factor subunit 1-2-like	mRNA surveillance pathway	unclear
Sc 15	TR279108|c0_g2	ribosome	bacterial contaminant	n/a
Sc 15	TR221473|c1_g2	hsp70-like	unknown, stress-related	probable
Sc 15	TR257120|c1_g2	ribosome	bacterial contaminant	n/a
Sc 15	TR206246|c8_g10	ribosome	bacterial contaminant	n/a
Sc 15	TR226472|c8_g12	ribosome	bacterial contaminant	n/a
Sc 15	TR225860|c0_g2	PREDICTED: Setaria italica probable mediator of RNA polymerase II transcription subunit 37c (LOC101785219)	transcription	probable
Sc 15	TR233889|c0_g2	ribosome	bacterial contaminant	n/a
Sc 15	TR279936|c1_g1	PREDICTED: Setaria italica BAG family molecular chaperone regulator 6 (LOC101763408)	regulates stress and apoptosis pathways	probable
Sc 15	TR268452|c1_g1	ribosome	bacterial contaminant	n/a
Sc 15	TR209047|c0_g2	Sorghum bicolor hypothetical protein, mRNA	unknown	unclear
Sc 15	TR200841|c0_g1	no significant similarty	unknown	unclear
Sc 15	TR278719|c22_g4	ribosome	bacterial contaminant	n/a
Sc 15	TR227064|c0_g2	Zea mays uncharacterized LOC100501983 (LOC100501983)	unknown	unclear
Sc 15	TR198428|c0_g1	ribosome	bacterial contaminant	n/a
Sc 15	TR282250|c0_g6	ribosome	bacterial contaminant	n/a
Sc 15	TR220167|c0_g1	PREDICTED: Setaria italica fructose-bisphosphate aldolase, chloroplastic-like (LOC101768485)	fructose and mannose metabolism	unclear
Sc 15	TR222595|c0_g1	PREDICTED: Setaria italica late embryogenesis abundant protein D-34-like (LOC101767768)	cellular dehydration tolerance	probable
Sc 15	TR214741|c0_g1	PREDICTED: Setaria italica 16.9 kDa class I heat shock protein 1-like (LOC101775696)	stress protection	probable

Supplementary Table: 5.3

Subcluster	Trinity transcript ID	best blast results	role/source	connected to salt exposure
Sc 15	TR274713|c0_g2	PREDICTED: Zea mays putative aconitate hydratase, cytoplasmic (LOC103647708)	fertility	unclear
Sc 15	TR221959|c5_g2	ribosome	bacterial contaminant	n/a
Sc 15	TR199490|c3_g12	ribosome	bacterial contaminant	n/a
Sc 15	TR276018|c0_g1	no signficant similiarty; PREDICTED: Setaria italica uncharacterized LOC101757776 (LOC101757776)	unknown	unclear
Sc 15	TR209246|c0_g1	PREDICTED: Setaria italica 70 kDa peptidyl-prolyl isomerase-like (LOC101769949)	heat shock protein (hsp) binding	probable
Sc 15	TR209243|c0_g1	PREDICTED: Setaria italica RING-H2 finger protein ATL72-like	unknown	unclear
Sc 15	TR27602|c0_g2	no signficant similiarity	unknown	unclear
Sc 15	TR69410|c0_g1	PREDICTED: Setaria italica GEM-like protein 5 (LOC101756330)	seed dormancy	unclear
Sc 15	TR269242|c0_g2	ribosome	bacterial contaminant	n/a
Sc 15	TR226472|c8_g5	ribosome	bacterial contaminant	n/a
Sc 15	TR197483|c0_g5	ribosome	bacterial contaminant	n/a
Sc 15	TR272101|c1_g1	Zea mays clone 994766 pyruvate dehydrogenase E1 component alpha subunit mRNA	metabolism	unclear
Sc 15	TR281116|c4_g9	ribosome	bacterial contaminant	n/a
Sc 15	TR6083|c0_g1	Zea mays uncharacterized LOC100281089 (IDP708), AMP-binding protein mRNA	unknown	unclear
Sc 15	TR265375|c0_g1	PREDICTED: Setaria italica uncharacterized LOC101776632 (LOC101776632), lipid binding protein mRNA	unknown	unclear
Sc 15	TR198917|c0_g1	Zea mays aspartic proteinase nepenthesin-1 precursor	proteinase	unclear
Sc 15	TR220540|c4_g1	ribosome	bacterial contaminant	n/a
Sc 15	TR235525|c0_g1	PREDICTED: Setaria italica non-specific lipid-transfer protein-like (LOC101774390)	unknown	unclear
Sc 15	TR226845|c0_g1	PREDICTED: Setaria italica non-specific lipid-transfer protein 2-like (LOC101779829)	unknown	unclear
Sc 15	TR217899|c2_g4	PREDICTED: Setaria italica probable xyloglucan endotransglucosylase/hydrolase protein 23 (LOC101772149)	cell wall biogenesis	unclear
Sc 15	TR226727|c1_g1	PREDICTED: Setaria italica late embryogenesis abundant protein, group 3-like (LOC101759437),	cellular dehydration tolerance	probable
Sc 15	TR237573|c6_g5	ribosome	bacterial contaminant	n/a
Sc 15	TR256672|c0_g1	no signficant similiarty	unknown	unclear
Sc 15	TR226473|c0_g4	no signficant similiarty	unknown	unclear
Sc 15	TR256830|c0_g1	Zea mays mRNA for dehydrin (dhn1 gene)	water stress related	probable
Sc 15	TR239073|c2_g3	ribosome	bacterial contaminant	n/a
Sc 15	TR198654|c2_g10	ribosome	bacterial contaminant	n/a
Sc 15	TR215052|c0_g4	PREDICTED: Setaria italica fructose-bisphosphate aldolase, chloroplastic-like (LOC101768485)	glycolosis	unclear
Sc 15	TR73413|c0_g1	no signficant similiarty	unknown	unclear
Sc 15	TR256594|c5_g8	ribosome	bacterial contaminant	n/a
Sc 15	TR221982|c0_g3	PREDICTED: Setaria italica bidirectional sugar transporter SWEET12-like (LOC101766631),	sugar transport	unclear
Sc 15	TR205090|c1_g1	PREDICTED: Setaria italica pollen-specific leucine-rich repeat extensin-like protein 1	cell wall organization (in pollen)	unclear
Sc 15		Saccharum hybrid cultivar SP80-3280 drought responsive protein 1 (dr1) mRNA	stress response	probable
Sc 15	TR217755|c0_g1	PREDICTED: Oryza sativa Japonica Group RING-H2 finger protein ATL72 (LOC4338915)	protein ubiquitination	probable
Sc 15	TR266272|c0_g1	PREDICTED: Setaria italica probable nucleoredoxin 2 (LOC101779892)	thiol-disulfide oxidoreductase, redox	unclear
Sc 15	TR260564|c0_g1	PREDICTED: Setaria italica phosphosulfolactate synthase (LOC101781083);	PEP metabolism	unclear
Sc 15		PREDICTED: Oryza brachyantha protein HEAT-STRESS-ASSOCIATED 32	unknown, stress-related	unclear
Sc 15	TR273310|c4_g16	ribosome	bacterial contaminant	n/a

Supplementary Table: 5.3

Subcluster	Trinity transcript ID	best blast results	role/source	connected to salt exposure
Sc 15	TR257996|c1_g2	ribosome	bacterial contaminant	n/a
Sc 15	TR205103|c0_g2	Zea mays 2C-type protein phosphatase protein (PP2C11)	starch biosynthesis, modification	unclear
Sc 15	TR210938|c3_g17	ribosome	bacterial contaminant	n/a
Sc 15	TR44680|c1_g1	no significant similairty	unknown	unclear
Sc 15	TR272254|c0_g1	PREDICTED: Setaria italica agglutinin isolectin 3-like (LOC101756270)	carbohydrate processing	unclear
Sc 15	TR6936|c0_g1	ribosome	bacterial contaminant	n/a
Sc 15	TR205203|c10_g7	ribosome	bacterial contaminant	n/a
Sc 15	TR244322|c2_g8	ribosome	bacterial contaminant	n/a
Sc 15	TR275405|c0_g1	ribosome	bacterial contaminant	n/a
Sc 15	TR209243|c0_g2	PREDICTED: Setaria italica RING-H2 finger protein ATL72-like (LOC101755108)	protein ubiquitination	probable
Sc 15	TR218836|c1_g3	Barley root-specific lectin mRNA;PREDICTED: Brachypodium distachyon agglutinin isolectin 3-like (LOC100836672)	carbohydrate processing	unclear
Sc 15	TR259575|c1_g1	Zea mays ASF/SF2-like pre-mRNA splicing factor SRP30 (srp30) gene	alternative splicing modulator	probable
Sc 15	TR276965|c1_g2	ribosome	bacterial contaminant	n/a
Sc 15	TR237573|c3_g1	ribosome	bacterial contaminant	n/a
Sc 15	TR58370|c0_g1	ribosome	bacterial contaminant	n/a
Sc 15	TR225860|c0_g1	PREDICTED: Setaria italica heat shock 70 kDa protein (LOC101756252),	stress response	probable
Sc 15	TR226750|c3_g3	PREDICTED: Setaria italica probable galactinol--sucrose galactosyltransferase 2 (LOC101763493)	carbohydrate synthesis	unclear
Sc 15	TR202908|c2_g2	PREDICTED: Setaria italica GEM-like protein 5 (LOC101756330); ABA-responsive protein	seed dormancy	unclear
Sc 15	TR273312|c6_g6	ribosome	bacterial contaminant	n/a
Sc 15	TR218459|c7_g1	PREDICTED: Setaria italica basic 7S globulin-like (LOC101765955)	cell wall protection	unclear
Sc 15	TR282270|c0_g1	PREDICTED: Setaria italica 1,4-alpha-glucan-branching enzyme, chloroplastic/amyloplastic-like (LOC101778660),	starch biosynthesis, modification	unclear
Sc 15	TR244322|c2_g1	ribosome	bacterial contaminant	n/a
Sc 15	TR226472|c7_g2	ribosome	bacterial contaminant	n/a
Sc 15	TR269556|c5_g17	ribosome	bacterial contaminant	n/a
Sc 15	TR247549|c0_g1	Zea mays clone 354643 mRNA sequence	unknown	unclear
Sc 15	TR214574|c2_g1	PREDICTED: Setaria italica acetate/butyrate--CoA ligase AAE7, peroxisomal-like (LOC101758375)	glyoxylate cycle inititation	unclear
Sc 15	TR205090|c0_g1	no significant similairty	unknown	unclear
Sc 15	TR263272|c0_g1	no significant similairty	unknown	unclear
Sc 15	TR223498|c0_g2	no significant similairty	unknown	unclear
Sc 15	TR221473|c2_g3	PREDICTED: Setaria italica heat shock cognate 70 kDa protein 2 (LOC101764302),	stress response	probable
Sc 15	TR249407|c0_g2	ribosome	bacterial contaminant	n/a
Sc 15	TR223032|c0_g1	PREDICTED: Setaria italica glucose-1-phosphate adenylyltransferase small subunit (LOC101755337)	starch biosynthesis	unclear
Sc 15	TR250703|c0_g4	PREDICTED: Zea mays protein ROOT HAIR DEFECTIVE 3 homolog 2-like (LOC103654528)	vesicle trafficing	probable
Sc 15	TR203996|c3_g1	PREDICTED: Setaria italica protein EXORDIUM-like (LOC101770010),	growth and development under stress	probable
Sc 15	TR230374|c9_g2	ribosome	bacterial contaminant	n/a
Sc 15	TR203317|c7_g2	PREDICTED: Setaria italica probable mediator of RNA polymerase II transcription subunit 37c (LOC101785219)	transcription	unclear
Sc 15	TR237566|c8_g3	ribosome	bacterial contaminant	n/a
Sc 15	TR229460|c0_g2	no significant similairty	unknown	unclear
Sc 15	TR238115|c6_g7	ribosome	bacterial contaminant	n/a

CHAPTER 6

CONCLUSIONS

Chapter 2 Diversity Analysis

Overview

The data and results presented in chapter two report findings on the relative DNA content of members of a Paspalum germplasm panel, estimates of ploidy level, a haploid genome size estimate for *Paspalum vaginatum* Sw. and identified patterns consistent with population structure in the diploid germplasm of both *P. vaginatum* Sw. and lines putatively *P. distichum* L.

Limitations

Though polyploid accessions were identified by flow cytometry, some of which were confirmed with chromosome counts, they were not included in the genetic diversity analysis with the diploids. This limits our ability to relate subpopulation 2, which contained at least two accessions (out of 4) with pubescent glumes, identifying them as diploid *P. distichum* accessions, with the polyploids, which are also putatively *P. distichum*. To what extent *P. distichum* germplasm (of any ploidy) contains alleles distinct from those in *P. vaginatum* germplasm is still unclear. Allelic variation between the two species could provide a genetic explanation for the source of the morphotypical distinctions between the species that have traditionally been considered as a result of polyploid syndrome.

Research Highlights

The identification of diploid *P. distichum* lines was unexpected and indicates that cytotype alone is not a distinguishing factor for *P. distichum* and *P. vaginatum*. The fact that one of the *P. distichum* lines, PI 612771, was found to have exclusively glabrous glumes also calls into question the validity of glume pubescence as a universal demarcation for distinguishing the species. It appears that morphotype: course versus non-course texture is the only valid distinguishing feature to remain.

The level of clonal redundancy detected with the genetic markers was higher than expected based on the geographic origin of the accessions, as was the lack of clonal identity among a number of lines thought to be identical, but maintained as separate collections. This suggests that line maintenance has been compromised in some cases. As a consequence of this, even though geographic origins as identified in the narratives of these lines do not implicate geography or general distribution patterns as important for contributing to genetic structure in the germplasm of the species, this source information associated with many of these lines may not actually be correct. The absence of a geographic associated patterning among the USDA sourced accessions was surprising, given that the lines in GRIN expectation that seashore paspalum distribution is thought to have only recently (~500 years ago) become pan-global.

Future Directions

Though genotyping by sequencing approaches have largely replaced the use of microsatellites, a small set of microsatellites can still be very powerful. Schröder, Bahri et al. (2016) found that a panel of 11 microsatellites resolved genetic relationships in a panel of Setaria lines to degree similar to 10,000 SNPs. Additionally, microsatellites are still a potentially valuable resource for germplasm characterization from herbarium specimens as well as for labs which do not have the

financial resources or computational infrastructure required to utilize next generation sequencing data. Collaborating with laboratories in areas with access to *in situ* populations of seashore paspalum could contribute greatly to expanding our understanding of the genetic diversity and structure of the species and these microsatellites would have value in those contexts.

Expanding the paspalum germplasm collection that is locally accessible is another important future direction. Receiving new lines from across the global range would provide insights into whether the genetic patterns observed in this study, which was limited to 90 individuals, are conserved more broadly. Having access to more germplasm would also benefit paspalum breeding, as the level of clonality present in the collections suggests much less diversity than previously thought.

Chapter 3 Genetic Map Construction and Comparative Genomics Analysis

Overview

Chapter three reports just over two thousand GBS-derived sequence tags containing parent specific SNPs which are segregating in an F1 mapping population. These SNPs were separated into two subsets for genetic map construction. Maps of the SNPs segregating in the paternal and maternal parents were each comprised of more than one thousand GBS-derived genetic markers. In both maps, all ten of the expected linkage groups were resolved unambiguously. Putative orthologs in the *Sorghum bicolor* and *Setaria italica* genomes were identified for ~25% of the paspalum markers. This comparative information was used to improve the marker orders in the absence of informative recombination events as well as to establish the relationship of the *P. vaginatum* genome with the sorghum and Setaria genomes. These analyses confirm a largely syntenic relationship between the *P. vaginatum* and *S. bicolor* genomes, while also confirming

the existence of a number of chromosomal rearrangements between *P. vaginatum* and *S. italica*; all rearrangements had previously been shown to have occurred in *S. italica*.

Limitations

The number of recombination events available with which to order markers was limited by the number of progeny screened. Additionally, the number of markers available was limited by the enzyme combination employed (*PstI*/*NdeI*+*MspI*). By using a triple digest, the resulting fragment pool contained a smaller number of unique fragments than if alternative combinations like *PstI/NdeI* or *PstI/MspI* had been used. Enzyme combination selection strikes a balance between the number markers generated, the read depth that will be achieved and the total number of lines which can be simultaneously screened. Additionally, because mapping was done in a F1 population, two genetic maps were required to provide robust marker orders. Integration of both maps to generate a consensus map is in progress.

Research Highlights

Although genetic maps have been developed previously in *Paspalum notatum*, a member of the Paspaleae lineage, these are the first genetic maps with that level of marker density for both *P. vaginatum* and the Paspaleae lineage. The two parental maps were similar with regard to their genomic coverage with the exception of linkage group 2 in the maternal parent and linkage group 6 in the paternal parent. A section of a chromosome arm on LG 2 in the paternal map was not represented in the maternal map. Similarly, a section of a chromosome arm on LG 6 in the maternal map was absent from the paternal map. In addition, our research provides the first evidence that the Paspalum genome is largely colinear with that of *S. bicolor*.

Future Directions

The utility of a robust genetic map is unparalleled for validating genome assembly as well as essential for mapping traits of agronomic importance. This particular mapping population was generated to study salt tolerance, but it will also have utility for studying: flowering time, plant height. The maps represent the first step towards the implementation of marker-assisted breeding in seashore paspalum.

The *P. vaginatum* genome sequence, produced by the Joint Genome Institute, currently consists of more than 100,000 scaffolds. The maps generated as part of my study will find utility in anchoring the genome sequence, especially as the genome assembly improves. However, for anchoring purposes, it might be useful to increase the resolution of the map by genotyping more progeny and to increase the marker density by re-genotyping using a less conservative enzyme combination to produce many more mappable markers.

Genotyping additional mapping populations is also an important future direction as is would provide an opportunity for additional trait mapping and for identifying structural differences that may be present among the different genomes. Mapping parents could be selected from different subpopulations (Chapter 2) to maximize genetic diversity. It would be particularly interesting to generate crosses between *P. vaginatum* and the diploid putative *P. distichum* accessions, assuming, of course, that these crosses produce viable F1 hybrids. Another direction would be to develop additional mapping populations from crosses between individual F1 lines developed for this study. All F1 sibs carry one HI33 and one 509022 chromosome, and the resulting F2 population would allow for studying recombination events occurring between the genomes of the original parents (HI33 and 509022). If such a population was created and

genotyped, it would enable the construction of a consensus map that could combine the markers that as of now are isolated to one of each of the separate, parent-specific genetic maps.

Chapter 4 QTL Mapping

Overview

Three QTL were identified in the paternal map. One QTL controlling sodium accumulation in green leaves was detected on linkage group 9. This QTL explained 9-13% of the phenotypic variation. QTL for potassium accumulation and K^+/Na^+ ratio co-localized to a region on linkage group 4. These QTL explained between 9-23% and 7-19% of the variation. A search for candidate genes in the orthologous regions of sorghum identified HKT6 as a strong candidate for K^+ accumulation and K^+/Na^+ ratio.

Limitations

Although significant QTL were found for two salt tolerance traits segregating in this F1 population, the pseudo testcross method we employed to generate the maps limits the detectable QTL to those that are heterozygous in only one parent (Wu, Yang et al. 2010). Traits that are segregating between the two parents are also not assessed in an F1 population as all F1 sibs will carry one maternal allele and one paternal allele at all loci. For this reason, other loci could be involved in salt tolerance in seashore paspalum that were not detectable by the genetic and QTL mapping approach used.

For each of the QTL detected, several candidate genes were identified in the QTL intervals. A candidate gene approach relies on the fact that (1) the gene that underlies the QTL will be conserved across species, and (2) this gene has previously identified as being associated with the trait and hence has been functionally annotated. One or both of these assumptions may

not be valid. Nevertheless, some strong candidate genes were identified. It is, however, impossible to determine which, if any, of these candidates is responsible without doing additional genetic and/or functional analyses.

Because of the structure of the population, even if other phenotypes of interest are identified as being different between the parents (whether associated with salt tolerance or not), they would necessarily segregate among the progeny. As such, it would be prudent to conduct pilot screens on a small number of progeny to detect whether this population will have utility for their study.

Research Highlights

It was interesting to find that the paternal parent was heterozygous for both of the salt tolerance traits which resulted in QTL in this experiment. Perhaps this should not be surprising as 'HI33' is an accession that has successfully colonized a coastal region outside of the species' native range. The mother, on the other hand, is said to have been collected from an inland area of northern Argentina. As such, it might be expected that 'HI33,' in order to be able to thrive in a coastal area, would as a consequence have superior salt tolerance to an inland adapted line. Our preliminary experiments suggested HI33 was the tolerant parent.

Another surprising result was to observe that filtering the ion data for consistency of rank improved the LOD score of QTL peaks detected. This suggests that the filtering process removed spurious or otherwise problematic values.

Future Directions

Repeating the screen to confirm the QTL is a priority, though detecting QTL in all three replicates collected at different time points is strong evidence that these QTL are robust. Repeating the QTL analysis on an integrated genetic map using a software package such as

FsQtlMap (Tong, Zhang et al. 2012) that takes into account that QTL, as genetic markers, can segregate 1:1 (*q1q1* x *q1q2* or *q1q2* x *q1q1*), 1:2:1 (*q1q2 x q1q2*) or, potentially, 1:1:1:1 (*q1q2* x *q3q4*) may also increase the power of QTL detection. Evaluating the population for other salt tolerance traits including those that involve osmotic tolerance (root elongation and leaf elongation rates) may lead to the detection of additional QTL that could help to clarify seashore paspalum's tolerance mechanisms and lead to the identification of the genes which confer them. Other screens for ionic tolerance traits such as heavy metal tolerance many uncover other loci as well. Screening additional progeny from the same population or developing additional populations from lines of interest from the original F1 progeny array would likely help reduce the size of the QTL interval which would reduce the number of candidate genes.

References

Endo, N., K. Yoshida, M. Akiyoshi, Y. Yoshida and N. Hayashi (2005). "Putative UDP-galactose epimerase and metallothioneine of *Paspalum vaginatum* enhanced the salt tolerance of rice, *Oryza sativa* L. from transplanting to harvest stages." Breeding Sci 55: 163 - 173.

Munns, R. and M. Tester (2008). "Mechanisms of salinity tolerance." Annu Rev Plant Biol 59: 651-681.

Schröder, S., B. A. Bahri, D. M. Eudy, D. J. Layton, E. A. Kellogg and K. M. Devos (2016). "Genetic diversity and origin of North American green foxtail [*Setaria viridis* (L.) Beauv.] accessions." Genetic Resources and Crop Evolution: 1-12.

Tester, M. and R. Davenport (2003). "Na+ tolerance and Na+ transport in higher plants." Ann Bot 91(5): 503-527.

Tong, C., B. Zhang, H. Li and J. Shi (2012). "Model selection for quantitative trait loci mapping in a full-sib family." Genetics and Molecular Biology 35(3): 622-631.

Wu, S., J. Yang, Y. Huang, Y. Li, T. Yin, S. D. Wullschleger, G. A. Tuskan and R. Wu (2010). "An Improved Approach for Mapping Quantitative Trait Loci in a Pseudo-Testcross: Revisiting a Poplar Mapping Study." Bioinformatics and Biology Insights 4: 1-8.