GENETIC MAPPING AND QUANTITATIVE TRAIT LOCUS (QTL) ANALYSIS OF ROOT-KNOT NEMATODE RESISTANCE IN PEARL MILLET

by

LIMEI LIU

(Under the direction of Peng W. Chee)

ABSTRACT

Pearl millet, a cereal that originated in the Sahel zone of west Africa, can tolerate dry conditions and low soil fertility. It is an important dry land crop, predominantly in Africa and Asia where it is a staple food grain. In the US, pearl millet is primarily used as a forage crop. A new genetic map of pearl millet was constructed based on 180 recombinant inbred lines (RILs) derived from an intraspecific cross between Tift 99B and Tift 454. The map contains 468 molecular markers (361 AFLP and 107 SSR), spans 757 cM, and covers all 7 linkage groups that represent the 7 chromosomes of pearl millet. In addition to using publicly available SSRs, we are also reporting 144 new pearl millet SSRs developed from expressed sequences tags (ESTs) for which probable functions have been recorded.

Root-knot nematodes, *Meloidogyne* spp, are significant pests of cotton and peanut in the southeastern United States. Because pearl millet are potential rotation crops for cotton and peanuts, knowledge of nematode resistance and its inheritance is important for breeding pearl millet with root-knot nematode resistance. In this research, quantitative trait loci (QTL) analysis was conducted to locate the genes for resistance to the southern root-knot nematode [*Meloidogyne incognita* (Kofoid and White) Chitwood]. One major QTL, *QMi-LG2*, has a LOD

of 14 and explains 32.0% of the phenotypic variance. This QTL for nematode resistance was located in a distal region of pearl millet LG2. An expressed sequence tagged simple sequence repeat (EST-SSR) marker ICMP 3029 (ori) was found to be tightly linked to the QTL and can be useful in marker-assisted selection for nematode resistance in pearl millet breeding.

Using this map, we also mapped quantitative trait loci (QTLs) for the agronomic traits of plant height, hundred seed weight, heading date, panicle length, and panicle width to verify the utility of the genetic map.

INDEX WORDS: AFLP, amplified fragment length polymorphism; RIL, recombinant inbred lines; PCR, polymerase chain reaction; EST-SSR, expressed sequence tagged simple sequence repeat; marker-assisted selection.

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DEDICATION

To my husband Muliang Peng

To my sons: Hao Peng and Frank H. Peng

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CHAPTER 1

INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) can tolerate drought, acid soil, and low soil fertility, yet has a higher and more reliable yield than other dry land crops such as sorghum or maize (Andrews et al., 1993). An important cereal crop, pearl millet is cultivated on 29 million hectares, predominantly in Africa and Asia as a staple food grain (http://www.icrisat.org/crop-pearlmillet.htm). It is a forage crop in the United States, Australia, southern Africa, and South America (Poncet et al., 2000).

In Georgia (United States), pearl millet has been cultivated as a forage crop for more than 100 years. Recently pearl millet was suggested as a potential feed grain for poultry (UGA, 2005). In 2006, Georgia was the largest broiler producing state in the United States and accounted for 15% of total broiler production (UGA, 2011). Although these broilers consume ~3 million tons of feed annually, less than 10% of the feed grain is produced in Georgia. To meet the demand for feed, corn and soybean are imported into the region. If pearl millet could replace corn as feed for broilers, then pearl millet would have a great market in Georgia. At present, bobwhite quail producers purchase virtually all pearl millet produced within Georgia.

Nematodes are a constraint to pearl millet production (Lee et al., 2004) and cause grain yield loss in the southeastern United States (Timper et al., 2002), but the primary concern about nematode susceptible hybrids are the effects on susceptible rotation crops. Crop rotation is an effective and low cost method to control soil-borne diseases and pests (Brown, 1987). Root-knot nematodes (RKN) (*Meloidogyne* spp.) are significant pests of cotton and peanut in the

southeastern United States. RKN-resistant pearl millet hybrids were effective in reducing RKN populations in soil and increasing peanut yield (Timper et al., 2007), thus it is a valuable crop in the crop rotation system. Different pearl millet varieties express different levels of RKN resistance. Timper et al. (2002) reported variation among grain hybrids for resistance to RKN in naturally infected fields and in greenhouse tests. Several pearl millet cultivars from West and East Africa were evaluated for RKN resistance and results showed that cultivars express a range of resistance levels from susceptible to highly resistant (Timper and Wilson, 2006).

Nematode resistance screening in greenhouse or field is tedious, expensive (Glover et al., 2004), and destructive. Timing is another concern for the screening method. If one cannot get the screening results before flowering; no targeted individual plant can be identified before backcrossing. Furthermore, nematode resistance is confounded with other diseases on the same plants because nematode infection can increase susceptibility to other diseases (Deberdt et al., 1999), so it is inconvenient in a classical disease pyramiding breeding program. Marker-assisted selection is of great interest in breeding pearl millet for nematode resistance because it can eliminate many of the difficulties in breeding for pest resistance. But, a genetic map with a dense set of genotypic markers is needed as a foundation to be successful.

The pearl millet genome has been genetically mapped (Devos et al., 2000; Liu et al., 1994; Qi et al., 2004), but these maps have big gaps that need to be filled. New markers were added to this existing linkage map as new pearl millet mapping populations were studied, and it has been used recently for skeleton linkage and QTL mapping (Gulia, 2004; Yadav, 2005). The recent studies have also increased the total length of pearl millet genetic linkage map to above 700 cM. Unfortunately, in most studies including the consensus map constructed by Qi et al. (2004), there still existed large gaps on both distal ends of each linkage group with most markers

concentrated in pericentromeric regions. A PCR-based linkage map published in 2010 was based on only 196 PCR-based markers (Pedraza-Garcia et al., 2010) with an average genetic distance between markers of 9.2 cM. Another recent genetic map was based on 258 Diversity Arrays Technology (DArT) and 63 SSR markers with an average genetic distance between markers of 3.7 cM (Supriya et al., 2011).

Marker-assisted selection is playing an important role in the breeding process to develop resistance that is complex and quantitative, and to pyramid many genes into one cultivar. Pyramiding is a process that incorporates multiple disease and pest resistance genes, or quantitative trait loci (QTLs) into one plant cultivar (Dubcovsky, 2004). The basis of marker-assisted selection is that we can infer the presence of a gene of interest through the presence of one or more DNA markers that are tightly linked to the gene (Kumar, 1999). As early as 1995, marker-assisted selection was successfully used in pyramiding resistance genes for crop breeding (Kelly et al., 1995; Yoshimura et al., 1995). Marker-assisted selection has also been used in quantitative trait breeding and QTL pyramiding (Eathington et al., 1997; Guo et al., 2005; Lecomte et al., 2004; Stuber, 1994; Young, 1999).

In order to identify the relationships of nematode resistance genes or QTLs and the tightly linked molecular markers for marker-assisted selection, a recombinant inbred line (RIL) mapping population based on the cross Tift 454 × Tift 99B was developed and used for genetic mapping, especially for the dissection of quantitative traits and mapping of quantitative trait loci for resistance to RKN. RILs are inbred and can be maintained, making them especially valuable for genetic mapping, especially for the dissection of quantitative traits and mapping of quantitative trait loci (Bailey, 1971; Goramaslak et al., 1991; Jinks and Pooni, 1976; Lark et al., 1995).

The objectives of this study are: 1) construct a skeleton genetic map of pearl millet; 2) align the skeleton map with the map constructed by Qi et al. (2004) and add new markers to the old map; and 3) detect RKN resistance QTLs and agronomic traits QTLs in pearl millet.

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CHAPTER 2

LITERATURE REVIEW

Introduction of Pearl Millet

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is in the genus *Pennisetum* of the Poaceae (grass) family. Although the current officially accepted name is *Pennisetum glaucum* (L.) R. Br. (Chase, 1921; Hitchcock and Chase, 1951; Stuntz, 1914; USDA, 1994), other commonly used synonyms for pearl millet include *Pennisetum americanum* (L.) K. Schum, *Pennisetum typhoides* (Burm. F.) Stapf et Hubb., *Pennisetum typhoideum* Rich, *Pennisetum spicatum* (L.) Koern (Jauhar, 1981). Pearl millet is a diploid (2n = 14) annual species (Rau, 1929) with the haploid (1 C) DNA content of about 2.45 pg (Bennett, 1976), which is equal to about 2.26 billion bases (0.965 billion bases per pg DNA). Pearl millet has a symmetrical karyotype with six metacentric or submetacentric chromosomes and one subterminal chromosome. The latter is the shortest chromosome (about two thirds of the longest chromosome) with a satellite on its short arm (Jauhar, 1981). In addition to abundant phenotypic variation within the species (Brunken et al., 1977), many wild and cultivated relatives with 2n = 14 can intercross with pearl millet (Jauhar, 1968; Jauhar, 1981), offering additional scope for breeding and genetic research.

Pearl millet originated in the Sahel zone of west Africa (Brunken et al., 1977) and was domesticated in Africa and India more than 3,000 years ago (Allchin, 1969; Davies, 1968). Because pearl millet can tolerate dry conditions (Brunken, 1977) and low soil fertility, it has a higher and more reliable yield than other dry land crops such as sorghum or maize under these conditions (Andrews et al., 1993). As an important cereal crop, pearl millet is cultivated on 29

million hectares predominantly in Africa and Asia as a staple food grain (http://www.icrisat.org/crop-pearlmillet.htm).

Pearl millet was introduced into the United States in the nineteenth century as a forage crop (Maiti and Wesche-Ebeling, 1997). In the United States, the estimated planted area of pearl millet is 0.6 million hectares, mostly for forage (Myers, 1999). Since the early 1990s, pearl millet has been suggested as a new feed grain crop for the southeastern and mid-southern regions of the United States where the soils are acidic, soil fertility is low, and drought is common (Andrews et al., 1993; Davis et al., 2003; Kennedy et al., 2002; Myers, 1999). Pearl millet has a higher protein content (Adeola et al., 1994; Amato and Forrester, 1995; Burton et al., 1972; Sullivan et al., 1990) and a more balanced essential amino acid profile than corn (Adeola et al., 1994; Amato and Forrester, 1995; Sullivan et al., 1990), and a higher oil content than other common cereal grains (Adeola et al., 1994; Hill and Hanna, 1990; Rooney, 1978; Sullivan et al., 1990). For these reasons, pearl millet is competitive with corn as a feed ingredient for broilers (Amato and Forrester, 1995; Collins et al., 1994; Davis et al., 2003; Sharma et al., 1979; Smith et al., 1989), laying hens (Collins et al., 1997; Elrazig and Elzubeir, 1997; Kumar et al., 1991; Singh and Barsaul, 1976), ducks (Adeola et al., 1994), and beef cattle (Hill and Hanna, 1990). There also exists a potential pearl millet food grain market due to immigrants from Asia and Africa. Pearl millet is suitable for ethanol production in drought prone regions of the southeastern United States where other crops do not thrive and/or their production is not economical to resource-limited farmers (Gulia et al., 2007a).

Genetic Maps of Pearl Millet and QTL Analyses

Molecular mapping plays an important role in investigating genome and chromosome organization, comparative mapping, gene tagging and gene isolation, and marker-assisted

selection in breeding (Nagamura et al., 1997). Liu et al. (1994) constructed the first detailed genetic map of pearl millet using restriction fragment length polymorphism **(**RFLP) markers on an F_2 population. This map contained 181 loci covering seven linkage groups and extended 303 cM with an average map distance between loci about 2 cM. The significant characteristic of this map is that the map length is shorter than those of other members of the cereal family. Even so, there were large intermarker gaps that needed to be filled in the distal regions of chromosomes.

A comparative map of pearl millet was constructed based on the same population as the first genetic map of Liu and his coworkers, including many anchor markers from foxtail millet and rice (Devos et al., 2000). Comparison with the foxtail millet and rice genomes revealed three features of the pearl millet genome. First, the pearl millet genome has been rearranged with some linkage groups that do not show simple conservation. For example, Linkage Group (LG) 1 of pearl millet is homologous to fragments of foxtail millet LGs 3, 6, 7, and 8, and rice LGs 5, 11, and 12. Second, two regions of duplication appeared on LGs 1 and 4. One of these two duplication regions corresponds to the ancient duplication previously identified between rice chromosome arms 11 S and 12 S, and the other duplication region is likely a pearl millet-specific duplication. Third, the first genetic map reported by Liu et al. (1994) was short because the map was incomplete due to the limited number of markers.

An integrated genetic map was constructed based on four F_2 populations with additional RFLP markers and with simple sequence repeat **(**SSR) markers (Qi et al., 2004). This map, sharing similar marker order and distribution with the first map of Liu et al. (1994), contains 242 loci and has a length of 473 cM, more than 170 cM longer than the first map. The greater length of the map was attributed to the 12 additional markers in the distal regions of six of the linkage groups, all chromosomes except for LG 5. Even though the map was longer and more markers

were used, the largest gaps were bigger than those of the first map. Qi and his coworkers (2004) suggested that the big gaps were due to higher recombination in the distal regions. They also confirmed the two duplications between LGs 1 and 4 reported by Devos and her coworkers (2000).

Thomas et al. (1984) constructed a restriction endonuclease map of the chloroplast genome of pearl millet in 1984. The size of the chloroplast DNA of pearl millet was about 127 to 138 kilobase pairs.

Some other skeleton genetic maps of pearl millet have been constructed with markers from the genetic map of Liu et al. (1994) in order to conduct quantitative trait loci (QTL) analysis (Bidinger et al., 2007; Jones et al., 1995; Jones et al., 2002; Poncet et al., 2000; Poncet et al., 2002; Yadav et al., 2002; Yadav et al., 2004; Yadav et al., 2003), to determine gene location (Azhaguvel et al., 2003), and to solve other genetic problems (Busso et al., 1995; Liu et al., 1996b). Recently, linkage maps have been constructed by selecting markers based on the integrated consensus map (Qi et al., 2004) with new markers being added (Gulia, 2004; Gulia et al., 2007b; Sehgal et al., 2012; Senthilvel et al., 2008; Yadav, 2005).

A pearl millet genetic map was constructed by Pedraza-Garcia et al. (2010) based on sequence-related amplified polymorphisms (SRAP), random amplified polymorphic DNA (RAPD), inter-simple-sequence repeats (ISSRs) and SSR markers on a RIL population. The markers of this map were distributed evenly on 9 groups, however with only 196 loci, the average distance between markers was more than 9 cM apart (Pedraza-Garcia et al., 2010).

A pearl millet genetic map was developed by Supriya et al. (2011) with Diversity Arrays Technology (DArT) markers and SSR markers using a RIL population. This map contained 321 loci (258 DArTs and 63 SSRs) on 7 linkage groups and the genetic map length is 1148 cM. The

average distance between adjacent-markers was 3.7 cM. About 43% of markers concentrated in LGs 1 and 2, leaving the other LGs with fewer markers.

A skeleton genetic map was constructed based on 33 RFLP markers to locate two dwarfing genes and one foliage color gene (Azhaguvel et al., 2003). The dwarfing D_1/d_1 gene was mapped on LG 1 and dwarfing D_2/d_2 and the foliage color gene P were mapped on LG 4, 92 cM apart.

The first QTL analysis in pearl millet was conducted to identify the genes resistant to downy mildew caused by *Sclerospora graminicola* (Jones et al., 1995). A skeleton map was constructed based on 22 RFLP markers. Twenty-four QTLs, distributed among five linkage groups, were detected, corresponding to four different pathotypes. Jones et al. (2002) extended their research on downy mildew resistance by reporting two QTLs, including one that was also detected in 1995, based on two screening environments (field and glasshouse). Later, working on different pearl millet mapping populations at ICRISAT, Azhaguvel (2001) constructed another linkage map for downy mildew using RFLPs. Gulia (2004) and Gulia et al. (2007b) constructed linkage maps using both SSR and RFLP markers and identified QTLs responsible for controlling downy mildew disease resistance. They constructed a genetic linkage map of 749 cM using 46 marker loci including RFLPs and SSRs from 172 F_2-derived F_4 mapping population progenies based on the cross of ICMB 89111B-P6 × ICMB 90111-P6 and identified 9 major putative QTLs that control downy mildew resistance. They added three new markers to LG 5 beyond what was available on the consensus map constructed by Qi et al. (2004).

Poncet and coworkers (2000) constructed a skeleton map using 32 RFLP markers to map QTLs for domestication traits in pearl millet. Overall, 46 QTLs were detected, corresponding to 27 traits. Comparative mapping of QTL regions involved in domestication showed that two

common regions that appear in LGs 6 and 7 control most of the key morphological traits of pearl millet (Poncet et al., 2002).

Yadav et al. (2002, 2003, 2004) conducted extensive research on QTLs associated with traits that determine grain and stover yield under drought stress for different years and seasons. They first reported 88 QTLs related to grain and stover yield on six of the seven pearl millet linkage groups, finding no QTLs on LG 5 under terminal drought-stress conditions (Yadav et al., 2002). In 2003, they reported 20 QTLs related to grain and stover yield traits under different seasons and locations. Some QTLs were not affected by environment, while others were (Yadav et al., 2003). They also reported that 19 QTLs were associated with grain yield and aspects of post-flowering drought tolerance across stress environments and tester backgrounds (Yadav et al., 2004). Nepolean (2002) and Yadav (2005) also constructed linkage maps and identified QTLs for grain yield and for restoration of fertility systems, respectively. Morgan et al. (1998) mapped pearl millet rust (*Puccinia substriata* var. *indica*) and pyricularia leaf spot (*Pyricularia grisea*) resistance genes. A major terminal-drought tolerance QTL of pearl millet was found to have relationship with salt tolerance (Sharma et al., 2011).

A combination of genome scanning and association mapping was used to locate genes responsible for pearl millet environmental adaptation (Mariac et al., 2011). One gene was found to have relationship with flowering-time variation and annual rainfall.

Liu et al. (1994), Gulia (2004), Yadav (2005), Gulia et al. (2007b), Pedraza-Garcia et al. (2010), and Supriya et al. (2011) reported the presence of segregation distortion in various mapping populations of pearl millet, but no study has been conducted to identify the cause for its occurrence. This phenomenon was also observed in barley (*Hordeum vulgare*) (Heun et al., 1991), tomato (*Solanum lycopersicum*) (Devicente and Tanksley, 1991), and other mapping

projects. Later researchers also reported the effect of gender (Busso et al., 1995), and genome and sex (Liu et al., 1996a) on recombination and segregation distortion. Busso et al. (1995) examined the gender effect on segregation distortion by comparing map lengths based on two populations derived from reciprocal three-way crosses among three cultivated pearl millet varieties. No difference was observed at the whole genome level, but recombination increased by 10% in the male population in individual linkage intervals. They attributed the result to post-gametic selection for individual genes or chromosomal regions (Busso et al., 1995). Similarly, Liu et al.(1996a) investigated the effects of genome and gender on recombination rates by comparing lengths of four maps based on four populations derived from reciprocal three-way crosses among three cultivated pearl millet species and two wild sub-species of pearl millet. The F_1s were obtained by hybridizing the cultivated and wild species (Liu et al., 1996a). In contrast to the results of Busso and his coworkers (1995) with a 10% increase in individual linkage intervals for the male, Liu and his coworkers (1996a) showed that the genetic maps were 10 % longer in the male, although no significant differences were found in individual linkage intervals, probably because of gametophytic selection.

Molecular Markers and the Recombinant Inbred Lines

DNA markers are widely used in high density genetic linkage map construction because more DNA markers segregate at the same time in a population than do classical morphological markers, and DNA markers usually do not affected by environment factors. The most common types of DNA markers used in genetic mapping are those based on Polymerase Chain Reaction (PCR). The PCR-based molecular markers in pearl millet include genomic SSR, expression sequence tagged (EST)-SSR, sequence-tagged-sites (STS), single-strand conformation polymorphism (SSCP), single nucleotide polymorphism (SNP), conserved intron scanning

primers (CISPs), inter simple sequence repeats (ISSR), sequence-related amplified polymorphism (SRAP), Diversity Arrays Technology (DArT) markers, random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) markers. Except for RAPD, AFLP, and DArT markers, the development of other molecular markers needs genome sequence information. SSR markers were supplemented with AFLP markers for this research because there were not enough SSR markers available for pearl millet to construct an adequately dense map. The two marker systems are discussed below.

Amplified Fragment Length Polymorphism (AFLP): AFLPs were first reported in 1993 (Zabeau and Vos, 1993) and were used in DNA fingerprinting in 1995 (Vos et al., 1995). The procedure can be divided into three steps: 1) digestion of total genomic DNA using restriction enzymes and ligation of oligonucleotide adaptors to all restriction fragments, 2) selective amplification of some of these fragments using PCR, and 3) electrophoretic separation and visualization of the banding pattern. The restriction enzymes used for digesting DNA are usually a combination of two enzymes, a frequent cutter such as *Mse*I and a rare cutter such as *Eco*RI or *Pst*I. Adapters that connect to the two sides of DNA fragment include a core sequence and an enzyme specific sequence. PCR primers consist of an adapter sequence and a selective extension. The amplification procedure is divided into two parts. The first step is pre-amplification with primers that have one selective nucleotide (sometimes with a nonselective nucleotide). The second step is final selective amplification with the diluted pre-amplification product as template and primers with two (or sometimes three) selective nucleotides. The two-step amplification procedure is used because the primer selectivity is good for primers with one or two selective nucleotides and three-selective-nucleotide primers are also acceptable. The AFLP technique has many advantages, such as high reproducibility (Blears et al., 1998; Jones et al., 1997; Powell et

al., 1996), simultaneous identification of multiple loci, no need for sequence information, and only small amounts of DNA are required (Vos et al., 1995). The AFLP technique has been widely used in genetic mapping (Vuylsteke et al., 1999), gene location and QTL analysis (Powell et al., 1997), and genetic diversity analysis (Lubberstedt et al., 2000).

When AFLPs are used as markers in plant genetic mapping, marker clustering was observed when *Eco*RI/*Mse*I was used (e.g., Menz et al., 2002), while no marker clustering was observed with *Pst*I/*Mse*I (e.g., Zhu et al., 1998). The marker clustering was assumed to be associated with centromeres. For example, in *Arabidopsis* species, Alonso-Blanco et al. (1998) found *Eco*RI/*Mse*I AFLP markers clustered around the centromeric regions of the chromosomes, and established the centromere position of Chromosome 3. In soybean, one *Eco*RI/*Mse*I AFLP marker cluster per linkage group was found and the researchers concluded that the marker clusters may show genetic locations of centromeres (Young et al., 1999). Similar clustering of *Eco*RI markers around centromeres has been observed in other plant species such as maize (Vuylsteke et al., 1999), barley (Becker et al., 1995; Powell et al., 1997; Qi et al., 1998), sorghum (Boivin et al., 1999), soybean (Keim et al., 1997), potato (Isidore et al., 2003), and tomato (Haanstra et al., 1999).

The causes for the clustering might be due to the structure of centromeres and the enzymes used in AFLP analysis. In eukaryotes, the centromere regions are constitutively heterochromatic and contain tandem satellite DNA (Bulazel et al., 2006; Schueler et al., 2001; Sumner, 2003). The sequence of the X chromosome centromere of *Macropus rufogriseus* revealed more than 60% A+T rich regions (Bulazel et al., 2006). In *Arabidopsis*, pericentromeric heterochromatin, which fluoresces brightly when stained with the fluorochrome DAPI, shows A+T rich regions (Ross et al., 1996). Centromeric DNAs are also heavily methylated on their

cytosine residues. Methylation may affect four functions of the centromere: 1) reducing recombination rate (Bender, 1998; Vongs et al., 1993; Yoder et al., 1997), 2) forming heterochromatin (Bender, 1998; Vongs et al., 1993; Yoder et al., 1997), 3) keeping the chromosome stable by keeping centromeric condensation (Hansen et al., 1999; Xu et al., 1999), and 4) binding centromere-specific proteins (Mitchell et al., 1996). Interestingly, the centromeric region is highly strand-biased (Luo and Preuss, 2003), suggesting that most centromeric loci are semimethylated. The commonly used restriction enzymes in AFLP analysis are *Eco*RI and *Pst*I combined with *Mse*I. *Mse*I, which recognizes 5'-TTAA-3', is unaffected by methylcytosine and therefore cuts plant DNA completely (McClelland et al., 1994). *Eco*RI, which recognizes 5'-GAATTC-3', can digest hemimethylcytosine DNA even though the rate is reduced (McClelland et al., 1994). *Pst*I, which recognizes 5'-CTGCAG-3', is sensitive to methylcytosine (McClelland et al., 1994), has fewer cut sites in heavily methylated regions than in euchromatic regions. Therefore, *Eco*RI/*Mse*I will be expected to have more restriction sites in centromere regions with A+T-rich and hemimethylated regions than *Pst*I/*Mse*I will.

Centromeric marker clustering may also relate to suppression of recombination of the centromere, and this phenomenon was also found using RFLP markers in mapping (Gill et al., 1996; Tanksley et al., 1992), even though the centromere suppression varies from chromosome to chromosome and did not totally depend on the distance from the centromeres in wheat (Gill et al., 1996).

Simple sequence repeats (SSR): Simple sequence repeats (SSRs; also called microsatellites) get their name because they usually consist of 1 to 6 base pairs, which are tandemly repeated in the genome (e.g., Tautz, 1989). Although SSRs are found in both coding and noncoding DNA, most SSRs distribute in noncoding regions in eukaryotic organisms (e.g.,

Moran, 1993). For example, only 7 to 10% of SSRs are found in protein coding regions in higher plants (Varshney et al., 2002; Wang et al., 1994).

The structure, function, and evolution of SSRs have been reviewed by Li et al. (2004) and Chistiakow et al. (2006). In many species, SSRs were chosen to be molecular markers for genome mapping because they are: 1) PCR-based, 2) usually co-dominant, 3) reproducible, and 4) have a high mutation rate and therefore are abundant in the genome (Saha et al., 2004). The portability of microsatellite markers among mapping populations, and across species or sometimes even across the genus level makes them potentially useful for studies of genome evolution and comparative genomics and marker-assisted selection (Fredholm and Wintero, 1995; Reddy et al., 2001; Tanksley and McCouch, 1997; Zardoya et al., 1996). The high level of polymorphism, the relatively low cost per analysis, and the ease of use have made SSRs excellent molecular markers for genetic mapping (e.g., Dib et al., 1996).

The process of genomic SSR development is time and labor intensive, involving the construction of a genomic library followed by screening of clones containing repetitive DNA sequences and sequencing the SSR region. The first two sets of pearl millet genomic SSRs were reported by Allouis et al. (2001) and Qi et al. (2001). These 50 genomic SSR markers were developed from bacterial artificial chromosome (BAC) libraries without subcloning and the SSR sequences were identified using suppression PCR (Allouis et al., 2001; Qi et al., 2001). The second set of genomic SSRs (18 pairs) was reported by Budak et al. (2003). These genomic SSRs were derived from a small insert genomic library and the SSR regions were selected using ^{32}P-labeled $(CT)_{15}$ oligonucleotide probe. The third set of genomic SSRs (44 pairs) was report by Qi et al. (2004). These primers were developed from a $(CA)_n$-enriched small-insert library and SSR regions were selected using biotinylated $(GT)_{15}$ oligonucleotide probes. A set of STS

primers derived from RFLP markers were also developed by Tracy Money, John Innes Centre, Norwich, UK. (Personal communication).

In 2003, the first set of 2494 pearl millet EST sequences was submitted to the National Center for Biotechnology Information (NCBI) GenBank data base by MK Reddy, and the total number of ESTs currently available there is 2920 sequences (NCBI, http://www.ncbi.nlm.nih.gov/nucest?term=pearl%20millet). Availability of EST sequences significantly accelerates the development of pearl millet molecular markers such as EST-SSR, expressed sequence tag single nucleotide polymorphism (EST-SNP) and conserved intron spanning primer (CISP), which in turn has caused the process to be both less costly and less time-consuming. Another advantage of markers derived from EST sequences is that these markers are associated with functional genes. Twenty-five, 58, 16, and 19 EST-SSR primers were generated using data mining of expressed sequences by Senthilvel et al. (2004), Senthilvel et al. (2008), Mariac et al. (Mariac et al., 2006), and Yadav et al. (Yadav et al., 2007), respectively. Bertin et al. (2005) developed a set of SSCP-SNP markers by aligning the pearl millet EST sequences with rice genome sequences to obtain the homologues of single-copy rice genes in which the intron positions could be precisely predicted. The SSCP-SNP primers can detect SNPs and sequence polymorphisms caused by indels or SSRs when the PCR products are separated on SSCP gels. By targeting conserved intron positions, Feltus et al. (2006) developed a set of CISP primers that can also detect the sequence polymorphisms caused by indels and SSRs. Even though most of these primers were derived from the conserved intron positions of rice genes by aligning the sorghum EST sequence with the rice genome, the CISP primers have been successfully used in pearl millet (Thudi et al., 2010).

More recently, new molecular markers such as those based on ISSR, SRAP, and DArT

markers were developed and used in pearl millet genetic maps (Pedraza-Garcia et al., 2010; Supriya et al., 2011). These markers are not widely used in pearl millet at this point.

Recombinant inbred lines (RILs): Recombinant inbred lines in plants are developed by hybridizing two inbred lines, followed by several generations of self-fertilization via single-seed descent. Within-line homozygosity at each locus is 99.2% for an F_8 population. For any two linked loci, Haldane and Waddington (1931) calculated the proportion of different zygotic types in every generation of RILs. Hospital et al. (1996) further developed a general algorithm to calculate multilocus genotype frequencies of RILs. Because every RIL is an inbred strain and can be maintained as a purebred, RILs are valuable for genetic mapping and for the dissection of quantitative trait loci (Bailey, 1971; Goramaslak et al., 1991; Lark et al., 1995).

RILs have been widely used in genetic mapping and QTL analysis. As stated by Broman (2005), "a panel of RILs has many advantages for genetic mapping: one need genotype each strain only once; one can phenotype multiple individuals from each strain to reduce individual, environmental, and measurement variability; multiple invasive phenotypes can be obtained on the same set of genomes."

When RILs are used in QTL analysis, the first question is the selection of the number of RILs and the number of replications. According to Knapp and Bridges (1990), the variance of a given trait in a QTL model contains three components: 1) the variance explained by the QTL in the model, 2) the variance explained by QTLs not in the model, and 3) nongenetic variance. Increasing the number of lines will decrease the first and second components while increasing the number of replicates will decrease the third component (Knapp and Bridges, 1990). It is better to use more lines than more replicates when one trait is controlled by many QTLs (Zou et al., 2006). After the phenotypic data are collected for each RIL, the average among the replicates

usually is used in QTL analysis. This widely-used method is called the "line means model" while with every replicate included, it is called the "full data model (Zou et al., 2006). The line means model is generally suitable for univariate QTL mapping (Zou et al., 2006).

Nematode Resistance in Plants

Nematoda is a large phylum of animals that include parasites of all kinds of plants and animals, and free-living species (Maggenti, 1981). Sedentary plant parasitic nematodes of the family Heteroderidae, including the cyst nematodes and the root-knot nematodes, feed on the cytoplasm of plant root cells and stunt host plant growth, wilt leaves, make the plant susceptible to other pathogens, and reduce yields.

The root-knot nematodes (*Meloidogyne* spp.), which cause root galls or root knots on their hosts, infect many plant species and cause severe yield losses for many crops throughout the world. The second-stage juvenile penetrates the root and migrates to a site near the vascular tissue to establish a permanent feeding site. Egg production begins at about 3 to 6 weeks after the initial infection (Williamson and Hussey, 1996). Gender is determined epigenetically with males increasing in frequency under conditions of crowding or poor nutrition (Triantaphyllou, 1973).

Plants are resistant to nematodes when the nematodes have reduced levels of reproduction (Trudgill, 1991). The expression of resistance to nematode resembles that in other pathogen-resistant plants (Dangl et al., 1996; HammondKosack and Jones, 1996). Many nematode resistance genes have been identified. Some are dominant or semidominant (Ballvora et al., 1995; Lorieux et al., 1995; Messeguer, 1991; Pineda, 1993; Salentijn et al., 1992; Webb et al., 1995; Williams et al., 1994), some are recessive (Wang and Goldman 1996), and some are quantitatively inherited (Faghihi et al., 1995; Kreike, 1993; Trudgill, 1991; Wang and Goldman, 1996). Two cyst nematode resistance genes in wheat were mapped at homeologous loci on the

long arms of Chromosome 2B and 2D (de Majnik et al., 2003). The cyst nematode resistance gene in rice was mapped on Chromosome 11 (Lorieux et al., 2003). Several crop species show nematode resistance that has been used in breeding programs (Roberts, 1992). For instance, a single dominant major gene *H1* conferring resistance to cyst nematode *Globodera rostochiensis* in potato (Jones, 1985), and a single dominant gene *Mi-1* conferring resistance to root-knot nematode *Meloidogyne incognita, M. javanica, M. arenaria* in tomato (Roberts and Thomason, 1989) have been used in breeding for more than 50 years.

Seven nematode resistance genes have been cloned from plants (Williamson and Kumar, 2006). Two soybean cyst resistance genes (Hauge et al., 2001; Lightfoot and Meksem, 2002) and one sugar beet cyst resistance gene code for membrane proteins, while products of the tomato cyst resistance gene, tomato root-knot resistance gene, and two potato cyst resistance genes (Paal et al., 2004; van der Vossen et al., 2000) code for cytoplasmic proteins. Some genes confer wide spectrum resistance against nematode species, while other genes confer a narrow resistance spectrum. For example, the genes *Mi-1* and *Hero A* give broad-spectrum resistance against several root-knot nematode species in tomato (Milligan et al., 1998; Vos et al., 1998) and potato (Ernst et al., 2002), respectively.

Nematodes are a constraint to pearl millet production (Lee et al., 2004), and cause grain yield loss in the southeastern United States (Timper et al., 2002), but the primary concern about nematode susceptible hybrids are the effects on susceptible rotation crops. Timper et al. (2002) reported variation among grain hybrids for resistance to root-knot nematode (*Meloidogyne* spp.) in naturally infested fields and in greenhouse tests. Several pearl millet land races from west and east Africa were evaluated for resistance to *M. incognita* (Timper and Wilson, 2006). All land races showed some level of resistance with individual plants expressing a range of resistance

levels from susceptible to highly resistant. Two dominant genes were thought to control the nematode resistance in the cultivar "Zongo" (Timper and Wilson, 2006).

Marker-Assisted Selection in Plants

A plant breeding process mainly involves creation of variation and selection from that variability with subsequent field testing. Selection from variability, or simply selection, is a key part of the breeding process because selection requires the most time and effort of the whole breeding process. In the traditional selection method, also known as phenotypic selection, breeders select individuals according to their observable characteristics or traits, such as morphological, developmental, and biochemical properties that can be seen, measured, or scientifically tested. Since a phenotype is the expression of a genotype, a phenotypic selection can be considered as an indirect selection of the genotype of an individual. Theoretically, genotypic selection is usually more precise and more efficient than phenotypic selection.

With increased availability of genetic markers, especially DNA markers, gene and/or QTL mapping, and genomic sequence information, Marker-assisted selection has played an important role in the breeding process. The basic principle of marker-assisted selection is the application of Linkage Disequilibrium between markers and QTL or gene of interest (Hospital, 2009). The presence of a gene of interest can be inferred through the presence of one or more DNA markers that are tightly linked to the gene (Hospital, 2009; Kumar, 1999). The closer that the markers are to the QTL or gene, the more efficient that marker-assisted selection is. Marker-assisted selection has been widely used in backcrossing, genes or QTLs pyramiding, breeding material evaluation, and early generation selection (Collard and Mackill, 2008).

Marker-assisted selection can greatly increase selection efficiency in backcrossing; therefore, marker-assisted back-cross breeding is thought to be one of the most successful cases

in which the marker-assisted selection experiments meet theory (Hospital, 2009). Backcrossing is an important breeding technique for plants and was especially popular between 1930s and 1960s (Stoskopf et al., 1993). Backcrossing is also widely applied in hybrid breeding using cytoplasmic male sterility to produce sterile lines and restore lines in pearl millet (Khairwal, 1999) and rice (Virmani, 1997). Backcrossing is used to improve an adapted or elite variety by integrating one or a few traits or introduce new traits from wild relatives to a cultivated species (Behura et al., 2011). Backcrossing involves crossing between a donor parent that contains one gene or a few genes that need to be transferred into another line and a recurrent parent that needs to be improved. It requires several repetitions of crossing between the hybrids and the recurrent parent to produce a new line in which most of the genetic materials come from the recurrent parent and a few new traits from donor parent (Gupta and Varshney, 2004).

Depending on the targets of selection, marker-assisted selection includes target gene selection or foreground selection (Hospital and Charcosset, 1997), recombinant selection to control linkage drag (Collard and Mackill, 2008), and background selection to recover recurrent parent (Collard and Mackill, 2008). Marker-assisted target gene or QTL selection (foreground selection) is especially important for traits that have laborious and time-consuming phenotypic screening procedures, i.e. disease or pest resistance. For selection of reproductive-stage traits, marker-assisted selection can be conducted in the seedling stage so the best plants can be selected for backcrossing (Collard and Mackill, 2008). Marker-assisted recombinant selection can effectively reduce the linkage drag by using the markers flanking both sides of a target gene (Collard and Mackill, 2008). Marker-assisted background selection can help to recover the recurrent parent background rapidly by eliminating two to four backcrossing generations (Frisch et al., 1999; Hospital and Charcosset, 1997; Visscher et al., 1996).

In addition to successful uses of marker-assisted selection in backcrossing, marker-assisted gene pyramiding is another successful example of marker-assisted selection in the breeding process (Hospital, 2009; Collard and Mackill, 2008). Gene pyramiding means incorporating several target genes or QTLs into a single genotype. Marker-assisted selection is particularly helpful when trying to get a durable resistance by pyramiding multiple resistance genes that show the same phenotype because phenotypic screening cannot distinguish these genes with the same resistance reaction (Collard and Mackill, 2008). For example, Huang and coworkers (1997) successfully used marker-assisted pyramid method to combine 2 dominant and 2 recessive rice bacterial blight (*Xanthomonas oryzae* pv. *Oryzae*) resistance genes into one genotype and obtained a new line that showed a wider and higher level of resistance. Castro et al. (2003) also pyramided one qualitative resistance gene and resistance QTLs to barley stripe rust (*Puccinia striiformis* f.sp. *hordei*) and obtained a series of double haploid lines containing qualitative resistance gene and one to three QTLs that have higher levels of resistance than a single gene or QTL. More success stories were reviewed by Collard and Mackill (2008).

Compared to the number of markers that are now known to be linked to QTLs and genes, the number of markers that have been used in marker-assisted selection is surprisingly low (Xu and Crouch, 2008). The primary challenge for marker-assisted selection is that the selection efficiency is lower than expected for some traits controlled by multiple QTLs, especially when QTL × QTL, QTL × genetic background, and QTL × environment interactions are involved (Hospital, 2009). For example, Steele et al. (2006) reported their marker-assisted introgression of four QTLs controlling root length and thickness and one recessive QTL for aroma from an upland *japonica* rice to an upland *indica* rice. Only one QTL was found to increase the root length significantly under both irrigated and drought stress environments and the other three

QTLs did not work in novel genetic background. Interestingly, the QTL that worked is not the major one which explained the largest phenotypic variance in the original mapping efforts. Bouchez et al. (2002) reported the effect of a QTL controlling corn dry grain yield was reversed in a new genetic background compared to the original QTL mapping results.

In addition to the low selection efficiency due to the complex nature of polygenic inheritance, other limitations also restrict the use of marker-assisted selection in breeding process; for example, linked markers to the target gene or QTL that lack polymorphism in other breeding materials and thus require a new mapping effort to find new linked markers before continuing to use marker-assisted selection.

Despite the difficulties of marker-assisted selection used with traits controlled by multiple QTLs, marker-assisted selection can be successfully used in disease and pest resistance introgression and pyramiding. So marker-assisted selection as well as QTL mapping for disease and pest resistance will continue to play important roles in plant breeding.

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CHAPTER 3

A HIGH-DENSITY GENETIC MAP OF PEARL MILLET [*PENNISETUM GLAUCUM* (L.) R.BR.] BASED ON AFLP AND SSR MARKERS WITH QTL ANALYSES OF AGRONOMIC TRAITS[1]

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Abstract

Pearl millet, a cereal that originated in the Sahel zone of west Africa, can tolerate dry conditions and low soil fertility. It is an important dry land crop, predominantly in Africa and Asia as a staple food grain and also in the United States and Brazil as an important forage crop. A new genetic map of pearl millet was constructed based on 180 recombinant inbred lines (RILs) derived from an intraspecific cross between Tift 99B and Tift 454. The map utilizes 468 molecular markers (361 AFLPs and 107 SSRs), spans 757 cM, and covers all 7 linkage groups that represent the 7 chromosomes of pearl millet. In addition to using the publicly available SSRs, we are also reporting 144 new pearl millet SSRs developed from expressed sequences tags (ESTs) for which putative functions have previously been determined; 29 of which were used in the mapping effort. To demonstrate the utility of this map, we mapped putative quantitative trait loci (QTLs) for plant height, hundred seed weight, heading date, and panicle length and panicle width.

Key words: pearl millet, genetic map, QTL, EST-SSR, AFLP

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] originated in the Sahel zone of west Africa (Brunken et al., 1977) and was domesticated in Africa and India more than 3,000 years ago (Allchin, 1969; Davies, 1968). Because pearl millet can tolerate extremely dry conditions (Brunken, 1977) and low soil fertility, it has a higher and more reliable yield than other dry land crops such as sorghum or maize under these conditions (Andrews et al., 1993). It is an important dry land crop, predominantly in Africa and Asia where it is a staple food grains and also is an important forage crop in the United States and Brazil.

Genetic mapping plays important roles in both genomic research and breeding. The first pearl millet genetic linkage map was developed by Liu et al. (1994), containing 181 restriction fragment length polymorphism **(**RFLP) loci, and extending 303 cM. One significant characteristic of this map is that 46% of the markers (83 out of 181) were found on Linkage Group (LG) 1 and 2. A comparative map (Devos et al., 2000) of pearl millet was constructed based on the same mapping population as the first genetic map. Some anchor markers from foxtail millet and rice were included in this map (Devos et al., 2000). This map revealed that the pearl millet genome is highly rearranged compared to those of rice and foxtail millet. In addition, the map also confirmed that the first genetic map reported by Liu et al. (1994) was short because it was actually incomplete due to the limited number of markers. An integrated genetic map was constructed with 353 RFLP markers and 65 simple sequence repeat **(**SSR) markers combining four pearl millet maps (Qi et al., 2004). Since 85% of the markers in the consensus map were clustered proximally, covering one third of the total map length, there were large gaps on distal regions (Qi et al., 2004). Qi et al. (2004) suggested that the big gaps were due to higher recombination in the distal regions. A new pearl millet genetic map was constructed by Pedraza-Garcia et al. (2010) based on sequence-related amplified polymorphisms (SRAP), random amplified polymorphic DNA (RAPD), inter-simple-sequence repeats (ISSRs) and SSR markers. The markers of this map distributed evenly on 9 groups; however, with only 196 loci, the average distance between markers was more than 9 cM apart (Pedraza-Garcia et al., 2010). More recently, a genetic map was constructed by Supriya et al. (2011) based on 158 Diversity Arrays Technology (DArT) markers and 63 SSR markers with a RIL population of 140 individuals. This map spans 1,148 cM and the average marker interval is 3.7 cM. One characteristic of this map is

that 44% (141 out 321) markers are distributed in LG1 and LG2 (Supriya et al., 2011). Seventy-five gene-based markers were mapped recently by Sehgal et al. (2012).

The previous pearl millet genetic maps were either incomplete due to a low density of markers (Liu et al., 1994; Pedraza-Garcia et al., 2010) or most of markers were clustered in the central regions of the LGs (Qi et al., 2004) or the markers were unevenly distributed among LGs (Supriya et al., 2011). To develop more information of the pearl millet genome and add more molecular markers which will be useful in pearl millet research and breeding, we constructed a detailed pearl millet genetic map, aligned the available SSR markers to previous genetic maps, and developed and mapped EST-SSR markers. To demonstrate the utility of this map, we used it to locate a number of quantitative trait loci (QTLs) for agronomic traits important for pearl millet production.

Materials and Methods

Plant Materials

The parental inbreds used in this study, Tift 454 and Tift 99B, were co-developed and released by USDA-ARS and the University of Georgia-Tifton Campus in Tifton, GA; Tift 454 and Tift 99A are parents of TifGrain 102, and Tift 99B is a male fertile maintainer line for Tift 99A (Hanna et al., 2005a; Hanna et al., 2005b). The parental line Tift 454 is highly resistant to root-knot nematode specie [*Meloidogyne incognita* (Kofoid &White) Chitwood] (Hanna et al., 2005a). The F_1 plants derived from a single cross of parents were assessed for resistance to this specie of root-knot nematodes (Timper, personal communication). Seeds from a single highly resistant F_1 plant were selfed to F_7 by the single-seed descent method in the greenhouse to generate a recombinant inbred mapping population of 180 lines.

DNA Extraction

DNA extraction for SSR analysis followed the SDS-potassium-acetate method (Li et al., 1995). DNA used for AFLP analysis was obtained using Qiagen's DNeasy Plant DNA Extraction Mini Kit (QIAGEN, Valencia, CA). DNA was quantified using the Fluorocount instrument (GMI Inc., Ramsey, MN) before dilution to an appropriate working solution for SSR and AFLP analyses.

AFLP Analysis

The detailed procedure for AFLPs followed the manufacturer's instructions for the 4300 DNA Analysis System (LI-COR Inc., Lincoln, NE). Approximately 100 ng of DNA was first digested with *Eco*RI and *Mse*I, and then restriction site-specific adaptors were ligated to both sides of the DNA fragments. Pre-amplification was carried out using single selective base primers carrying adaptor-specific sequences. The pre-amplification product was then segregated on a 2% agarose gel to check the effect of enzyme digestion and pre-amplification. The pre-amplification products were diluted 20 to 40 fold before used in the last two-base-selective amplification with one primer labeled with IRdye 700. The amplification products were separated and visualized using LI-COR 4300. All 64 primer combinations were used to screen the parents to choose the best combinations to genotype the mapping population. The polymorphic bands were scored manually and fragment lengths were estimated according to a 50-700 bp sizing standard from LI-COR Inc. (Lincoln, NE). The names of the AFLP markers in the map were named beginning with AFLP instead of the name of the primer combination and segment length. The names in map and their correspondent original bands were reported in the supplementary tables (Table S3.1).

SSR Analysis

PCR amplifications were carried out on a PTC-200 Thermo Cycler (MJ Research Inc., Waltham, MA) or MyCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). The amplification was done in a 10 μl volume consisting of 10–15 ng genomic DNA as template, 50 pmol of each primer, 1 mM $MgCl_2$, 0.1 mM of each dNTP, 1 × PCR reaction buffer, and 0.2 U *Taq* polymerase. The PCR cycles began with 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 54 °C for 45 s, 72 °C for 60 s, ended at 72 °C for 10 min, and store at 4 °C. PCR products were separated on a 10% polyacrylamide gel. Electrophoresis instruments, running conditions, and silver staining methods were described by Zhang et al. (2002).

Linkage Analysis and Map Construction

The marker data were scored in dominant or codominant form according to the definition of JoinMap 3.0 (Van Ooijen and Voorrips, 2001). The linkage analyses were conducted with a LOD score of 3.0, a recombination frequency of 0.4 to provide evident linkage, and the Kosambi map function (Kosambi, 1944) was used to convert recombination frequencies to map distances. The Chi-squared test was performed on all markers to test for segregation distortion. The names of linkage groups and the orientation of the chromosome arms were determined through comparison of the location of previously mapped SSR markers (Qi et al., 2004; Senthilvel et al., 2008). The graphs of the linkage groups was created using MapChart 2.2 (Voorrips, 2002).

EST-SSR Marker Development, the Mean Polymorphism Information Content (PIC) Values Calculation, and the EST Function Prediction

The BC_8 ovule transcriptome SSR data were obtained from Zeng et al. (2011). The SSR finder program written by S. Cartinhour (ftp://ftp.gramene.org/pub/gramene/software/scripts/ssr.pl) was run against 26,576 contigs derived from ovule RNA amplified by T7-based in-vitro transcription, sequenced by 454-Technology (454 Life Sciences, Branford, CT), and assembled using MIRA (http://www.chevreux.org/projects_mira.html). The search was restricted to motifs having at least 18 bp long (*i.e.*, di-nucleotide≥ 9; tri-nucleotide≥ 6; tetra-nucleotide ≥ 5). From the SSR containing EST sequences, primer design was conducted with Vector NTI/Contig express 7.0 (Invitrogen, Life Technologies Corporation, Grand Island, NY). The rate was 153-171. The expected amplicon lengths for most primers were about 100 to 160 bases. The polymorphic information content (PIC) values were calculated using the software PowerMarker 3.25 (Liu and Muse, 2005).

EST-SSR functions were predicated by using NCBI BLASTN search against the rice transcripts (CDS+UTR) at the Rice Annotation Project (RAP) database (http://rapdb.dna.affrc.go.jp/tools/blast/). Only those rice genes that returned an e-value of e-5 or less during the BLASTN search were considered putative homologs. BLASTN searches were carried out in June 2010.

Marker Selection

Besides the AFLPs and the SSRs from above, additional SSR primers (Table 3.1) were used in screening the two parents for polymorphisms. These additional markers came from eight sources and included 122 genomic SSRs (PSMP and CTM prefixes), 55 RFLP-STSs (PSMP prefix), 131 EST-SSRs (ICMP and CUMP prefixes), 102 single strand conform polymorphism (SSCP) (PSMS prefix), and 139 conserved intron primers (SRSC and PCISP prefixes).

Phenotype Data Collection

Recombinant inbred lines were planted in a randomized complete block design with two replications in 2006 and 2007 in Tifton, Georgia. For each replication, a single row of 18 foot long plots were planted using a two-row cone planter at a rate of approximately 0.50 g of seed.

The heading dates were recorded according to average panicle date of 10 plants per plot. Plant heights were measured on 10 individual plants within each plot with a meter stick to the tip of panicle. After harvest, the panicle width was measured with a digital caliper (ATD-8656) to the nearest mm and the panicle length was measured with a meter stick to the nearest cm. The panicles were threshed using an ALMACO (Nevada, IA) single panicle thresher model SVPT. The threshed grain was screened with a #8 sieve and placed in individual envelopes. The grain was dried at 38 $^{\circ}$C for 24 hours to get uniform moisture content. After drying, one hundred grains were counted and weighed with two replications per line.

QTL Analysis

Heading date, head width and head length were analyzed with SAS 9.2 [SAS Institute, Inc. (2008)] GLM Procedure. Hundred seed weight and plant height were analyzed with Microsoft Excel 2003 (Microsoft Corporation, Redmond, WA). The mean values of each trait were used for QTL analysis. Multiple QTL mapping analysis was conducted using MapQTL 5.0 (Van Ooijen, 2004) with mapping step size of 1 cM and five neighboring markers. The LOD thresholds, which were used to declare a significant QTL, were determined by permutation test in MapQTL 5.0 with a genome-wide significance level of $P<0.05$ and n=1,000. The LOD thresholds were 3.1, 2.9, 2.9, 3.0, 3.0, 3.0, and 3.0 for heading date of 2006, heading date of 2007, plant height of 2006, plant height of 2007, head width of 2007, head length of 2007, and hundred seed weight 2007, respectively. Additive effects were defined with respect to the alleles of Tift 99B. Thus, positive genetic effects indicated the alleles of Tift 99B increased the phenotypic value, and negative values indicated that the alleles of Tifton 99B decreased the phenotypic value.

Results

EST-SSR Development Results

From a total of 26,576 EST sequences analyzed, 221 sequences were identified to contain SSRs of at least 18-20 nucleotides in length, including 14 of which had more than one SSR. The relative abundance of di-, tri-, and tetra-nucleotide repeats was 52 (23.5%), 130 (58.8%), and 39 (17.6%), respectively. The largest section of tri-nucleotide motifs was GCC/GGC (11.5%),

followed by CGC/GCG (10.7%) while the most abundant di-nucleotide was AG/CT motifs (21.1%).

Overall, 169 primer pairs were developed from the 221 SSR-containing EST sequences and were designated with the prefix UGTP (Table S3.2). Out of 169 primer pairs, 80 primers produced a simple product; 15 produced longer fragment lengths than expected; and 18 did not give clear amplification products which were not used in mapping. Out of 15 primers that gave longer fragments, 9 gave 50- to 500 bases longer than the expected length.

Thirty-two primer pairs showed polymorphism between the two parents of the mapping population. We were able to map 29 primer pairs including one which produced 2 loci. Out of 30 mapped loci, 25 were codominant loci, and 5 were dominant loci. The PIC value for EST-SSRs ranged from 0 to 0.84 (Table S3.2)

The EST function blast showed that only 57 ESTs (33.7%) had homologues on the rice genome, and 7 out of the 29 mapped EST had functions identified (Table S3.3). Positions of rice RAP homologs of newly mapped previous developed EST-SSR and their putative annotation were also identified (Table S3.4).

SSR and AFLP Screening Results

Of the SSR markers used for the mapping (Table 3.1), the genomic SSRs produced the highest rate of polymorphism among all the primers used, 31.07% and 26.32% for PSMP primers and CTM primers, respectively. On the other hand, the polymorphic rates for conserved intron primers designed from ESTs of other cereal crops were the lowest, being only 4.65% and 5.21% for PCISP and SRSC primers, respectively.

Of the 64 primer combinations for the AFLP markers used to screen the two parents, 37 primer combinations produced more polymorphic bands between the two parents and were chosen for testing the RIL population. The other 27 primer combinations had unclear banding patterns or very few bands and were discarded. Only segments between 65 to 650 base pairs were scored. Overall, 369 markers were selected for mapping, and 361 (97.8%) markers were successfully placed in the linkage map. An average of 9.7 markers was mapped per primer combination.

Genetic Mapping

From a total of 487 segregating markers, which include 369 AFLP and 118 SSR markers, 468 (96%) markers comprising of 361 AFLP and 107 SSR markers were assigned to 7 main LGs and 2 small LGs each with only 2 loci. Since pearl millet has n = 7 chromosomes, the 7 LGs likely represent the collinear chromosomes of this species. Of the two small LGs, one was assigned to LG2 on the basis of homology according to the previously mapped location assigned by Qi et al. (2004). The total length of the map was extended to 757 cM and the average distance between markers was 1.62 cM although 3 gaps bigger than 20 cM were present in the distal regions of some LGs. The average number of markers in the 7 main LGs was 69; however, the number varies from 25 in LG4 to 103 in LG3 (Table 3.3 and Fig.3.1). One common feature of the 7 LGs is clustering of markers in the proximal regions of each LG and the presence of gaps (larger than 15 cM) in each linkage group and some gaps around 20 cM in the distal regions of some of LGs. A total of 63 new SSR loci were present in the map, including 52 of which were derived from EST sequences. Three large EST-SSR marker clusters, containing 6, 6, 9 markers were located at the distal region of LG2, LG3 and LG6, respectively.

The present map was aligned with previously published maps to assist in assigning the name and marker orders of each LG. For convenience, the previously mapped data including the consensus map (Qi et al., 2004), the map published by Senthilvel et al. (2008), and the unpublished PSMS map data (Devos, unpublished data) was redrawn as a revised map using the original consensus map (Qi et al., 2004) as the back bone. Markers from Senthilvel et al. (2008) and unpublished PSMS map data (Devos, unpublished data) were added to the consensus map (Qi et al., 2004) if the markers had neighbors on the consensus map and map position could be inferred.

Previously mapped SSR loci were used as anchor markers to align the new map with the revised consensus map. Alignment results showed that the order of the anchor markers was mostly consistent with the consensus map (Qi et al., 2004). There were a few inconsistencies and most involved markers that were not in the original consensus map but mapped later and added to consensus map according to the relative marker position (Fig. 3.2 and Fig. S3.1). There were reversals of the marker order that involved markers PSMP2070 and PSMP2267 (3.2 cM apart) on LG3, markers CTM21 and PSMS22 (0.6 cM apart) on LG2, ICMP3078 and CTM25 (8.5 cM apart) on LG5, and ICMP3043 and CTM08 (5.0 cM apart) on LG7. Except for PSMP2070 and PSMP2267, the other six markers were new markers added to the consensus map. Other inconsistencies include PSMS08, PSMS36, and PSMS78 that were mapped in different linkage groups in our map. ICMP3029 (ori) was mapped in LG 2, but ICMP3029 was mapped in LG4 by Supriya et al. (2011). Another interesting conflict includes the marker PSMP2229 that was mapped on LG3 on our map as a single marker, but was mapped previously as a double polymorphic marker on LG5 and LG7.

To see how our EST-SSR sequences matched with other related genetic maps, we performed BLASTN searches on these mapped EST-SSR sequences from above against the rice transcripts (CDS+UTR) at the Rice Annotation Project (RAP) database. Tables S3.3 and S3.4 showed the comparative positions of the pearl millet genes on rice genomes. Our results were consistent with that of Devos et al. (2000) in that 19 out of 25 mapped EST-SSRs (76%) that were found in the annotated rice genome were found in the expected map locations. Some new homologous relationships between pearl millet and the rice genome were found that included two markers [PSMS 89 and ICMP3029 (ori)] on LG2 corresponding to rice chromosome 8, CUMP 18 and ICMP3078 on LG5 corresponding to rice chromosome 7, one marker (UGTP051) on LG4 corresponding to rice chromosome 1, and one locus (ICMP129) on LG7 that corresponded to rice chromosome 1.

Segregation distortion appears to be common with 203 of 468 mapped loci (43.3%) significantly deviated from the expected 1:1 segregation ratio at the 1% level (Table 3.2, Fig. 3.3, Fig. S3.2) with 62% (126 out 203) skewed towards Tift 99B and 38% (77 out 203) towards Tift 454. These loci that deviated were mainly mapped on LG1, LG2, LG3, and LG5, of which loci on LG2 and LG3 favored the female parental allele Tift 99B, and those on LG1and LG5 favored the male parental allele Tift 454. The loci that caused the distortion were distributed along a large segment of the LG and did not appear to be biased by the positions within the linkage group. For example, a 54 cM distorted segment was located in the centromeric region of the LG2, conversely, two distorted regions were located in telomeric regions of the LG5 (Fig. S3.2**)**. Since the distorted regions contain both AFLP and SSR loci, the clustering of distorted loci did not appear to be caused by the different types of markers.

Identification of QTLs for Agronomic Traits

The ANOVA results showed that all the traits analyzed had significant difference among RILs at 1% significance level. QTL analysis of all agronomic traits shows that the LOD values were below the critical thresholds to declare the significant QTLs for the traits of interest. But we detected 5 putative QTLs with LOD values greater than two. We reported these putative QTLs positions and phenotype variances that were explained by each of them (Table 3.4).

The plant height of Tift 99B, the female parent, was about 30 cm shorter than Tift 454, the male parent, in both 2006 and 2007 (Fig. 3.4A). In the 2007 dataset, one putative QTL was detected in LG5 between marker AFLP056 and AFLP295 with a LOD score of 2.4, explaining 6.0% of the phenotypic variation (Fig. 3.1, Table 3.4). The same position in this linkage group also showed an association in the 2006 dataset, but the LOD score (1.94) was narrowly below 2.

The differences in heading dates between the two parents were one and three days for the 2006 and 2007 data, respectively (Fig. 3.4B), with Tift 454, showing a wider range than that of Tift 99B. In the RIL population, the heading dates were about 10 days longer in 2007 (55.5 days) than in 2006 (45.8). One putative QTL was detected on LG7 between AFLP237 and AFLP328 with a LOD score of 2.49, explaining 7.2% of the phenotypic variation (Table 3.4). The same position in this linkage group also showed a putative association in 2006 dataset, but the LOD score was 1.8.

The panicle width of Tift 454 was one millimeter bigger than Tift 99B (Fig. 3.4C). One putative QTL was detected on LG3 between marker AFLP229 and AFLP261 with the LOD score of 2.6, explaining of 6.7% phonotypic variance (Fig. 3.1, Table 3.4).

Tift 99B had a greater hundred seed weight than Tift 454 with the difference of 0.12 g (Fig. 3.4D). One putative QTL was detected on LG4 between marker AFLP303 and AFLP113 with the LOD score of 2.3, explaining 5.9% of phenotypic variance (Fig. 3.1, Table 3.4).

The panicle length of Tift 454 was eight centimeters longer than Tift 99B (Fig. 3.4E). One putative QTL was detected on LG3 between marker AFLP211 and AFLP033 with a LOD score of 2.0, explaining 5.5% of phonotypic variance (Fig. 3.1, Table 3.4).

Discussion

EST-SSR Marker Composition

The tri-nucleotide SSRs (58.8%) were most abundant in this study, similar to other cereals (Kantety et al., 2002), but different from the study of Senthilvel et al. (2008) in pearl millet where the di-nucloetide SSRs were most frequent. The most abundant di-nucleotide was AG/CT motifs (21.1%), consistent with previous studies in other cereal EST-SSR (Kantety et al., 2002) and pearl millet (Senthilvel et al., 2008). The CG motif was not found in this study and was also the rarest in other studies (Kantety et al., 2002; Senthilvel et al., 2008). The largest section of tri-nucleotide motifs was GCC/GGC (11.5%), followed by CGC/GCG (10.7%). This is not consistent with the results of Senthilvel et al. (2008), in which AGC/CGT was the most abundant repeat motif (26.4%).

Marker Distribution in Pearl Millet Genetic Maps

The present genetic map, which contained 468 markers, spans 757 cM, and had an average distance of 1.62 cM between markers, represented the most detailed map for pearl millet

to date with more EST-based PCR molecular markers than previously published pearl millet genetic linkage maps. The original consensus map with 418 loci (Qi et al., 2004) is the only pearl millet map that has a comparable marker density. The other maps contain fewer than 200 markers (Devos et al., 2000; Liu et al., 1994; Pedraza-Garcia et al., 2010). The markers in our map distribute more evenly than these of the consensus map. About 85% of the markers of the consensus map are located in the proximal parts of linkage groups with big gaps that occur in distal regions and occupy about two thirds of the total map length (Qi et al., 2004). This map only has three big gaps of about 20 to 22 cM appearing in LG5 and LG6 distal regions. Compared to the other published maps (Devos et al., 2000; Liu et al., 1994; Pedraza-Garcia et al., 2010), our map shows clearer chromosome structures with heterochromatic regions where markers cluster and euchromatic regions where markers are evenly distributed. The first pearl millet genetic map (Liu et al., 1994) has fewer markers to distinguish the chromosome structure; the consensus map (Qi et al., 2004) shows too many gaps in euchromatic region and in the more recent map (Pedraza-Garcia et al., 2010), the markers are distributed evenly so no clear centromeric regions are evident.

Besides the AFLP markers, 107 SSR markers were mapped in this study, the biggest set of SSR markers that have ever been mapped in this species. Previously, 65, 27 and 67 SSRs were mapped by Qi et al. (2004), Senthilvel et al. (2004) and Pedraza-Garcia et al. (2010), respectively. Out of the 107 SSR markers, 66 are EST based markers. Three large EST clusters were present on LG2, LG3, and LG6, spanning 42, 36 and 61 cM, respectively. Previously, 21 EST-SSRs were mapped to distal region by Senthilvel et al. (2008) and one EST-SSR was mapped by Pedraza-Garcia et al. (2010). This latest pearl millet genetic linkage map provides new insights

into pearl millet genome structure, make available more SSR markers for QTLs and gene mapping, aid in map-based gene cloning, and assist in molecular breeding.

The nonrandom patterns of marker distribution across seven main linkage groups, due to marker clusters in the middle, and an even distribution with gaps in some distal regions, are clearly presented in our map. On every linkage group, at least one clear marker cluster is evident, some of these possibly corresponding to the centromeric regions. This may offer some clues on pearl millet genome organization. The marker clustering on centromere regions when AFLPs are used as markers in plant genetic mapping was observed especially when *Eco*RI/*Mse*I was used (Menz et al., 2002). The marker clustering was found to be associated with centromeres in *Arabidopsis* species (Alonso-Blanco et al., 1998) and may be associated with centromeres in soybean (Young et al., 1999), maize (Vuylsteke et al., 1999), barley (Becker et al., 1995), sorghum (Boivin et al., 1999), potato (Isidore et al., 2003), and tomato (Haanstra et al., 1999).

Centromeric marker clustering may also relate to the suppression of recombination at the centromere, and this phenomenon was also found using RFLP markers in mapping (Gill et al., 1993; Tanksley et al., 1992). The centromere suppression varies from chromosome to chromosome and does not totally depend on the distance to the centromere in wheat (Gill et al., 1996).

From the present map, we can estimate the proximate positions of the centromere of each linkage group. LG3, harboring the largest number of markers but with the shortest chromosome length, may correspond to the shortest chromosome of pearl millet. The shortest chromosome of pearl millet is a subterminal chromosome with the satellite on its short arm (Jauhar, 1981). LG3 of this present map was shortest and had two marker clusters near the bottom of our linkage

group. Knowledge of possible centromere organization could be important for future genetic research. For example knowing centremere locations would permit us to determine if the different SSR markers are on the same chromosome arm and may be helpful for developing a physical map and whole genome sequence assembly.

In addition to the marker clustering in the centromeric regions, big gaps were also presented in the distal region of the new map. Overall, 6 gaps extending 10-15 cM and 5 gaps extending 15-22 cM appeared in LG2, 4, 5, 6, and 7 without counting the two potential gaps for the two unlinked small linkage groups. Big gaps in the pearl millet genetic linkage maps are also observed in the consensus map (Qi et al., 2004), the map constructed by Senthilvel et al. (2008), the map by Pedraza-Garcia et al. (2010) and the map by Supriya et al. (2011). Notably in the consensus map, about 85% of the markers are located on about 30% of the length of the chromosomes. Qi et al. (2004) thought that the gaps were caused by high recombination rather than the lack of markers (Qi et al., 2004). Aligning the new map with the consensus map shows that some gaps are filled by the newly mapped markers, most of which are EST-SSRs. For example, the upper part of LG2 and lower part of LG6 have more markers than the consensus map; three markers (ICMP3048, ICMP 3043, and CTM08) filled the gap in LG7 in our map and the map by Senthilvel et al. (2008); LG3 had no gap larger than 10 cM in our map. This indicates that the big gaps in our map may still be caused by the lack of markers, but the possibility of the recombination hot spots cannot be excluded. In addition to high recombination and lack of markers, the big gaps may also relate to the genetic distance of the two parents used to develop the map population. The parents Tift 99B and Tifton 454 are elite inbred lines used to develop hybrid pearl millet lines, and they share a common ancestor Tift 23D. They at least share the dwarf genes in Tift 23D. Some gaps may be due to the lower amount of genetic polymorphisms

between the two parents. The smallest number of markers on LG4 (25 loci) may also be associated with Tift 23D. Big gaps on the sorghum consensus map (Mace et al., 2009) and the potato ultradense genetic map (van Os et al., 2006) are also thought have some relationship with the homozygosity of the parents as well.

Qi et al. (2004) stated that more markers in the distal regions of LG3 and LG5 in the consensus map, which are extremely short (about 30 and 40 cM, respectively), are expected to expand the maps in future. Our data fulfill the predictions. One EST-SSR cluster spanning 30 cM appears on the upper part of the LG3 in our map and extends the map length to 71.5 cM. Similar to LG3, an EST-SSR group on the lower part of the LG5 extends the map length from about 70 to 112 cM. Still, one small EST-SSR-containing group cannot be linked it to any main group; it probably is a distal region which needs a bridge to connect to its main group. Another two-marker linkage group belonged to the LG2 distal region towards the lower part of the chromosome according to the anchor markers in consensus map, but it did not link to the main group due to lack of markers between them.

Marker Segregation Distortion in Pearl Millet Genetic Maps

Forty-three percent of the markers in the present map showed significant segregation distortion ($P \leq 0.01$) and covered about one third of the total map length distributed on LG1, 2, 3, and 5. Overall, every linkage group of the pearl millet genome has been independently reported in at least two mapping populations to show segregation distortion regions (Table S3.5). For example, LG4 in six populations, LG1, LG2, LG4 and LG5 in five populations, LG3, LG6, and LG7 in two populations from all mapping populations including our mapping population show

segregation distortion (Busso et al., 1995; Liu et al., 1996; Liu et al., 1994; Qi et al., 2004; Supriya et al., 2011).

More interestingly, most of the segregation distortion regions located on the same linkage group in different mapping populations showed overlap even though the sizes of overlap regions are different. For example, in LG1, a significant segregation distortion region ($P \leq 0.01$) in our new map is between marker AFLP265 and AFLP337; Busso et al. (1995) reported a significant segregation distortion region ($P \leq 0.01$) from marker PSM223 to PSM607 in mapping population [(81B × ICMP 451) × BKM], that corresponds to the segregation distortion region in our map, ranges above PSMP347; Liu et al. (1996) reported segregation distortion regions from PSM565 to PSM360 in two mapping populations which likely correspond to our distortion region around marker PSMP347; Liu et al. (1994) presented a segregation distortion region between markers PSM652 to PSM196.1 and the distortion region was further extend to PSM756 (Liu, personal communication) in mapping population LGD-1-B-10 × ICMP 85410, which approximately corresponds to PSMP347 and below the segregation distortion region in our map. Qi et al. (2004) suggested that the segregation distortion in pearl millet was cross specific, but the overlap of the distortion regions in different mapping populations may indicate that some genetic factors cause the severe segregation distortion in pearl millet and is less likely caused by sampling errors.

The common segregation distortion regions that appeared in different mapping populations were also observed in sorghum (Mace et al., 2009). Segregation distortion is common in plant and animal genetic mapping research (Song et al., 2006; Taylor and Ingvarsson, 2003). In plants, marker type and population type were thought to have a relationship with segregation distortion (Song et al., 2006). Dominant markers reportedly were affected more than

co-dominant markers from the segregation distortion (Lorieux et al., 1995a; Lorieux et al., 1995b). Our results showed that both dominant AFLP markers and co-dominant SSR markers can present severe segregation distortions. In the first pearl millet genetic map constructed with RFLP markers, most markers are co-dominant and showed significant segregation distortion in 47 % of the markers (Liu et al., 1994). So, AFLPs may have an effect on the segregation distortion in this mapping population, but are not solely responsible.

RILs usually have severe segregation distortion due to environmental and artificial selection over several generations of self- fertilization (Wang et al., 2003). But the genetic maps of pearl millet using the F_2 population also have displayed severe distortion (Busso et al., 1995; Liu et al., 1994; Liu et al., 1996; Qi et al., 2004) while the pearl millet genetic map by Pedraza-Garcia et al. (2010) and Supriya et al. (2011) that used RIL populations showed 10% and 35% of the markers with segregation distortion, respectively. Therefore, the RILs alone cannot be the main reason for segregation distortion. Our results show that 43.5% of the markers showed significant segregation distortion. Such severe segregation distortion usually appears in interspecific populations (Kianian and Quiros, 1992), but previous pearl millet maps (Busso et al., 1995; Liu et al., 1994; Liu et al., 1996; Pedraza-Garcia et al., 2010; Qi et al., 2004; Supriya et al., 2011) and our map were all intraspecific crosses.

Distorted marker distribution in the genetic map for Liu et al. (1994) all skewed towards the less fit parent which was smaller in biomass and earlier flowering, while in the Supriya et al. (2011) map, distorted makers skewed towards both parents but with one parent having more distorted markers than the another. The distorted markers in this study skewed towards both parents with the female parent Tift 99B having more distorted markers than male parent Tift454.

The result is similar to Supriya et al. (2011). The different traits of fitness of the parents Tift 99B and Tift 454 are similar, for example, the biomass and flowering date are similar for the two parents.

The distorted distributions of segregating markers in the present map are clustered in certain chromosome regions, not distributed randomly along the chromosomes. This type of segregation distortion was considered to be caused by segregation distortion loci or segregation distorters (Lyttle, 1991; Song et al., 2006; Taylor and Ingvarsson, 2003). Distorters can be located on sex chromosomes and autosomes (Lyttle, 1991). Both Liu et al. (1994) and Pedraza-Garcia et al. (2010) thought that the genes that had affected fitness (seed dormancy causing failure of seed germination, and short seed filling period) were the causes of their segregation distortion. Consequently, any genetic elements that affect the reproduction system and individual development could have caused segregation distortion in our pearl millet mapping population. Seed dormancy and the flowering characteristics of pearl millet may also have some effect on segregation distortion. Pearl millet is usually out-crossed because of the protogyny, in which stigmas emerge earlier than anthers. Genes control the length of time for stigma receptivity, the time period between emergence of stigma and anther, and other aspects on flowering and fertilization and thus may also produce segregation distortions.

Map Alignment with Consensus Map

Alignment of our pearl millet genetic map agreed with the revised consensus map (we have added markers to it) except for a few inconsistencies that involved LG2, LG3, LG5, and LG7. In LG2, the marker order inversion involved CTM 21 and PSMS22, 0.6 cM apart. In LG3, PSMP2070 and PSMP2267 were inverted, 3.2 cM apart. In LG5, a marker inversion was

between ICMP3078 and CTM25 even though they could not be put on the consensus map because there were not enough neighboring markers on consensus map. The separation of the two markers was 8.5 cM in LG5 on our map compared to 22.9 cM on the map developed by Senthilvel et al. (2008), but the mapping software was different so they should not be compared by way of the distances with each other. In LG7, the position of ICMP3043 and CTM08 on our new map was not consistent with Senthilvel et al.'s results (2008) either, even though the estimated chromosome region is broadly the same. ICMP3043 and CTM08 were found near the middle of LG7 on our map, whereas ICMP3043 was also in the middle of Senghivel et al.'s map, but CTM08 was the last marker on the end of the chromosome. The map distance between marker ICMP3043 and CTM08 was 97.5 cM on Senthilvel et al.'s map, but the distance on our map was only 7 cM; however, Senthilvel et al.'s map (187.2 cM) was much longer than ours (132.8 cM). All inconsistencies need to be confirmed in future, but these large inconsistencies noted here may lead to other very interesting genetic events. Closely linked marker flips were also observed when the alignment was conducted in different maps in pearl millet (Supriya et al., 2011) and in other crops (Feltus et al., 2006b; Mace et al., 2009; Wu and Huang, 2007). These marker order differences may be real, or may be due to scoring error in either of the populations being compared, or perhaps the map software cannot statistically distinguish the marker order at the cM-scale because of the small size of mapping populations (Mace et al., 2009).

The map position of PSMS08, PSMS36, and PSMS78 were mapped in LG2, LG3, and LG6 on our map but were previously mapped on LG3, LG7, and LG2, respectively (Devos, unpublished data). These markers are originally single-strand conformational polymorphism (SSCP) markers used to detect single nucleotide polymorphism and sequence-length polymorphism caused by insertion and deletion (Bertin et al., 2005). The electrophoresis system

we used cannot detect the SSCP, but can only detect DNA fragment size differences. Since we detected multiple sequence fragments for these markers, it was likely that our mapped loci were different from previously mapped loci (Devos, unpublished data).

EST-SSR marker ICMP3029 (ori) (Senthilvel et al., 2004) and ICMP3029 (Senthilvel et al., 2008) were developed from the same SSR-contained EST sequence, but the primer sequences are different. The ICMPA3029 (ori) in our map was mapped in LG2, but the ICMP3029 was mapped in LG4 by Supriya et al. (2011). Because ICMP3029 (ori) detect multiple bands, so it is probably that two map effects mapped different locus of the same EST sequence.

Our last inconsistency is very interesting, we only detected two co-segregating bands for PSMP2229 and the marker was mapped on LG3, but apparently two loci have been mapped on LG5 and LG7, respectively, on the consensus map (Qi et al., 2004). This marker position difference needs to be further clarified and confirmed.

Comparative Relationship with Rice Genome Predicted by EST-SSR Function

EST-SSR functions were predicted by executing BLASTN against the Rice Annotation Project (RAP) database. The homologous relationships between the pearl millet and rice genomes resolved here were largely consistent with the comparative mapping result shown by Devos et al. (2000). The conserved relationship among the grass family will greatly facilitate the research of an orphan crop like pearl millet using the information from other members of the grass family such as rice (Feltus et al., 2006a). But some EST-SSR markers showed new homologous loci that were not reported by Devos et al. (2000). For example, locus PSMS89 (E value=e^{-64}) and ICMP3029 (E value = e^{-110}) in LG2 corresponded to rice chromosome 8 and

was not presented on the Devos comparative map (2000); but the two loci are separated by other loci correspondent to another rice chromosome. This may reveal the real chromosome position because this phenomenon was further demonstrated by Devos et al. (2000), but it may simply be map error. CUMP18 (E value = $8e^{-30}$) and IMCP3078 (E value = $7e^{-65}$) in LG5 were homologous to rice chromosome 7, and these two loci are closely linked without any markers found in between them. UGTP051 (E value = $9e^{-7}$) in LG4 corresponds to rice chromosome 1, but is the only marker on the linkage group that shows this homologous relationship; and the E value is rather high , so the relationship needs to be further confirmed in the future. New homologous relationship was also noticed in other pearl millet genetic mapping (Sehgal et al., 2012). These new findings present further supporting evidence that the pearl millet genome is highly rearranged compared to other members of the grass family (Devos et al., 2000), and they also indicate that caution is needed in applying comparative information from the members of the grass family to pearl millet.

QTLs for Agronomic Traits

QTL analysis of all agronomic traits shows that the LOD values were below the critical thresholds determined by permutation test to declare the significant QTLs and phenotype variances that were explained (less than 10%) by each QTL were small (Table 3.4). The differences are minimal between the two parents for the traits presented here, but are within the ranges expected for cultivated pearl millet inbreds in the United States. For these traits, there is no major gene segregation between the two parents, and consequently, no major QTLs were detected. For example, both Tift 454 and Tift 99B possess the d_2 dwarfing gene came from the shared ancestor Tift 23D2 (Johnson Jr et al., 1968). The height differences are the result of a

QTL having a smaller effect than d_2, but having commercial significance since d_2 is fixed within most commercial breeding populations in the U.S. Even though these QTLs are only suggestive, they still provide some useful information for pearl millet genetic research and improvement.

The map position of the QTL for heading date (Table 3.4) was on a similar chromosome region on LG7 as a minor QTL detected by Poncet et al. (2000). The QTLs for plant height, panicle length, panicle width, and hundred seed weight are different loci from previously mapped QTLs. For example, the presently described QTL for plant height was mapped on LG5, but QTLs were previously mapped on LG6 and LG7 by Poncet et al. (2000), and a gene was also mapped on LG1 by Azhaguvel et al. (2003). The QTL that we presented for hundred seed weight was mapped in LG4, while QTLs for hundred seed weight were previously mapped in LG1, 2, 3, and 6 (Poncet et al., 2000). The QTLs for panicle length and panicle width were both mapped onto LG3 in this study, but QTLs for panicle length were previously found in LG1, 2, and 7, and QTLs for panicle width were previously mapped in LG2, 5, and 7 (Poncet et al., 2000).

Future research is needed to determine if the discrepancies in the QTL positions we identified with the previous research are mostly from population differences. Different parents could easily provide different QTLs for the traits of interest, which superficially would appear to be discrepancies. Given the parental relationships along with the percent of phenotypic variance explained and the LOD values, this population clearly demonstrates that QTL analysis can work in closely related intravarietal populations. It also demonstrates the utility of this map within the new consensus map in using the markers in marker-assisted selection.

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Table 3.1. SSR primers used for the pearl millet genetic map

Name	Screening pairs	Polymorphic pairs	Polymorphic loci	Mapped primers	Mapped loci	(%) Percent polymorphism	Primer type	Source
ICMP	112	15	16	13	14	13.39	EST-SSR	Senthilvel et al., 2004, 2008
UGTP	169	33	36	29	30	19.53	EST-SSR	This study
PSMP	55	9	10	9	9	16.36	RFLP-STS	Tracy Money (personal communication)
PSMP	103	32	34	30	32	31.07	GENOMIC SSR	Qi et al., 2004
CTM	19	5	5	5	5	26.32	GENOMIC SSR	Budak et al., 2003
PSMS	102	12	12	11	11	11.76	SSCP	Bertin et al., 2005
SRSC	96	5	5	3	3	5.21	CONSERVED INTRON	Feltus et al., 2006
PCISP	43	2	2	2	2	4.65	CONSERVED INTRON	Charlie T.Hash (personal communication)
CUMP	19	1	1	1	1	5.26	EST-SSR	Yadav et al., 2007
Total	718	114	121	102	107	14.84		

Table 3.2. Mapped markers for pearl millet linkage group 1 to 7

Chromosome	Total loci	Total SSR loci (anchor loci)	Newly mapped SSR loc*	Length (cM)	Average interval (cM)
LG1	77	14(6)	8	97.9	1.27
LG2	72	25(7)	18	97.2	1.35
LG3	103	14(3)	11	75.5	0.73
LG4	25	6(2)	4	80.3	3.21
LG5	61	12(8)	4	112.3	1.84
LG6	67	12(2)	10	138.9	2.07
LG7	59	21(14)	10	132.8	2.25
Unlinked A	2	1(0)	1	8.4	4.18
LG2 unlinked	2	2(2)	0	13.7	6.84
Total	468	107(44)	63	757.0	1.62

*Not previously mapped loci

Table 3.3. Marker segregation distortion for pearl millet genetic map

Linkage group	Marker number	Segregation distortion (α=0.01)		No segregation distortion	
		Number	%	Number	%
LG1	77	57	74.0	20	26.0
LG2	72	33	45.8	39	54.2
LG3	103	92	89.3	11	10.7
LG4	25	3	12.0	22	88.0
LG5	61	18	29.5	43	70.5
LG6	67	0	0.0	67	100
LG7	59	0	0.0	59	100
Unlinked A	2	0	0.0	2	100.0
LG2 unlinked	2	0	0.0	2	100.0
Total	468	203	43.3	209	56.7

Table 3.4 QTLs influencing heading date (Head), plant height (PH), head width (HW), head Length (HL), and hundred seed weight (HSW)

Trait*	Year	QTL	LG	Interval	Nearest SSR marker (cM away from the peak)	LOD	Additive†	% Var ‡
Head	2007	head7	7	AFLP237-AFLP328	PSMP2087(15.0)	2.49	-1.67	7.2
		Head5	5	AFLP132-CTM25	PSMP2274(0)	1.99	1.33	5.0
	2006	Head7	7	AFLP325-AFLP328	PSMP2087(11.0)	1.8	-1.08	4.7
PH	2007	ph5	5	AFLP056-AFLP295	PSMP2274(1.4)	2.41	3.88	6.0
	2006	ph5	5	CTM25-AFLP295	PSMP2274(2.5)	1.94	3.30	4.9
HW	2007	hw3	3	AFLP223-AFLP358	PSMP2229(0.8)	2.31	-0.71	6.1
HL	2007	hl3	3	AFLP033-AFLP375	PSMP2229(7.9)	2.27	-0.82	5.9
HSW	2007	hsw4	4	AFLP303-AFLP113	UGTP063(8.0)	2.32	0.04	5.9

*Width and length of only the primary heads were measured in 2007, and hundred seed weight only measured in only 2007.

† A positive sign of the additive effect indicates that the allele of Tift 99B is associated with increased value of the trait.

‡ Percent phenotypic variance explained by the QTL.

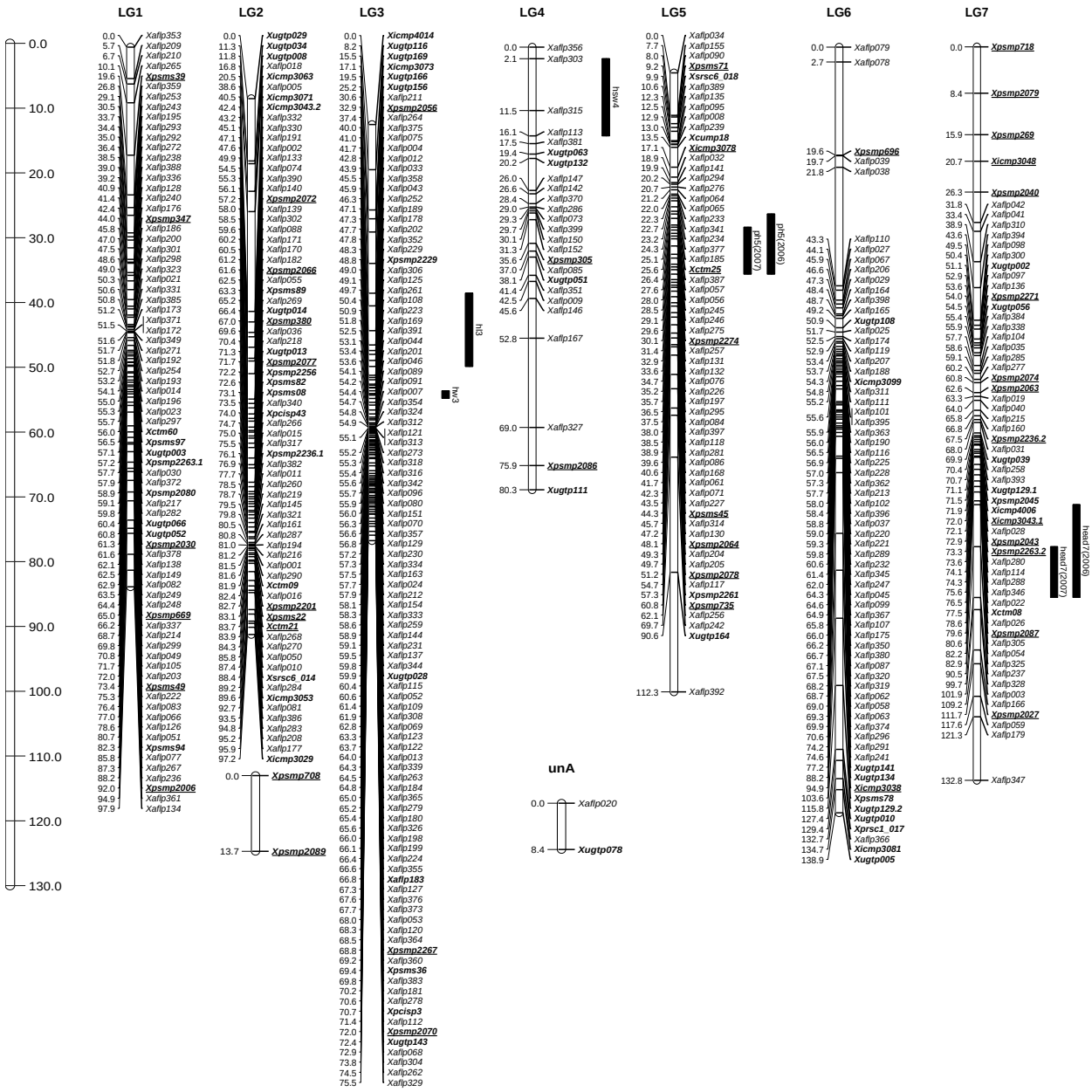

Figure 3.1. A genetic linkage map of pearl millet [*Pennisetum glaucum* (L.) R. Br.] developed from 180 RILs derived from inbred lines Tift 99B and Tift 454. Marker distances (cM) are described on the left of the linkage groups. SSR markers are indicated in bold with underlined loci being previously mapped chromosome anchor markers. The QTL 2 LOD intervals for the agronomy traits are shown as bars on the right side of the linkage group. The right bar shows the length of Linkage group in cM.

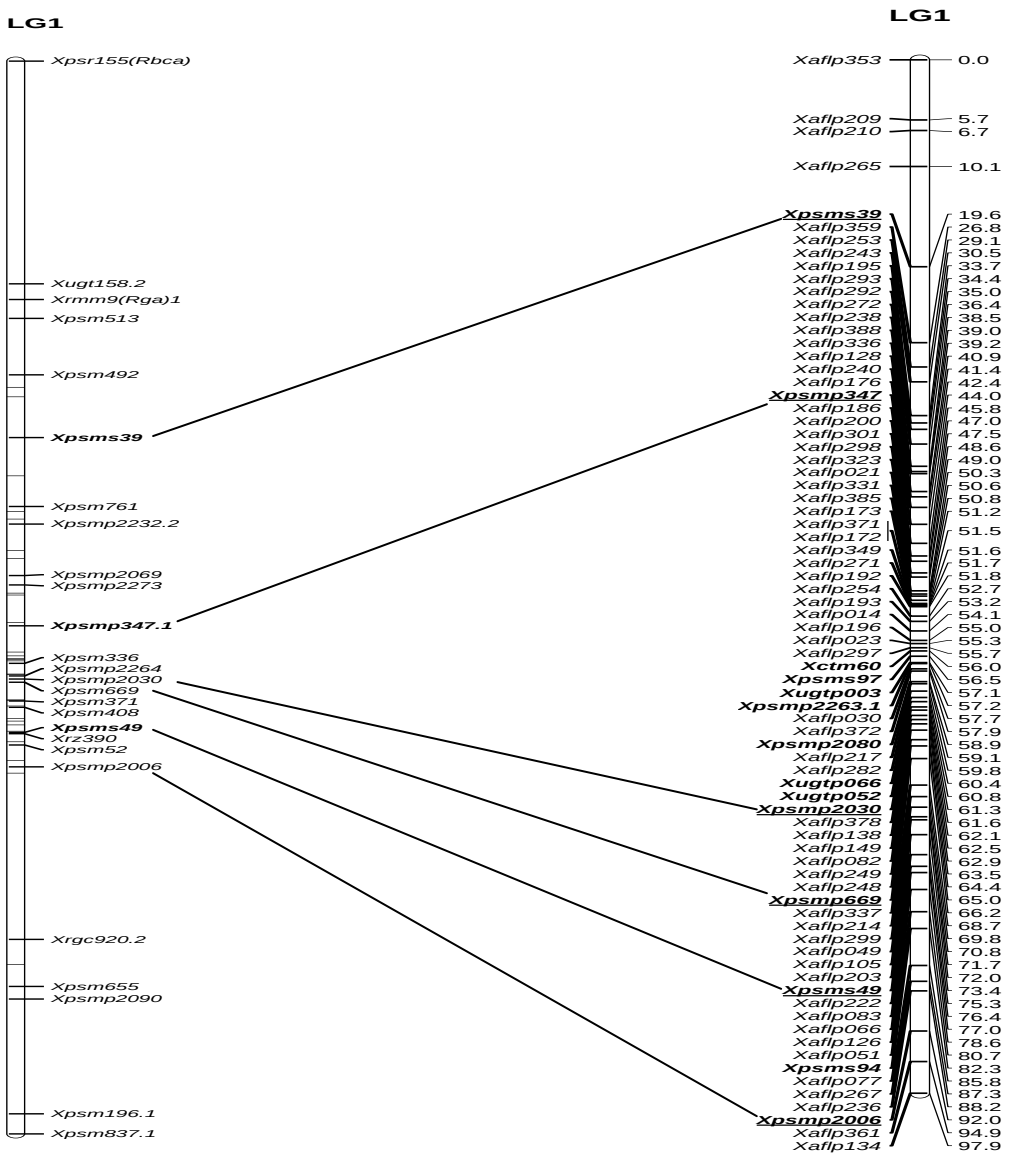

Figure 3.2. Alignment of newly developed pearl millet genetic map LG1 (right) with the pearl millet consensus map (left) (Qi et al., 2004). The consensus map LG1 was redrawn according to the original consensus map. Additional SSRs (in bold) not present in the consensus map were inferred back on the published data of Senthilvel et al. (2008) or from previously unpublished data of K. Devos et al.

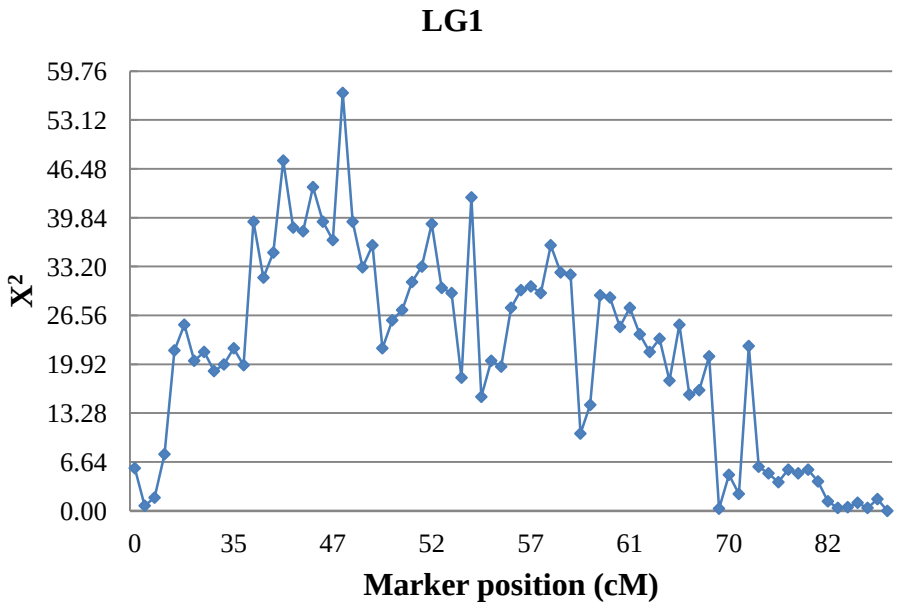

Figure 3.3. Segregation distortion in the LG1. X-axis shows marker position in cM, and Y-axis shows chi-squared values of the markers. χ^2 (0.01, 1) = 6.64. Chi-squared values greater than 6.64 mean significant distorted at 1% level.

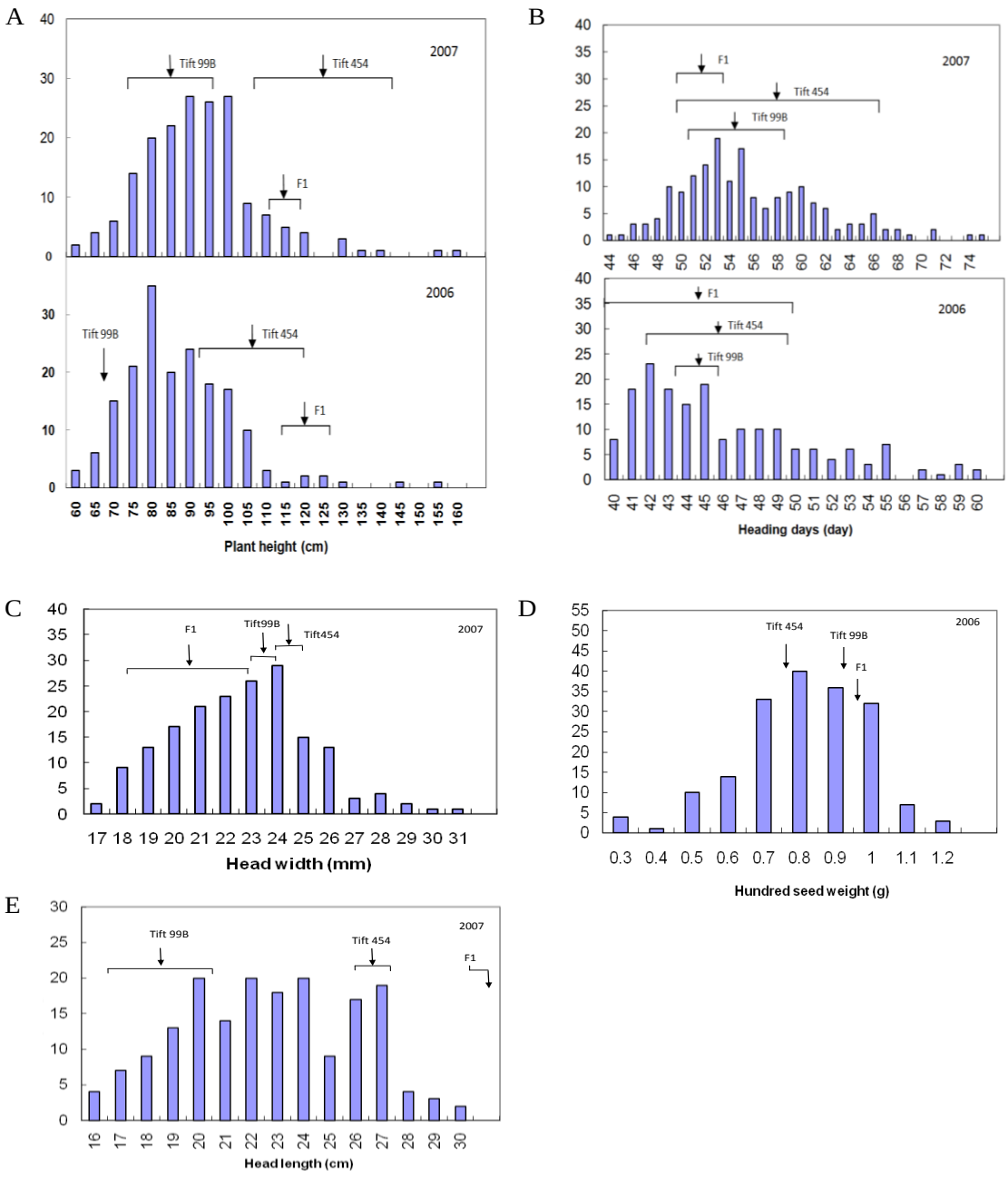

Figure 3.4. Frequency distribution of A: plant height, B: heading days, C: panicle width, D: hundred seed weight, and E: panicle length in the RILs mapping population derived from parent Tift 99B and Tift 454. Arrows show mean ± standard deviation

Supplemental tables and figures

Table S3.1. AFLP codes in genetic map correspond to original fragments

Code in map	Fragment	Code in map	Fragment
AFLP1	MCAT-EAAC-270	AFLP50	MCAT-EACG-379
AFLP2	MCAT-EAAC-345	AFLP51	MCAT-EACG-437
AFLP3	MCAT-EAAC-360	AFLP52	MCAT-EACG-443
AFLP4	MCAT-EAAC-455	AFLP53	MCAT-EACG-453
AFLP5	MCAT-EAAG-80	AFLP54	MCAT-EACG-486
AFLP6	MCAT-EAAC347	AFLP55	MCAT-EACT-104
AFLP7	MCAT-EAAG-110	AFLP56	MCAT-EACT-106
AFLP8	MCAT-EAAG-125	AFLP57	MCAT-EACT-108
AFLP9	MCAT-EAAG-190	AFLP58	MCAT-EACT-143
AFLP10	MCAT-EAAC348	AFLP59	MCAT-EACT-164
AFLP11	MCAT-EAAG-235	AFLP60	MCAT-EACT-175
AFLP12	MCAT-EAAG-250	AFLP61	MCAT-EACT-181
AFLP13	MCAT-EAAG-320	AFLP62	MCAT-EACT-218
AFLP14	MCAT-EAAG-440	AFLP63	MCAT-EACT-221
AFLP15	MCAT-EAAG-448	AFLP64	MCAT-EACT-266
AFLP16	MCAT-EAAG-525	AFLP65	MCAT-EACT-277
AFLP17	MCAT-EAAG-528	AFLP66	MCAT-EACT-326
AFLP18	MCAT-EACA-65	AFLP67	MCAT-EACT-354
AFLP19	MCAT-EACA-80	AFLP68	MCAT-EACT-356
AFLP20	MCAT-EACA-99	AFLP69	MCAT-EACT-384
AFLP21	MCAT-EACA-253	AFLP70	MCAT-EACT-204
AFLP22	MCAT-EACA-258	AFLP71	MCAT-EACT-173
AFLP23	MCAT-EACA-303	AFLP72	MCAT-EACT-75
AFLP24	MCAT-EACA-313	AFLP73	MCAT-EAGC-121

AFLP25	MCAT-EACA-330	AFLP74	MCAT-EAGC-123
AFLP26	MCAT-EACA-343	AFLP75	MCAT-EAGC-136
AFLP27	MCAT-EACA-393	AFLP76	MCAT-EAGC-235
AFLP28	MCAT-EACA-395	AFLP77	MCAT-EAGC-295
AFLP29	MCAT-EACC-78	AFLP78	MCAT-EAGC-400
AFLP30	MCAT-EACC-90	AFLP79	MCAT-EAGC-404
AFLP31	MCAT-EACC-138	AFLP80	MCAT-EAGC-440
AFLP32	MCAT-EACC-154	AFLP81	MCAT-EAGG-86
AFLP33	MCAT-EACC-175	AFLP82	MCAT-EAGG-106
AFLP34	MCAT-EACC-193	AFLP83	MCAT-EAGG-324
AFLP35	MCAT-EACC-213	AFLP84	MCAT-EAGG-340
AFLP36	MCAT-EACC-216	AFLP85	MCAA-EAAC-88
AFLP37	MCAT-EACC-218	AFLP86	MCAA-EAAC-142
AFLP38	MCAT-EACC-235	AFLP87	MCAA-EAAC-175
AFLP39	MCAT-EACC-251	AFLP88	MCAA-EAAC-211
AFLP40	MCAT-EACC-286	AFLP89	MCAA-EAAC-218
AFLP41	MCAT-EACC-342	AFLP90	MCAA-EAAC-257
AFLP42	MCAT-EACC-346	AFLP91	MCAA-EAAC-289
AFLP43	MCAT-EACC-364	AFLP92	MCAA-EAAC-303
AFLP44	MCAT-EACC-410	AFLP93	MCAA-EAAC-305
AFLP45	MCAT-EACC-509	AFLP94	MCAA-EAAC-325
AFLP46	MCAT-EACC-523	AFLP95	MCAA-EAAC-329
AFLP47	MCAT-EACG-219	AFLP96	MCAA-EAAC-344
AFLP48	MCAT-EACG-236	AFLP97	MCAA-EAAC-520
AFLP49	MCAT-EACG-361	AFLP98	MCAA-EAGC-82
AFLP99	MCAA-EAGC-86	AFLP149	MCAC-EACA-212
AFLP100	MCAA-EAGC-180	AFLP150	MCAC-EACA-360
AFLP101	MCAA-EAGC-184	AFLP151	MCAC-EACA-361

AFLP102	MCAA-EAGC-185	AFLP152	MCAC-EACA-362
AFLP103	MCAA-EAGC-190	AFLP153	MCAC-EACA-384
AFLP104	MCAA-EAGC-211	AFLP154	MCAC-EACA-386
AFLP105	MCAA-EAGC-250	AFLP155	MCAC-EACA-429
AFLP106	MCAA-EAGC-280	AFLP156	MCAC-EACA-459
AFLP107	MCAA-EAGC-294	AFLP157	MCAC-EACA-388
AFLP108	MCAA-EAGC-426	AFLP158	MCAA-EAGC-433
AFLP109	MCAA-EAGC-428	AFLP159	MCAA-EAGC-434
AFLP110	MCAA-EAGC-252	AFLP160	MCAC-EACC179
AFLP111	MCAA-EAGC-262	AFLP161	MCAC-EACC213
AFLP112	MCAC-EAAC-75	AFLP162	MCAC-EACC243
AFLP113	MCAC-EAAC-85	AFLP163	MCAC-EACC248
AFLP114	MCAC-EAAC-104	AFLP164	MCAC-EACC317
AFLP115	MCAC-EAAC-218	AFLP165	MCAC-EACC349
AFLP116	MCAC-EAAC-228	AFLP166	MCAC-EACC351
AFLP117	MCAC-EAAC-232	AFLP167	MCAC-EACC400
AFLP118	MCAC-EAAC-271	AFLP168	MCAC-EACC464
AFLP119	MCAC-EAAC-273	AFLP169	MCAC-EACC479
AFLP120	MCAC-EAAC-321	AFLP170	MCAC-EACC485
AFLP121	MCAC-EAAC-390	AFLP171	MCAC-EACC490
AFLP122	MCAC-EAAC-416	AFLP172	MCAC-EACC508
AFLP123	MCAC-EAAC-417	AFLP173	MCAC-EACC510
AFLP124	MCAC-EAAC-272	AFLP174	MCAC-EACC594
AFLP125	MCAC-EAAC-448	AFLP175	MCAC-EACC600
AFLP126	MCAC-EAAC-461	AFLP176	MCAC-EACC602
AFLP127	MCAC-EAAC-482	AFLP177	MCAC-EACT94
AFLP128	MCAC-EAAC-483	AFLP178	MCAC-EACT124
AFLP129	MCAC-EAAC-504	AFLP179	MCAC-EACT192

AFLP130	MCAC-EAAC-590	AFLP180	MCAC-EACT226
AFLP131	MCAC-EAAC-621	AFLP181	MCAC-EACT251
AFLP132	MCAC-EAAC-646	AFLP182	MCAC-EACT257
AFLP133	MCAC-EAAG-88	AFLP183	MCAC-EACT260
AFLP134	MCAC-EAAG-121	AFLP184	MCAC-EACT362
AFLP135	MCAC-EAAG-127	AFLP185	MCAC-EAGC141
AFLP136	MCAC-EAAG-213	AFLP186	MCAC-EAGC163
AFLP137	MCAC-EAAG-241	AFLP187	MCAC-EAGC215
AFLP138	MCAC-EAAG-257	AFLP188	MCAC-EAGC224
AFLP139	MCAC-EAAG-324	AFLP189	MCAC-EAGC274
AFLP140	MCAC-EAAG-326	AFLP190	MCAC-EAGC290
AFLP141	MCAC-EACA-79	AFLP191	MCAC-EAGC319
AFLP142	MCAC-EACA-106	AFLP192	MCAC-EAGC354
AFLP143	MCAC-EACA-152	AFLP193	MCAC-EAGC400
AFLP144	MCAC-EACA-161	AFLP194	MCAC-EAGC404
AFLP145	MCAC-EACA-165	AFLP195	MCAC-EAGG146
AFLP146	MCAC-EACA-182	AFLP196	MCAC-EAGG200
AFLP147	MCAC-EACA-191	AFLP197	MCAC-EAGG203
AFLP148	MCAC-EACA-193	AFLP198	MCAC-EAGG221
AFLP199	MCAC-EAGG222	AFLP250	MCAG-EAGC92
AFLP200	MCAC-EAGG231	AFLP251	MCAG-EAGC145
AFLP201	MCAC-EAGG247	AFLP252	MCAG-EAGC272
AFLP202	MCAC-EAGG425	AFLP253	MCAG-EAGC281
AFLP203	MCAC-EAGG478	AFLP254	MCAG-EAGC398
AFLP204	MCAC-EAGG610	AFLP255	MCAG-EAGC504
AFLP205	MCAC-EAGG623	AFLP256	MCAG-EAGC668
AFLP206	MCAG-EAAC102	AFLP257	MCAG-EAGG79
AFLP207	MCAG-EAAC115	AFLP258	MCAG-EAGG121

AFLP208	MCAG-EAAC224	AFLP259	MCAG-EAGG131
AFLP209	MCAG-EAAC273	AFLP260	MCAG-EAGG136
AFLP210	MCAG-EAAC299	AFLP261	MCAG-EAGG178
AFLP211	MCAG-EAAC301	AFLP262	MCAG-EAGG249
AFLP212	MCAG-EAAC382	AFLP263	MCAG-EAGG397
AFLP213	MCAG-EAAC422	AFLP264	MCTA-EACT79
AFLP214	MCAG-EACA56	AFLP265	MCTA-EACT87
AFLP215	MCAG-EACA86	AFLP266	MCTA-EACT92
AFLP216	MCAG-EACA110	AFLP267	MCTA-EACT115
AFLP217	MCAG-EACA240	AFLP268	MCTA-EACT120
AFLP218	MCAG-EACA302	AFLP269	MCTA-EACT168
AFLP219	MCAG-EACA316	AFLP270	MCTA-EACT257
AFLP220	MCAG-EACA366	AFLP271	MCTA-EACT269
AFLP221	MCAG-EACA380	AFLP272	MCTA-EACT280
AFLP222	MCAG-EACA520	AFLP273	MCTA-EACT300
AFLP223	MCAG-EACC68	AFLP274	MCTA-EACT401
AFLP224	MCAG-EACC171	AFLP275	MCTA-EAGC115
AFLP225	MCAG-EACC275	AFLP276	MCTA-EAGC149
AFLP226	MCAG-EACC319	AFLP277	MCTA-EAGC167
AFLP227	MCAG-EACC437	AFLP278	MCTA-EAGC169
AFLP228	MCAG-EACC447	AFLP279	MCTA-EAGC187
AFLP229	MCAG-EACC515	AFLP280	MCTA-EAGC197
AFLP230	MCAG-EACC583	AFLP281	MCTA-EAGC278
AFLP231	MCAG-EACC727	AFLP282	MCTA-EAGC298
AFLP232	MCAG-EACC735	AFLP283	MCTA-EAGC306
AFLP233	MCAG-EACG87	AFLP284	MCTA-EAGC375
AFLP234	MCAG-EACG98	AFLP285	MCTA-EAGC495
AFLP236	MCAG-EACG112	AFLP286	MCTA-EAGC530

AFLP237	MCAG-EACG202	AFLP287	MCTA-EAGC572
AFLP238	MCAG-EACG238	AFLP288	MCTA-EAGC584
AFLP239	MCAG-EACG268	AFLP289	MCTA-EAGC625
AFLP240	MCAG-EACG316	AFLP290	MCTA-EACC138
AFLP241	MCAG-EACG354	AFLP291	MCTA-EACC196
AFLP242	MCAG-EACG382	AFLP292	MCTA-EACC286
AFLP243	MCAG-EACG475	AFLP293	MCTA-EACC301
AFLP244	MCAG-EACG505	AFLP294	MCTA-EACC334
AFLP245	MCAG-EACG512	AFLP295	MCTA-EACC574
AFLP246	MCAG-EACG514	AFLP296	MCTA-EAAG81
AFLP247	MCAG-EAGC68	AFLP297	MCTA-EAAG90
AFLP248	MCAG-EAGC77	AFLP298	MCTA-EAAG115
AFLP249	MCAG-EAGC81	AFLP350	MCTC-EACT105
AFLP300	MCTA-EAAG332	AFLP299	MCTA-EAAG174
AFLP301	MCTA-EAGG107	AFLP351	MCTC-EACT113
AFLP302	MCTA-EAGG122	AFLP352	MCTC-EACT189
AFLP303	MCTA-EAGG155	AFLP353	MCTC-EACT223
AFLP304	MCTA-EAGG168	AFLP354	MCTC-EACT245
AFLP305	MCTA-EAGG193	AFLP356	MCTC-EACT302
AFLP306	MCTA-EAGG213	AFLP357	MCTC-EACT304
AFLP307	MCTA-EAGG296	AFLP358	MCTC-EACT319
AFLP308	MCTA-EAGG302	AFLP359	MCTC-EACT406
AFLP309	MCTA-EAGG311	AFLP360	MCTG-EAAC76
AFLP310	MCTA-EAGG313	AFLP361	MCTG-EAAC101
AFLP311	MCTA-EAGG347	AFLP362	MCTG-EAAC139
AFLP312	MCTA-EAGG383	AFLP363	MCTG-EAAC230
AFLP313	MCTA-EAGG502	AFLP364	MCTG-EAAC243
AFLP314	MCTA-EAGG513	AFLP365	MCTG-EAAC253

AFLP315	MCTA-EAGG523	AFLP366	MCTG-EAAC296
AFLP316	MCTC-EAAC62	AFLP367	MCTG-EAAC300
AFLP317	MCTC-EAAC207	AFLP368	MCTG-EAAC385
AFLP318	MCTC-EAAC247	AFLP369	MCTG-EAAC387
AFLP319	MCTC-EAAC289	AFLP370	MCTG-EAAC401
AFLP320	MCTC-EAAC292	AFLP371	MCTG-EAAC431
AFLP321	MCTC-EAAC301	AFLP372	MCTG-EAGG105
AFLP322	MCTC-EAAC438	AFLP373	MCTG-EAGG145
AFLP323	MCTC-EAAC641	AFLP374	MCTG-EAGG225
AFLP324	MCTC-EACA60	AFLP375	MCTG-EAGG278
AFLP325	MCTC-EACA94	AFLP376	MCTG-EAGG460
AFLP326	MCTC-EACA115	AFLP377	MCTG-EAGG507
AFLP327	MCTC-EACA136	AFLP378	MCTT-EAGC83
AFLP328	MCTC-EACA145	AFLP379	MCTT-EAGC89
AFLP329	MCTC-EACA160	AFLP380	MCTT-EAGC115
AFLP330	MCTC-EACA161	AFLP381	MCTT-EAGC180
AFLP331	MCTC-EACA228	AFLP382	MCTT-EAGC233
AFLP332	MCTC-EACA281	AFLP383	MCTT-EAGC267
AFLP333	MCTC-EACA350	AFLP384	MCTT-EAGC333
AFLP334	MCTC-EACA495	AFLP385	MCTT-EAGC376
AFLP335	MCTC-EACA560	AFLP386	MCTT-EAGC567
AFLP336	MCTC-EACC80	AFLP387	MCTT-EAGC612
AFLP337	MCTC-EACC82	AFLP388	MCTT-EAGG106
AFLP338	MCTC-EACC209	AFLP389	MCTT-EAGG123
AFLP339	MCTC-EACC265	AFLP390	MCTT-EAGG172
AFLP340	MCTC-EACC337	AFLP391	MCTT-EAGG184
AFLP341	MCTC-EACC374	AFLP392	MCTT-EAGG194
AFLP342	MCTC-EACG193	AFLP393	MCTT-EAGG223

AFLP344	MCTC-EACG238	AFLP394	MCTT-EAGG231
AFLP345	MCTC-EACG264	AFLP395	MCTT-EAGG260
AFLP346	MCTC-EACG362	AFLP396	MCTT-EAGG261
AFLP347	MCTC-EACG364	AFLP397	MCTT-EAGG264
AFLP348	MCTC-EACG495	AFLP398	MCTT-EAGG364
AFLP349	MCTC-EACT79	AFLP399	MCTT-EAGG456

Table S3.2. Detailed information of new development pearl millet ESR-SSRs

SSR name	contig name	Forward sequence (5'- 3')	Reverce sequence (5' – 3')	SSR motif	Expected size (bp)	Tm(℃)		PIC
						Forward	Reverse	
UGTP001	BC8_c571	GAACGACACAATTCAAAGTAGATTA	CGGCTTTTCTGTATGTATTGTAGG	(acat)6	106	58	61.2	0.247
UGTP002	BC8_c11022	AGTTGCTCCGGGTTTGTTGTT	GCATCCATAATCAGTCACTTTCA	(aatg)5	128	60.6	59.2	0.6404
UGTP003	BC8_c23193	AGGTTGCTAAAGCTACTGATGTTA	GCCTCTGTGTGATATGTTATTTGTC	(tga)11	167	59.4	61.3	0.372
UGTP004	BC8_c7308	TGTAGGCTATCAATATTATGAGTGG	AACGACAAACACTCTTCATTCAT	(tgaa)5	96	59.7	57.4	0.000
UGTP005	BC8_c4325	TCTTTTTTGGTAAAATGTGTGTACA	AACCGAGTCTTTCTTTCTTTCTAC	(tg)9	106	56.4	59.4	0.222
UGTP006	BC8_c6277	ACACTAAATCTAAACAACACAAGGG	TTTTTTACATCGATCCATGGA	(atcc)6	148	59.7	54.8	0.351
UGTP007	BC8_c9591	GTCTATTTGAGCTCAGCATTTCA	CACACACATATACTCAGCAGTCAG	(atc)6	147	59.2	62.9	0.332
UGTP008	BC8_c19602	CACTTAGGAACGTAGCATCTATGT	CTGCTGAACATGATAAATCAATATG	(cat)7	97	61.2	58	0.482
UGTP010	BC8_c11699	GCCTTTTGATTCGACCGT	AAACGTACCGGTTTCGAATT	(tgta)6	92	57.6	56.3	0.513
UGTP011	BC8_c3554	CATCCGCATATCGAAAGATT	TTGAGATGCATCAGGCGA	(attc)11	100	56.3	57.6	0.314
UGTP013	BC8_c7848	CAAGGTTGTACCACGCTTTG	CACAACAATCACTTGCATCATC	(atg)6	104	60.4	58.9	0.305
UGTP014	BC8_c8675	ATGGAATGGACGGTATCGGA	CGTACTACGTACCATAACAACAACA	(tgt)6	102	60.4	61.3	0.332
UGTP015	BC8_c8555	GTACAGTGGTCAGCAGCAGATGT	CCGAAGGCTAATCAACAATCA	(tg)11	133	64.6	58.7	0.332
UGTP018	BC8_c10024	TGTGAGGAGTTTGCTTTCCC	GCAAATCTTTAGTGCACACACG	(atcc)7	99	60.4	60.8	0.000
UGTP019	BC8_c11861	CTTCCTTTGGCCACCTCTTT	CAAGCCATCAAAGAACACATTC	(tttc)5	82	60.4	58.9	0.332
UGTP020	BC8_c5248	AGACCCAAGACCGACCGGT	GCCCCCACCAGCTAGCTA	(agct)7	107	64.5	64.5	0.372
UGTP021	BC8_c154	GTGCTGCTGGTGTGTTGTAAG	CATCATCCTGAGATTAATCGACC	(tgc)6	116	62.6	55.8	0.000

UGTP022	BC8_c20318	CTGCTGCTTCTCAGACTGAGA	CCACATTAGCATCATCCTGAGAT	(tgc)6	83	62.6	61	0.332
UGTP023	BC8_c18657	AACCTTCCTTCCTTCCTTCC(51.3)	GATGAACCTGCACACCGA(51.3)	(cctt)5	114	60.4	59.9	0.346
UGTP024	BC8_c497	GCTTACGGCGGCCGTGGA	TCCTCCAGTTCCCACCGTCG	(gcg)6	103	66.7	66.6	0.000
UGTP025	BC8_c17400	GGAAGAAATTAGGAGGACAAGG	GACAGGTACACATACACCCCAT	(tgg)7	150	60.8	62.7	0.000
UGTP026	BC8_c6899	CACTTCCTTCCTTGCTCTTC	CAGAAGGAGGAGAAGGTTGATG	(tct)6	88	60.4	62.7	0.000
UGTP027	BC8_c5875	CTGTTCGTGGTGATGTGATTAGCG	CGAAAGTTAAGCTCATTGGAGTCTA	(tgt)6	109	64.4	61.3	0.000
UGTP028	BC8_c1563	GATGAGGGGCCGGTCCTT	TCCTCCGCCTTCTTCGCCTC	(gtc)7	117	64.5	66.6	0.375
UGTP029	BC8_c19567	ATGACAGTGTGCATCGGAGA	CAATGCAAATTGCAAGCAAG	(agat)5	77	60.4	56.3	0.351
UGTP030	BC8_c8570	GGTGCTAAATAGGACGGAGTCT	GGATCTATCTCCATGCACAATAGTA	(ttat)5	97	62.7	61.3	0.305
UGTP031	BC8_c748	GAGGACGAGGACGATGCT	CCACGAGCAGAGACAGAG	(ct)11	82	62.2	62.2	0.000
UGTP033	BC8_c2498	CACAGCATGTAGATCTCGTCAC	CTCTAGAGAGGGGTAACACAGCTA	(tg)9	114	62.7	64.4	0.000
UGTP034	BC8_c24625	GAACCACTCGCATCAAGAAG	GGTGAGCAAATGCGATGA	(tgc)6	116	60.4	57.6	0.164
UGTP035	BC8_c17826	AGCTGCTAGGACGCGACCA	TCACAGGGCACCTGGATG	(ta)11	105	64.5	62.2	0.000
UGTP037	BC8_c22088	CAGGGTTTAGCCGCCTCCGA	AGCTGTGCCGCTTCCGGTAC	(ccg)6	109	66.6	66.6	0.000
UGTP038	BC8_c8402	TGACACCATGGCCGTACG	TGCTGCAGGAGGAGGAGGAG	(cgc)6	94	62.2	66.6	0.000
UGTP039	BC8_c9109	CCTTTTTCCGCTTTCCTG	GTTTTGGCGGTAGTTCTGG	(acc)8	96	57.6	60.2	0.286
UGTP040	BC8_c11683	ATGAGTATGTGCACGCTGCA	TGCATCAATCGTCGTCCC	(atac)5	104	60.4	59.9	0.164
UGTP041	BC8_c7460	GAGGAAGTTACTTACCCACGAGG	AGTGGAGTGAGTTGTGTGTGTG	(ac)14	87	64.6	63.6	0.410
UGTP042	BC8_c4937	ACCGGAACGCAAACGCAA	GCACGAGTGAAGAAGGGACAAT	(gctc)5	119	59.9	62.7	0.000
UGTP043	BC8_c8302	TCTTTTCCTCCCCATCCA	GCGGGGGTAGTTTCAGTAATCT	(ca)9	114	57.6	62.7	0.269
UGTP044	BC8_c12501	TTCCGGTTTGTCTCTCCTTG(52.4)	AACGGTTTCACCCTCGAA(50.8)	(gat)6	128	60.4	57.6	0.372
UGTP045	BC8_c22308	TTCTTTCCCTTGGCGTGG	GCAAGCCCAAGGATGATAAC	(ctt)6	130	59.9	60.4	0.000

UGTP046	BC8_c17684	CCATCTCCTCCTCCTCCTC	AAGTACACGCGCGGGTTG	(ctc)7	140	64.5	62.2	0.000
UGTP047	BC8_c17684	CCATCTCCTCCTCCTCCTC	AAGTACACGCGCGGGTTG	(ggc)6	140	64.5	62.2	0.000
UGTP048	BC8_c8578	CGAAGACGGTGGCTAAGTAG	ACGCCGAGTACACAGATCAT	(tgt)6	147	62.4	60.4	0.365
UGTP049	BC8_c18643	GTCACCTCGCTTGAGGAGTT	GGCCATTCTGCAAGCATT	(gag)6	92	62.4	57.6	0.000
UGTP051	BC8_c23283	GCCAAGTCCCCCAAGAAAGCTT	TGGGGCAACACAACCGAA	(gct)6	129	64.5	59.9	0.269
UGTP052	BC8_c20257	CGGAAGAACACCACGACA	TGCTCTGTACTGGTAGAGAGAGAG	(tc)9	85	59.9	64.6	0.305
UGTP054	BC8_c14296	GCTTGGCTTTGCGTACGT	CCCGTACTACAATAACCAGGAGAG	(ct)9	106	59.9	62.6	0.375
UGTP055	BC8_c20677	GTTACTAAGTTGCAACAGCCCACG	GCCTGGTTAGTTGCTGGGAATA	(ac)9	111	64.6	62.7	0.000
UGTP056	BC8_c9144	ACCGGAGGAGGTGCATAG	AAGGAGCAGTGCCACTAGAGA	(tgga)5	117	62.2	62.6	0.375
UGTP057	BC8_c6748	ACCCACGCGAGCAGCAAA	GCCCGCCTCATCCCAAAA	(agaa)5	141	62.2	62.2	0.351
UGTP059	BC8_c5887	AACTTTATCCCTCCCCTTCC	TTTCTTCCTCAAGGGAGGC	(ctg)6	119	60.4	60.2	0.000
UGTP060	BC8_c22687	GAGGCGGAACCCTCTTACTGTAAG	GCCACGGAGACGATGTCAG	(ggc)6	139	66.3	64.5	0.000
UGTP063	BC8_c3860	CCTTTCGGATGCCTGGAA	CACGTTCAGGACCGTAAAAC	(tga)6	91	59.9	60.4	0.559
UGTP064	BC8_c18385	CTGGCCCAAGACAGTTTCG	CTGGGTCTCAGCATAGCGT	(gca)8	118	62.3	62.3	0.365
UGTP065	BC8_c788	CAGAGATCATCTGCATTGCC	TGAGGATGGCGTGGCATT	(aag)6	100	60.4	59.9	0.492
UGTP066	BC8_c2127	TCTTGCTCCCTCGTGCTG	CGACACGTATGTCCGGAACT	(ag)9	105	62.2	62.4	0.489
UGTP068	BC8_c497	CCATGAGTTTATCCCTGTCTCTGC	CGAACACACAAGCTTGCGAT	(ttg)6	96	64.6	60.9	0.314
UGTP069	BC8_c16223	CATCCATCTCCATCAGCCT	AAATCGAATCAACCCCGC	(ctt)6	117	60.2	57.6	0.000
UGTP070	BC8_c12086	CGAGGGGGAATCCGATTC	GGGATTTCGACGATCTCG	(gtc)6	89	62.2	59.9	0.365
UGTP071	BC8_c1915	CAACTACAACCAATGCCTCC	GCACCGCAGTTGGTTATTAGTC	(agcc)5	112	60.4	62.7	0.000
UGTP072	BC8_c1547	AGTCCCGTGGTGCATCCA	GCGGGGGGTATTTTCAGT	(ca)9	145	62.2	59.9	0.000
UGTP073	BC8_c2360	CGACGAGGGGAGGAGGACGAAA	TGGGAACCATGGCCGCGTA	(acc)6	119	68.3	64.5	0.000

UGTP075	BC8_c1935	TAACGAGTCGATCTCAAGTGCG	CTCTCTATCTCTGCAGCTATGCTG	(tg)11	123	62.7	64.6	0.269
UGTP076	BC8_c8867	CCAGGTTATCTGGCAGCAAGCATG	AGCAGCATCCAGGGGATGG	(cag)7	124	66.6	64.5	0.365
UGTP077	BC8_c9882	TAATGCCGGCGCAACTAC	ATGGAAACGGGGAACTTG	(tgt)6	127	59.9	57.6	0.164
UGTP078	BC8_c1918	AGTGCTGGTGCGAGTTACAA	TTGCGGGTCTGTTCTGTTAC	(ca)9	135	60.4	60.4	0.375
UGTP079	BC8_c7201	GCTTAGAGAAAGAGAGAGGGAGAG	GCAGAAGCAGCAAAACGA	(ga)11	126	64.6	57.6	0.482
UGTP081	BC8_c1727	CTACCAAAAAAACAGCGAGGGC	CCTCTAGAACTGCTGCTGCTG	(agc)6	120	62.7	64.5	0.000
UGTP082	BC8_c24173	CACACTCCACAATGCAACACAC	CGCTTCGTTCGATCTGTGAT	(ac)9	81	62.7	60.4	0.000
UGTP083	BC8_c8637	TGGCTGCTCATGTGTTGC	AAAGCAGGCGAGCACGGT	(tggc)5	106	59.9	62.2	0.346
UGTP084	BC8_c5436	ACGCACGAACGTGCATTT	CAATGCACCCACAACCAGAA	(gct)6	106	57.6	60.4	0.000
UGTP085	BC8_c8471	GCAAATTAGGAGTAGCAGAGGC	CCGCCCTATTGATTTACTGG	(ac)9	112	62.7	60.4	0.351
UGTP087	BC8_c3856	CTCTAGGGTTTTCGCCGC	TCTTGGTGTCTCGGTGGG	(cct)7	98	62.2	62.2	0.559
UGTP088	BC8_c15765	CAGCGAGTTTGTGGATGTG	CGCCACGTGTTCTTTCTTCT	(ag)15	103	60.2	60.4	0.000
UGTP089	BC8_c17829	TCAAGAACCGAACTCTGATCTCCG	CGGTTGATGAGATTGAGGAAGAGG	(cat)9	146	64.6	64.6	0.000
UGTP090	BC8_c15548	GGTGTCAGCTATACCTGATGTCAGC	ATGCTCCAGCAGGCCAAG	(gcg)6	154	66.6	62.2	0.000
UGTP091	BC8_c24263	CTTCTTCTTCTTTGCCGCAG(52.7)	GCAAACCCTAGCTCCCTAGC	(gcc)7	77	60.4	64.6	0.222
UGTP092	BC8_c12426	GGTGCTCTTGCTCACAACC	CGGTTTGCTTTGCTTTGC	(ac)9	94	62.3	57.6	0.000
UGTP093	BC8_c2662	CGGACAGACGGACAGAGAT	GAAAATGCAAGCTCGCCT	(cac)11	114	62.3	57.6	0.332
UGTP094	BC8_c25161	GTCGGCCTTGGACTCGAA	CTGCATCTCCTCCTCCTCC	(gca)9	105	62.2	64.5	0.000
UGTP095	BC8_c8682	AAGGTAGCTGAATGTGCCG	GGATGGTCAACGAGCAAGAA	(tggt)5	117	60.2	60.4	0.000
UGTP096	BC8_c14306	GCATCCAACTCCGGCGAGA	ATCTGCGCTGCCGAGAGA	(ggc)6	91	64.5	62.2	0.000
UGTP097	BC8_c5436	TGGATGGTGGTGGTTATTGG	GATCGAGAGCTAGCTTGCTTG	(tga)6	104	60.4	62.6	0.000
UGTP100	BC8_c11243	CCCCGTTAGCCGCTGAAA	CGTCGATGGTGAGCGTCT	(cgc)7	101	62.2	62.2	0.000

UGTP101	BC8_c788	CGTCAGGCAAATCTTCAGTG	GAGGAGTTTGCTTTCCCTTC	(gatg)7	102	60.4	60.4	0.844
UGTP102	BC8_c5880	CCTTCGCCGCCAGCCAAGAA	TTGTTGAGGAGCACGGCGG	(cgc)9	134	66.6	64.5	0.365
UGTP103	BC8_c2882	TGATCGTATAGGTTGGCCAC	GCGTGAAGTAGACCACCAGTG	(caa)7	118	60.4	64.5	0.000
UGTP104	BC8_c1349	CCCTTCAGTGGCTGTCTTAGG	GCTGCTGCCAATGGTGAC	(ttc)6	105	64.5	62.2	0.000
UGTP105	BC8_c14401	GCAAGGCCAATCCATGGA	CTCCTCAAACTCGAGCTCAA	(gca)6	126	59.9	60.4	0.000
UGTP106	BC8_c3505	GACTCCTATGTTGACATGGTGG	TGCTTGGCTTGCCAGAAT	(acgt)6	82	62.7	57.6	0.000
UGTP107	BC8_c5533	AACGGAAGGCGTGATTGC	CTCTACATAGTCCGCAGCAGTA	(ta)9	105	59.9	62.7	0.000
UGTP108	BC8_c5131	CCCTGTTGTGGGTCCTGTGTAA	GGAGAAAAACACTGACGTTGCGTC	(tg)9	109	64.5	64.6	0.332
UGTP109	BC8_c13079	CTAGCCTTCTAGGTACATTGGTGC	GAGAAGGCACTCGTAGAGTCATAG	(cta)6	136	64.6	64.6	0.269
UGTP111	BC8_c6763	TTTGCTTGCTTCATCGCC	GAGCATCCACCTCACGATTA	(cctt)7	124	57.6	60.4	0.269
UGTP112	BC8_c16538	GAGGGGGTGACGGAAAGCGA	AATCGAGATCGGCGGAGGCC	(ccg)7	94	66.6	66.6	0.000
UGTP113	BC8_c2569	CCAGAGTGTTAGGAAGGAAAGG	GGGGTTGCTTTACCTGATGT	(at)9	110	62.7	60.4	0.000
UGTP114	BC8_c774	TTCTCTTCTTGGTAGGTGGTTGGG	GGGATGATGACAATGAGGAGGAAG	(tcc)7	111	64.6	65.2	0.000
UGTP115	BC8_c1439	GTTCGTGGTGATGTGATTAGCG	GGGGGGGAAAACAAAGTT	(tgt)6	115	62.7	57.6	0.000
UGTP116	BC8_c14790	CCTCTTCTCCTTACACGATCG	GCCATGCTATGCCAGTGAAT	(ctg)6	124	62.2	60.1	0.269
UGTP117	BC8_c751	GCATCCTCTCTGCTTCAGTG	TGGAACCACTCGCATCAAG	(gtc)7	109	62.4	60.2	0.000
UGTP118	BC8_c1349	CTTCAGCGTCACCATTGGC	GCTGCAGATGGTGAAGCTG	(ctt)7	115	62.3	62.3	0.000
UGTP120	BC8_c4349	ATCAAGCCAACCGCAAAG	CCCCAGTTACCTTTGTACTCCTC	(tag)6	99	57.6	64.6	0.000
UGTP121	BC8_c4952	TCATCTTCGAGGAACATCAGGAGG	CCGACGATCAACAAGATGTTGC	(ggc)6	103	64.6	62.7	0.410
UGTP122	BC8_c13580	TTCATCTCTTGTGACAAGGGACCG	CCGAACCAATCCATGGAGCTTGTT	(aac)6	136	64.6	64.6	0.000
UGTP123	BC8_c3524	TAGCAACTGTGACATTCTGACCCG	CCGAGTTGGTTAGTGTTCGTCTTC	(catc)5	100	64.6	64.6	0.365
UGTP124	BC8_c19814	GCAGCAAACAAAGCTCCA	GAGGAGGAGGAAGAAGAAGAAG	(ttc)6	135	57.6	62.7	0.548

UGTP125	BC8_c1139	AACCCAGGAGCAAGAGAATG	CTGTCTTAGGTTCAGCATCCTC	(aag)6	113	60.4	62.7	0.000
UGTP126	BC8_c18392	GCACTTCTAGGTTAGGGTCATG	GGGAAGAAGACAGGCTACTACTAC	(ta)9	114	62.7	64.6	0.365
UGTP127	BC8_c633	ATTATGGGTCTGAGCTTCTGTGGC	TTGCGCAACAGGCTGACA	(gct)6	105	64.6	59.9	0.000
UGTP128	BC8_c7712	CTGGTCGATCATGGCGGCCA	ATCCTTCAGACTCGGACGGCGG	(gcc)7	118	66.6	68.3	0.000
UGTP129	BC8_c17749	GGCACGCTGGGGAAAGAGA	CGCAACACGCTCCATCTG	(gga)6	101	64.5	62.4	0.365
UGTP130	BC8_c7640	TTCCACTCAGTGACCGATTG	CAAGAAAATGAGCACGGAGC	(caa)7	95	60.4	60.4	0.426
UGTP131	BC8_c16261	CTCTGACTGACTCTTGACTGACG	GCGTGTCGTCGTCGTCTT	(caa)6	98	64.6	62.2	0.351
UGTP132	BC8_c5910	CTACATTCAGCGTTTCAGCC	CCGTAAAACATTGCCACCAC	(tga)6	106	64.4	60.4	0.466
UGTP133	BC8_c5339	TTGATACGGAGAGCGGGG	GTTCCCGCCGTATATGGA	(tcc)6	110	62.2	59.9	0.351
UGTP134	BC8_c9732	CGGGATGAACAGAACAAAGC	TGCGTCACCAGCCATTTT	(agc)6	114	60.4	57.6	0.586
UGTP135	BC8_c12083	GCGTGGTGGTCAAAGCCA	CGGTAATGAGTTCAGCAGCAG	(ctg)6	114	62.2	62.6	0.000
UGTP136	BC8_c5379	TTGCTCCTCCTTCTTCTGGC	CAACCTCTCCACCGACGAC	(gtc)7	156	62.4	64.5	0.000
UGTP141	BC8_c12233	GCTTGCTGCGTCTGCATT	TGTAAACCGGTCGTGTGC	(atgg)8	141	59.9	59.9	0.351
UGTP143	BC8_c6333	GAGTGCATCCTTCCCTCG	TGTACTCGCCGCCCTTTT	(tc)11	158	62.2	59.9	0.351
UGTP145	BC8_c1563	AGGTTGATGCAGGAGCAGC	AGCAACCAGATCGCCTCCG	(gcc)6	120	62.3	64.5	0.000
UGTP146	BC8_c10075	CGCTGCTGCTGCTTCTCA	GAAACCCTACGCTGCCTTA	(tgc)6	175	62.2	60.2	0.000
UGTP147	BC8_c22683	CCTAGCTAGGGTTTGAGCCG	CCATGTTGACGGAAGTGTCC	(gcc)6	70	64.5	62.4	0.000
UGTP148	BC8_c9778	GGCGTCCTGCGAAGGTTGT	TAGACGCGATCGCCGTTC	(gcg)6	111	64.1	62.2	0.000
UGTP149	BC8_c6378	GCTCTCAGCACTGCCCAT	CAGGAGAACCAGCTCTGGA	(gca)6	184	62.2	62.3	0.000
UGTP150	BC8_c12842	CTTCTGACTCTCGCTTTGGTTG	TGCACAGTCACACGGGTT	(ta)12	107	62.7	59.9	0.365
UGTP151	BC8_c18947	CAATCAGAACAGGTGGCAAG	GCAGGAACGTCTGTAGGAAGAT	(gat)8	108	60.2	62.7	0.000
UGTP152	BC8_c6145	CACCTGGGGAGGCCATCTA	CCGGCGGTATGGTAATACG	(agg)6	124	64.5	62.3	0.381

UGTP153	BC8_c18192	ATGGGAGCAAGAAGAAGCTG	GGCTTCAATCGTCGTCTCAT	(agg)6	126	60.4	60.4	0.365
UGTP154	BC8_c10564	CCGGGGGTGGTGTCTCTTT	GCGACAAGTCTGACGGCTT	(ctg)7	139	64.5	62.3	0.495
UGTP155	BC8_c1411	TAGACGCGATCGCCGTTC	CATTCAAAGTTCCCGCCG	(tcc)6	115	62.2	59.9	0.000
UGTP156	BC8_c6170	ACAGGCAGGACGTCCCAA	CCACAAGGACACGATGTCTCT	(ga)12	128	62.2	62.6	0.381
UGTP157	BC8_c2987	GGTTGTACATGACACGAACAGG	CCACAAGGACACGATGTCTCTA	(ag)26	121	62.7	62.7	0.000
UGTP158	BC8_c24625	GAAGCTGCCCATAAGACAAAGAGG	GCAGCAGCAGCAAGAGTGTAG	(gac)7	111	64.6	64.5	0.000
UGTP159	BC8_c3717	GCCACGCCGACCAAGAACTT	GGCCTCAACAAACACGTTACTACC	(gga)6	148	64.5	64.6	0.372
UGTP160	BC8_c1697	GAAGATCAAGGTTGAGGAAGCC	ACAGCCTCAGCTAATCCTGAC	(aga)6	102	62.7	62.6	0.351
UGTP161	BC8_c10897	CCCTCCATGTCGCCGTCAT	CGACAAGGGGAAGGGGAA	(cct)6	99	64.5	62.2	0.000
UGTP162	BC8_c16703	AGGAGCCTTCGTCGCCGTCA	GCCGCCATTGATGCTGCT	(agc)8	125	66.6	62.2	0.000
UGTP163	BC8_c10024	AATGATGAGGATGGCGTGG	CATCTGCATTGCCAACGC	(ttc)8	104	60.2	59.9	0.362
UGTP164	BC8_c5005	CTCAAAGCACACCAGATTCG	GCCCCTTCGTTTCATTCCTT	(ag)10	119	60.4	60.4	0.332
UGTP165	BC8_c3328	TACCGTACCGTCTGTGTTCAC	GGTGTTGCTCGTCGTGTG	(gag)7	99	62.6	62.2	0.000
UGTP166	BC8_c5604	CATCTGTTTGATCGATGGGG	TTGTTGGCGTCCCGGTTT	(tta)7	101	60.4	59.9	0.164
UGTP167	BC8_c2222	AATGCAGATCTGCTGACCCGGAG	GCGGGATATGATGGACTATTACCG	(gct)8	301	66.3	64.6	0.000
UGTP168	BC8_c24870	GCCACCGTGTACCGTTGCCA	AGGTCATCAAGGCGCCCGTG	(ggc)8	110	66.6	66.6	0.000
UGTP169	BC8_c9996	TCTGTTTGATCGATGGGKACG	GCAAAAGACATTGTGTGGGG	(tta)6	133	61.6	60.4	0.410

Table S3.3. Positions of rice RAP homologs of newly developed EST-SSR and their putative annotation

S. No	Name of SSR	Contig name	Map position	Homologous rice RAP accession [b]	Putative annotation of rice homolog	E-value
1	UGTP019	BC8_c11861		Os02t0244100-01	Zinc finger, RING-type domain containing protein.	5.00E-17
2	UGTP021	BC8_c154		Os08t0428800-00	Similar to High mobility group I/Y-2.	7.00E-25
3	UGTP024	BC8_c497		Os12t0632000-01	Similar to Glycine-rich RNA-binding protein 1 (Fragment).	6.00E-09
4	UGTP027	BC8_c5875		Os01t0933900-01	Similar to Glutathione transferase III(B) (EC 2.5.1.18).	2.00E-28
5	UGTP028	BC8_c1563	LG3	Os02t0826400-00	Conserved hypothetical protein.	1.00E-29
6	UGTP031	BC8_c748		Os07t0159800-01	MD-2-related lipid-recognition domain containing protein.	1.00E-41
7	UGTP034	BC8_c24625	LG2	Os03t0843300-02	Late embryogenesis abundant protein 2 family protein.	1.00E-17
8	UGTP037	BC8_c22088		Os08t0436800-01	Similar to 60S ribosomal protein L34.	4.00E-74
9	UGTP045	BC8_c22308		Os02t0105500-01	IQ calmodulin-binding region domain containing protein.	1.00E-07
10	UGTP046	BC8_c17684		Os02t0121300-02,	Cyclophilin 2.	4.00E-18
11	UGTP047	BC8_c17684		Os02t0121300-02,	Cyclophilin 2.	4.00E-18
12	UGTP051	BC8_c23283	LG4	Os01t0502700-00	Similar to Histone H2A.	9.00E-07
13	UGTP056	BC8_c9144	LG7	Os07t0571100-02	Hypothetical conserved gene.	2.00E-10
12	UGTP060	BC8_c22687		Os01t0266600-01	Thioredoxin fold domain containing protein.	1.00E-35
15	UGTP065	BC8_c788		Os10t0418000-01	Similar to Histone H2A.	4.00E-69
16	UGTP068	BC8_c497		Os12t0632000-01	Similar to Glycine-rich RNA-binding protein 1 (Fragment).	6.00E-09
17	UGTP070	BC8_c12086		Os04t0654600-01	Protein kinase, core domain containing protein.	1.00E-17

18	UGTP076	BC8_c8867	Os09t0507200-01	MADS box protein.	2.00E-21
19	UGTP079	BC8_c7201	Os01t0616300-01	Thioredoxin, core domain containing protein.	4.00E-06
20	UGTP083	BC8_c8637	Os03t0854200-01	Exoribonuclease domain containing protein.	3.00E-13
21	UGTP084	BC8_c5436	Os09t0459200-01	Conserved hypothetical protein.	6.00E-10
22	UGTP085	BC8_c8471	Os02t0620400-01	RmlC-like jelly roll fold domain containing protein.	4.00E-22
23	UGTP089	BC8_c17829	Os02t0244600-01	Nucleotide-binding, alpha-beta plait domain containing protein.	2.00E-20
24	UGTP090	BC8_c15548	Os05t0595800-00	Protein kinase-like domain containing protein.	3.00E-07
25	UGTP093	BC8_c2662	Os02t0520750-01	Similar to H0209H04.3 protein.	4.00E-16
26	UGTP094	BC8_c25161	Os07t0112800-01	Similar to Eukaryotic translation initiation factor 5A-4 (eIF-5A-4).	2.00E-17
27	UGTP096	BC8_c14306	Os05t0594900-01	Similar to U6 snRNA-associated Sm-like protein LSm8.	5.00E-17
28	UGTP097	BC8_c5436	Os09t0459200-01	Conserved hypothetical protein.	6.00E-10
29	UGTP100	BC8_c11243	Os01t0142200-01	Kinase binding protein CGI-121 domain containing protein.	2.00E-28
30	UGTP102	BC8_c5880	Os07t0112700-01	Cupredoxin domain containing protein.	9.00E-48
31	UGTP104	BC8_c1349	Os03t0352300-02	Similar to nucleolar protein Nop56.	9.00E-12
32	UGTP112	BC8_c16538	Os03t0835900-01	Similar to Ferredoxin III, chloroplast precursor (Fd III).	1.00E-25
33	UGTP113[a]	BC8_c2569	Os06t0183800-01	Similar to Chromatin-remodeling factor CHD3.	1.00E-14
34	UGTP115	BC8_c1439	Os01t0933900-01	Similar to Glutathione transferase III(B) (EC 2.5.1.18).	5.00E-10
35	UGTP117	BC8_c751	Os03t0843300-02	Late embryogenesis abundant protein 2 family protein.	1.00E-

						107
36	UGTP118	BC8_c1349		Os03t0352300-02	Similar to nucleolar protein Nop56.	9.00E-12
37	UGTP121	BC8_c4952		Os03t0675300-01	Hypothetical conserved gene.	1.00E-05
38	UGTP125	BC8_c1139		Os03t0352300-02	Similar to nucleolar protein Nop56.	2.00E-56
39	UGTP127	BC8_c633		Os07t0114300-01	Protein of unknown function DUF1909 domain containing protein.	6.00E-61
40	UGTP128	BC8_c7712		Os12t0571200-01	Similar to Mitochondrial import inner membrane translocase subunit Tim9.	4.00E-80
41	UGTP129	BC8_c17749	LG6,LG7	Os01t0949500-04	Similar to Calmodulin (CaM).	1.00E-49
42	UGTP130	BC8_c7640		Os01t0348600-03	Similar to glyoxysomal fatty acid beta-oxidation multifunctional protein MFP-a.	3.00E-57
43	UGTP133	BC8_c5339		Os04t0663800-01	Similar to Peptidyl-prolyl cis-trans isomerase 1 (EC 5.2.1.8) (Rotamase Pin1) (PPIase Pin1) (PIN1At).	3.00E-08
44	UGTP136	BC8_c5379		Os02t0826400-00	Conserved hypothetical protein.	4.00E-23
45	UGTP143	BC8_c6333	LG3	Os04t0421900-01	Conserved hypothetical protein.	1.00E-18
46	UGTP145	BC8_c1563		Os02t0826400-00	Conserved hypothetical protein.	1.00E-29
47	UGTP147	BC8_c22683		Os01t0834500-01(more on chr.3,10)	Similar to 40S ribosomal protein S23 (S12).	3.00E-37
48	UGTP151	BC8_c18947		Os09t0436400-01	PDZ/DHR/GLGF domain containing protein.	1.00E-10
49	UGTP152	BC8_c6145		Os02t0833400-01	Conserved hypothetical protein.	1.00E-06

50	UGTP155	BC8_c1411	Os04t0663800-01	Similar to Peptidyl-prolyl cis-trans isomerase 1 (EC 5.2.1.8) (Rotamase Pin1) (PPIase Pin1) (PIN1At).	8.00E-16
51	UGTP157	BC8_c2987	Os02t0805900-01	NUDIX hydrolase, core domain containing protein.	9.00E-35
52	UGTP158	BC8_c24625	Os03t0843300-02	Late embryogenesis abundant protein 2 family protein.	1.00E-17
53	UGTP160	BC8_c1697	Os07t0636000-01	Similar to H/ACA ribonucleoprotein complex subunit 4 (EC 5.4.99.-) (Nucleolar protein NAP57 homolog) (Nopp-140 associated protein of 57 kDa homolog) (AtNAP57).	1.00E-05
54	UGTP161	BC8_c10897	Os04t0395700-00	Conserved hypothetical protein.	3.00E-09
55	UGTP164	BC8_c5005	Os03t0118400-02	Similar to Cyclin-dependent kinase A-1.	3.00E-12
56	UGTP167	BC8_c2222	Os03t0137200-01	Protein phosphatase 2C domain containing protein.	4.00E-19
57	UGTP168	BC8_c24870	Os01t0957100-01	Protein kinase, core domain containing protein.	2.00E-19

[a]get the function against translated protein database. Others got from transcripts (CDS +UTR)

[b] best hit (lowest E-value) was selected if more than one hit was found

Table S3.4. Positions of rice RAP homologs of newly mapped previous developed EST-SSR and their putative annotation

S. No	Primer parie	Genebank EST ID	Map position	Homologous rice RAP accession [b]	Putative annotation of rice homolog	E-value
1	PSMS39	CD725872	LG1	Os11t0648100-01	Kinesin, motor region domain containing protein.	1.00E-49
2	PSMS97	CD726001	LG1	Os05t0401100-01	Protein of unknown function DUF477 family protein.	2.00E-52
3	PSMS49	CD725605	LG1	Os05t0208000-04	Similar to Mitochondrial 2-oxoglutarate/malate carrier protein.	3.00E-47
				Os11t0432400-01	Similar to 2-oxoglutarate/malate translocator.	1.00E-35
				Os11t0432301-00	Hypothetical gene.	2.00E-22
4	PSMS94	CD726569	LG1	Os05t0310800-01	Similar to Coatomer delta subunit (Delta-coat protein) (Delta-COP).	1.00E-101
				Os05t0311000-01	Clathrin adaptor, mu subunit, C-terminal domain containing protein.	1.00E-136
				Os08t0368000-01	Similar to Coatomer delta subunit (Delta-coat protein) (Delta-COP).	1.00E-128
				Os01t0833700-01	Clathrin adaptor, mu subunit, C-terminal domain containing protein.	3.00E-70
5	ICMP3063	CD725658	LG2	Os03t0836200-03	Similar to RNA-binding protein RZ-1.	1.00E-13
6	ICMP3043	CD724407	LG2	Os07t0204400-00	Conserved hypothetical protein.	2.00E-34
				Os03t0806800-02	Conserved hypothetical protein.	2.00E-28
7	PSMS89	CD725143	LG2	Os08t0282400-01	Similar to Alpha-SNAP (Fragment).	1.00E-49
8	PSMS08	CD726577	LG2	Os11t0455500-01	Similar to Adenosylhomocysteinase-like protein.	1.00E-45
9	PSMS82	CD725185	LG2	Os01t0965400-01	Similar to Uridylate kinase (EC 2.7.4.-) (UK) (Uridine monophosphate kinase) (UMP kinase).	1.00E-117

				Os04t0405800-01	Similar to Uridylate kinase (EC 2.7.4.-) (UK) (Uridine monophosphate kinase) (UMP kinase).	6.00E-62
10	PSMS22	CD726311	LG2	Os02t0230100-01	Leucine-rich repeat, SDS22 containing protein.	1.00E-120
11	ICMP3029	CD726384	LG2	Os08t0520300-01	Similar to Oligouridylate binding protein.	1.00E-110
12	PSMS36	CD725922	LG3	Os05t0138300-03	Hydrophobic protein LTI6B (Low temperature-induced protein 6B).	1.00E-41
				Os05t0138300-01	Similar to ICT protein (Fragment).	4.00E-26
				Os07t0635900-01	Hydrophobic protein LTI6A (Low temperature-induced protein 6A).	7.00E-25
				Os03t0370600-00	Similar to Hydrophobic protein LTI6A (Low temperature-induced protein 6A).	6.00E-16
				Os01t0287400-01	Similar to Hydrophobic protein LTI6A (Low temperature-induced protein 6A).	9.00E-12
				Os06t0184800-01	Similar to Low-temperature induced protein lt101.1 (Blt101) (Blt101.1).	9.00E-09
13	ICMP3078	CD726702	LG5	Os07t0529600-01	Similar to Thiazole biosynthetic enzyme 1-1, chloroplast precursor.	7.00E-65
				Os07t0529600-02	Similar to Pathogen-induced defense-responsive protein 8.	1.00E-59
14	PSMS71	CD725270	LG5	Os12t0623900-01	Similar to Ethylene-responsive methionine synthase (Fragment).	0.00E+00
15	CUMP18	BM084569	LG5	Os07t0616600-02	Similar to 40S ribosomal protein SA (p40) (Laminin receptor homolog).	8.00E-30
16	PSMS45	CD725783	LG5	Os03t0376600-01	Similar to 29 kDa ribonucleoprotein, chloroplast precursor (RNA-binding protein cp29).	4.00E-69
				Os03t0376701-00	Hypothetical gene.	9.00E-30

				Os07t0631900-01	Nucleotide-binding, alpha-beta plait domain containing protein.	3.00E-20
17	PSMS78	CD725244	LG6	Os06t0111500-01	Cytosolic 6-phosphogluconate dehydrogenase.	1.00E-139
				Os12t0616900-01	Similar to Pyruvate dehydrogenase E1 beta subunit (Fragment).	8.00E-13
				Os03t0645100-01	Similar to Pyruvate dehydrogenase E1 beta subunit (Fragment).	7.00E-10
18	ICMP3081	EB411027	LG6	Os05t0106600-01	Similar to Actin 97.	3.00E-44
				Os01t0964133-01	Similar to Actin.	7.00E-39
				Os03t0836000-01	Similar to Actin 7 (Actin 2).	4.00E-25
				Os10t0510000-01	Similar to actin.	1.00E-21
				Os11t0163100-01	Similar to Actin 7 (Actin 2).	1.00E-21
				Os12t0163700-01	Similar to Actin 7 (Actin 2).	6.00E-21
				Os03t0718100-01	Actin 1.	5.00E-15
19	ICMP3043	CD724407	LG7	Os07t0204400-00	Conserved hypothetical protein.	3.00E-28

Table S3.5 Marker segregation distribution of pear millet genetic maps

Map population	LG1	LG2	LG3	LG4	LG5	LG6	LG7
(81B X ICMP451) F_2				X			
(81B X ICMP451)X BKM1163 F_1	X			X			X
(LGD1-B-10 X ICMP85410) F_2	X	X		X	X		X
(PT732B X P1449-2) F_2		X		X	X		
(ICMP841 X 863B) F_2			X			X	
(H 77/83-2 X PRLT 2/89-33) RIL	X	X			X	X	
(TIFT99B X TIFT454) RIL	X	X	X		X		

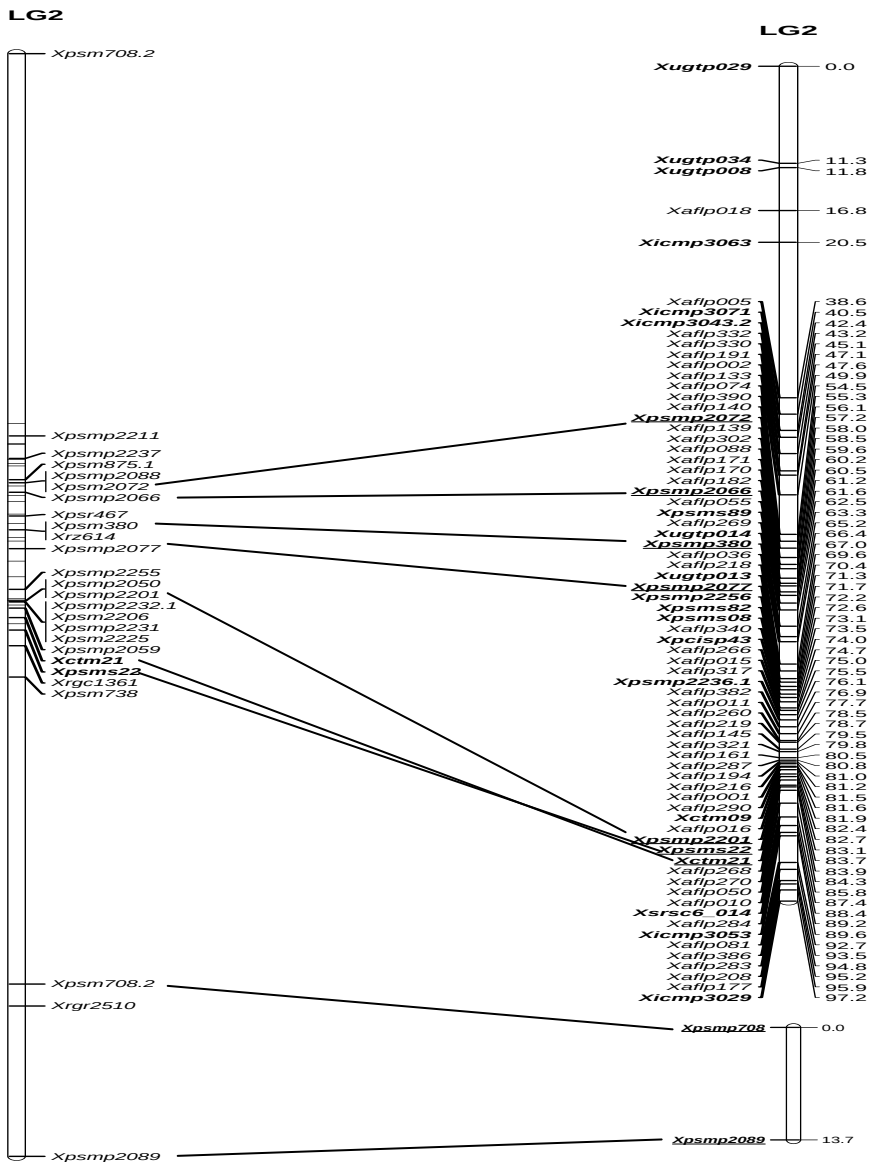

Figure S3.1a. Alignment of newly developed pearl millet genetic map LG2 (right) with the pearl millet consensus map (left) (Qi et al., 2004). The consensus map LG2 was redrawn according to the original consensus map. Additional SSRs (in bold) not present in the consensus map were inferred back on the published data of Senthilvel et al. (2008) or from previously unpublished data of K. Devos et al.

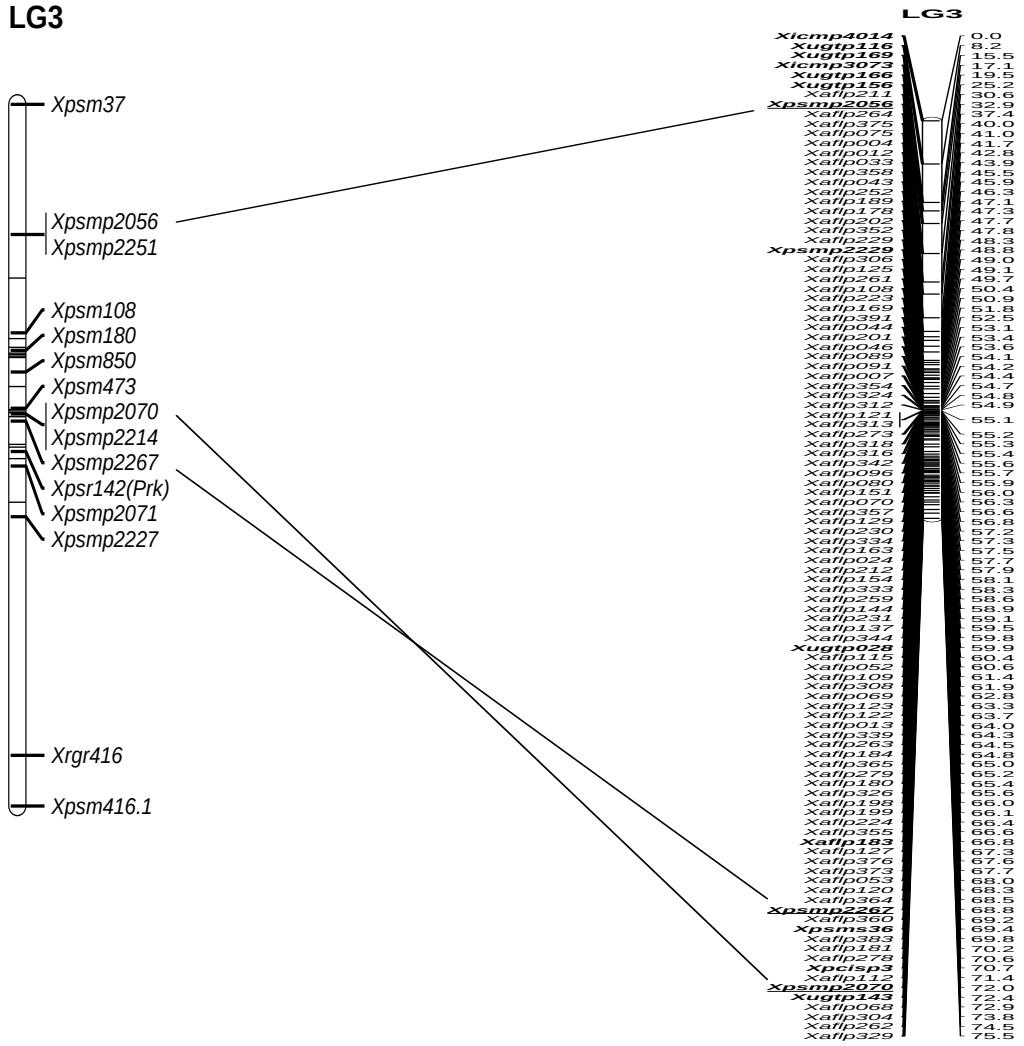

Figure S3.1b. (continued) Alignment of newly developed pearl millet genetic map LG3 (right) with the pearl millet consensus map (left) (Qi et al., 2004). The consensus map LG3 was redrawn according to the original consensus map. Additional SSRs (in bold) not present in the consensus map were inferred back on the published data of Senthilvel et al. (2008) or from previously unpublished data of K. Devos et al.

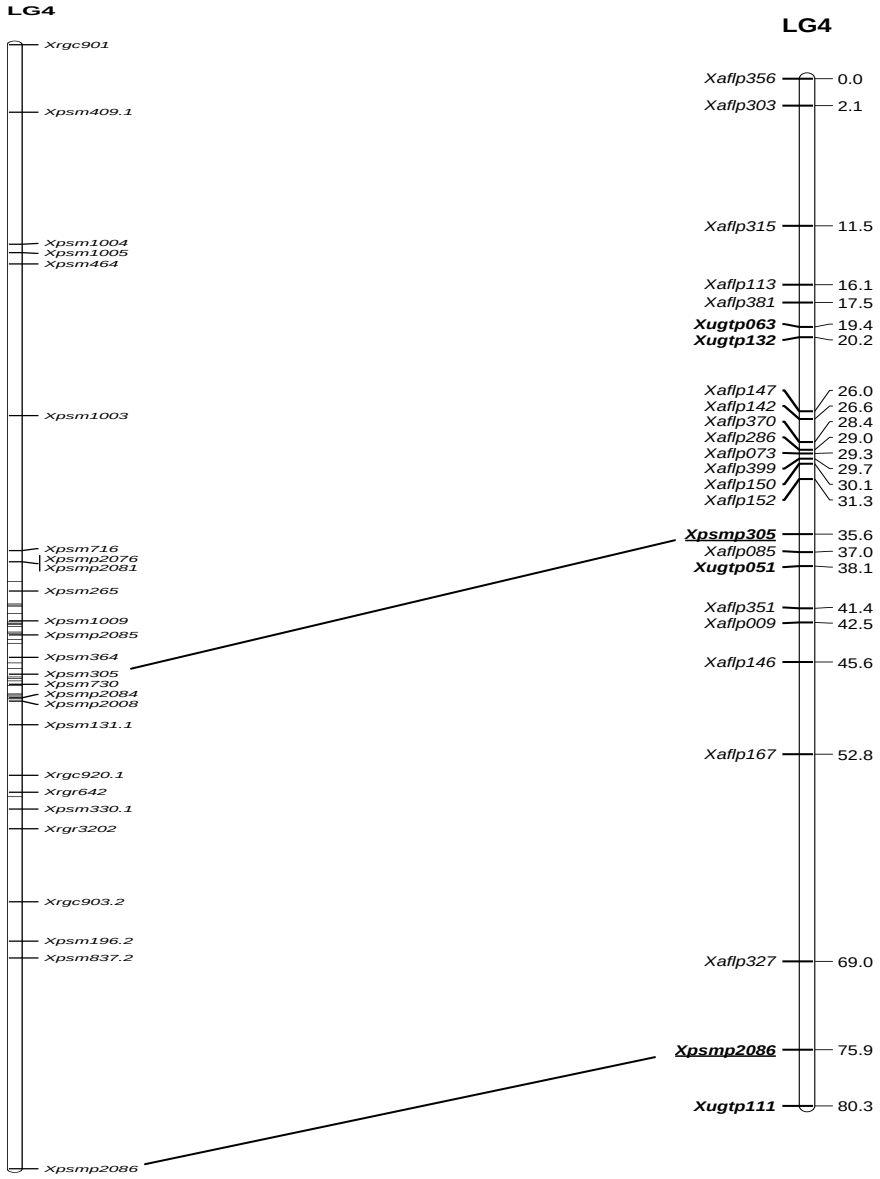

Figure S3.1c (continued) Alignment of newly developed pearl millet genetic map LG4 (right) with the pearl millet consensus map (left) (Qi et al., 2004). The consensus map LG4 was redrawn according to the original consensus map. Additional SSRs (in bold) not present in the consensus map were inferred back on the published data of Senthilvel et al. (2008) or from previously unpublished data of K. Devos et al.

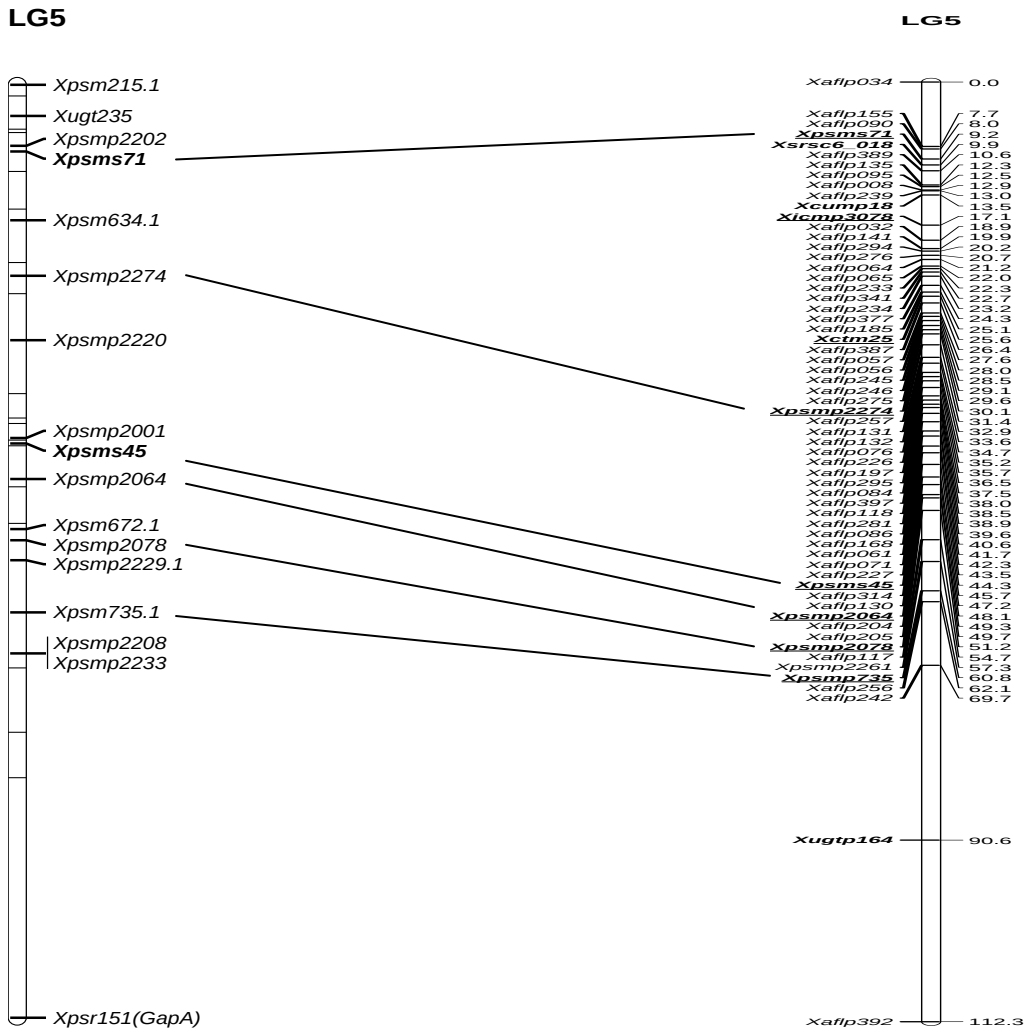

Figure S3.1d. (continued) Alignment of newly developed pearl millet genetic map LG5 (right) with the pearl millet consensus map (left) (Qi et al., 2004). The consensus map LG5 was redrawn according to the original consensus map. Additional SSRs (in bold) not present in the consensus map were inferred back on the published data of Senthilvel et al. (2008) or from previously unpublished data of K. Devos et al.

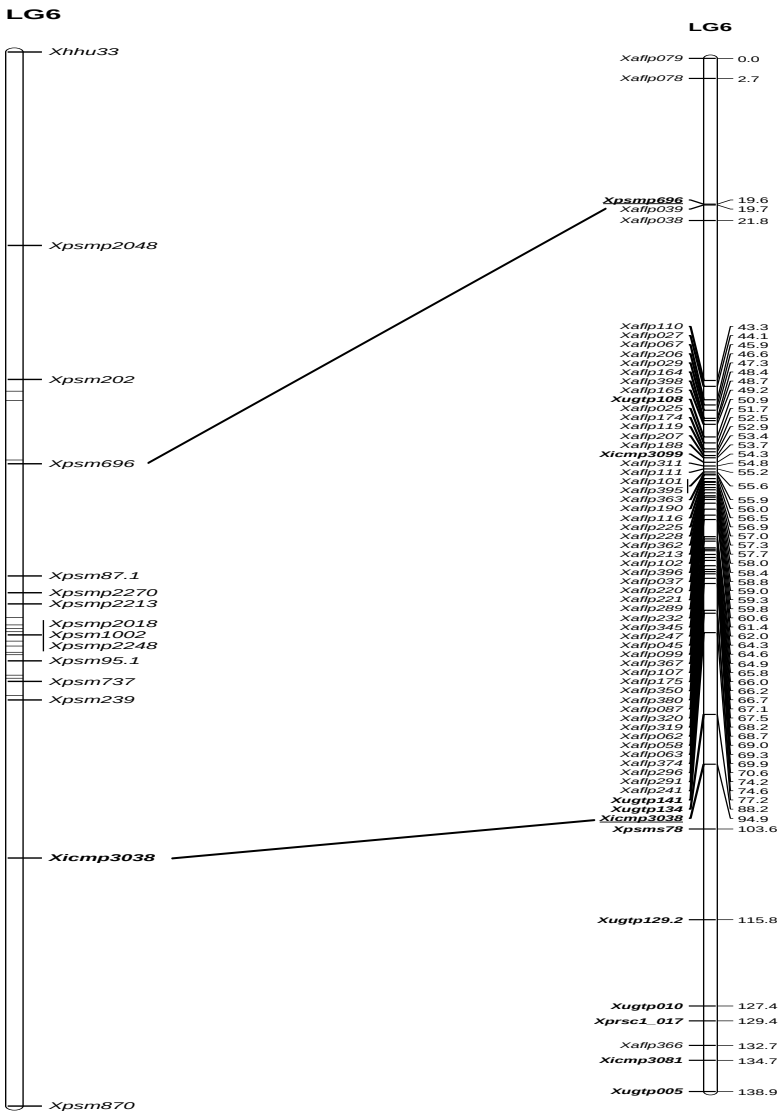

Figure S3.1e. (continued) Alignment of newly developed pearl millet genetic map LG6 (right) with the pearl millet consensus map (left) (Qi et al., 2004). The consensus map LG6 was redrawn according to the original consensus map. Additional SSRs (in bold) not present in the consensus map were inferred back on the published data of Senthilvel et al. (2008) or from previously unpublished data of K. Devos et al.

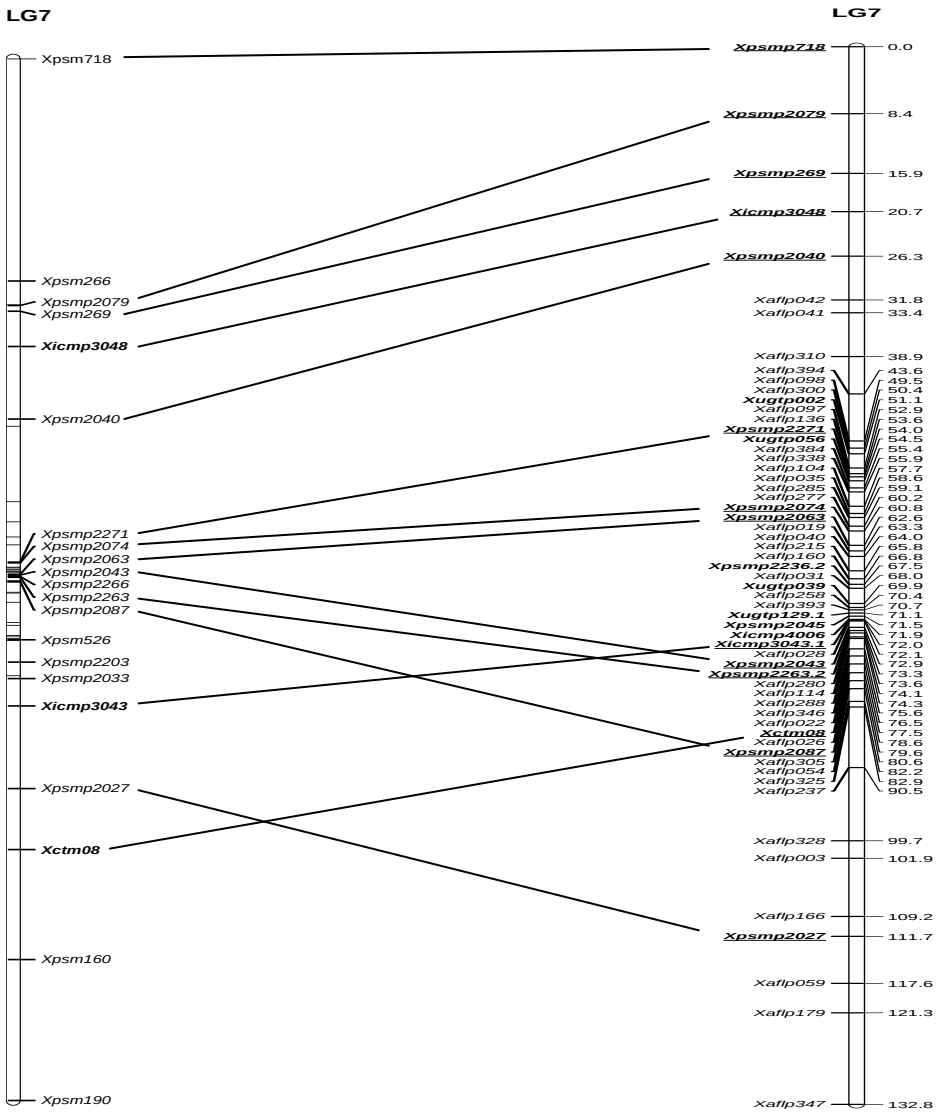

Figure S3.1f. (continued) Alignment of newly developed pearl millet genetic map LG7 (right) with the pearl millet consensus map (left) (Qi et al., 2004). The consensus map LG7 was redrawn according to the original consensus map. Additional SSRs (in bold) not present in the consensus map were inferred back on the published data of Senthilvel et al. (2008) or from previously unpublished data of K. Devos et al.

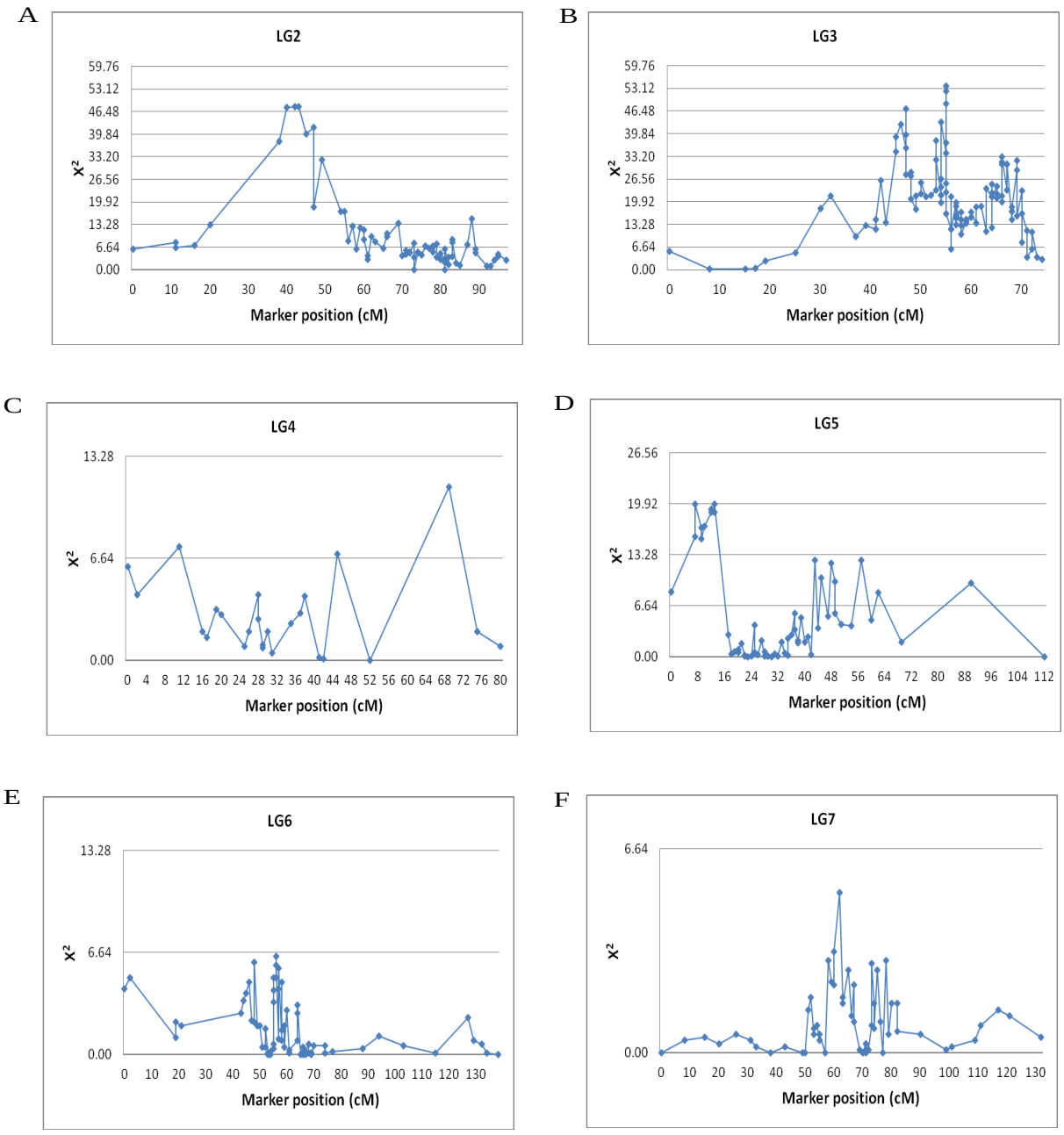

Figure S3.2. Marker segregation distortion of pearl millet newly developed genetic map A: LG2, B: LG3, C: LG4, D: LG5, E: LG6, F: LG7. X-axis shows marker position in cM, and Y-axis shows Chi-squared values of the markers. χ^2 (0.01, 1) = 6.64. Chi-squared values greater than 6.64 mean significant distorted at 1% level.

CHAPTER 4

GENETIC MAPPING OF ROOT-KNOT NEMATODE [*MELOIDOGNE INCOGNITA* (KOFOID &WHITE) CHITWOOD] RESISTANCE GENE IN PEARL MILLET [*PENNISETUM GLAUCUM* (L.) R. BR.][2]

[2] Limei Liu, Patricia Timper, Jeff Wilson, Gulia Surinder, Peggy Ozias-Akins, Joann A. Conner, Andrew H. Paterson, Katrien Devos, and Peng W. Chee To be submitted to *Crop Science*.

Abstract

Pearl millet (*Pennisetum glaucum)* is an important forage crop and a potential feed crop in the Southeastern United States. In this region, root-knot nematodes, *Meloidogyne* spp, are significant pests for field crop, including pearl millet. Because cotton and peanut have a long plant history in Georgia and other southeastern states, and root-knot nematodes cause severe yield loss for both of them. Rotation is an effective way to control the root-knot nematode damage. Pearl millet is a possible rotation crop with cotton and peanut in southeastern United States. Knowledge of root-knot nematode resistance and its inheritance will aid in breeding pearl millet with resistance to these nematodes. In this research, one major quantitative trait locus (QTL) was identified for resistance to the southern root-knot nematode through a genetic map constructed with AFLP and SSR markers, and 180 recombinant inbred lines (RILs) derived from an intraspecific cross between Tift 99B and Tift 454. The resistance QTL *QMi-LG2* has a LOD of 14 and explained 32.0% of the phenotypic variance. This nematode resistance gene locus was located in a distal region of pearl millet LG2. An expressed sequence tagged simple sequence repeat (EST-SSR) marker ICMP 3029 (ori) was found to be tightly linked to the gene and can be useful in marker-assisted selection for nematode resistance in pearl millet breeding.

Abbreviations: RILs, Recombinant inbred lines; PCR, polymerase chain reaction; QTL, quantitative trait locus; EST-SSR, expressed sequence tagged simple sequence repeat; marker-assisted selection.

Introduction

Pearl millet is indigenous to the Sahel zone of west Africa (Brunken et al., 1977) and was domesticated in Africa and India more than 3,000 years ago (Allchin, 1969; Davies, 1968). With high tolerance to dry conditions (Brunken, 1977) and low soil fertility, pearl millet has higher

and more reliable yields than other dry land crops such as sorghum or maize under these conditions (Andrews et al., 1993). Pearl millet is planted predominantly in Africa and the Indian subcontinent as a staple food.

Pearl millet is cultivated as both a forage crop and feed grain crop in the United States. Pearl millet was introduced into the United States in the nineteenth century as a forage crop (Maiti and Wesche-Ebeling, 1997). Still produced mostly for forage, pearl millet is estimated to be grown on 600 thousand hectares in the United States (Myers, 1999). Since the early 1990s, pearl millet has been suggested as a new feed grain crop for the southeastern and mid-southern regions of the United States where the soils are acidic, have low fertility, and are dry (Andrews et al., 1993; Davis et al., 2003; Kennedy et al., 2002; Myers, 1999). As a feed grain, pearl millet has a higher protein content, and a higher oil content than other common cereal grains as well as a more balanced essential amino acid profile than that of corn (Adeola et al., 1994). For these reasons, pearl millet is competitive with corn as a feed ingredient for broilers (Amato and Forrester, 1995; Davis et al., 2003), laying hens (Collins et al., 1997; Elrazig and Elzubeir, 1997; Kumar et al., 1991; Singh and Barsaul, 1976), ducks (Adeola et al., 1994), and beef cattle (Hill and Hanna, 1990). In addition to forage and feed grain crop, pearl millet is also suitable as a supplemental feedstock for ethanol production in drought prone regions of the southeastern United States where other crops do not thrive and/or their production is not economical (Gulia et al., 2007).

In the Southeastern United States, southern root-knot nematodes (RKN) [*Meloidogyne incognita* (Kofid and White) Chitwood)] can damage pearl millet roots and cause loss of grain yield (Timper et al., 2002); however, the primary concern about pearl millet susceptibility to RKN lies in its effects as a rotation crop for cotton and peanut, both of which are good hosts of

Meloidogyne spp.. Rotation is an effective and low cost method to control soil-borne diseases and pests (Brown, 1987). RKN-resistant pearl millet hybrids were shown to reduce RKN populations in soil and to increase peanut yield when grew in rotation (Timper et al., 2007). Therefore, it is a valuable crop in the crop rotation system.

Different pearl millet varieties express different levels of RKN resistance. Timper et al. (2002) reported variation among grain hybrids for resistance to RKN in a naturally infested field and in greenhouse tests. Several pearl millet land races from west and east Africa were evaluated for RKN resistance and results showed that individuals within these land races expressed a range of resistance levels from susceptible to highly resistant (Timper and Wilson, 2006). The mode of inheritance of the resistance trait among these sources is currently unknown.

Marker-assisted selection can play a more important role in plant breeding when the development and analysis of molecular markers are easier and cheaper. The basis of marker-assisted selection resides in the fact that we can infer the presence of a gene of interest through the presence of one or more DNA markers that are tightly linked to the gene (Kumar, 1999). As early as 1995, marker-assisted selection was successfully used in pyramiding resistance genes for crop breeding in beans (Kelly et al., 1995) and rice (Yoshimura et al., 1995). Marker-assisted selection has also been used in quantitative trait breeding and QTL pyramiding (Eathington et al., 1997; Guo et al., 2005; Lecomte et al., 2004; Stuber, 1994; Young, 1999). One successful story of variety development using marker-assisted selection in pearl millet was the development of the downy mildew resistant pearl millet hybrid "HHB 67 improved" (http://www.researchintouse.com/nrk/RIUinfo/PF/PSP24.htm). Many other successful stories on marker-assisted selection in backcross and gene or QTLs pyramiding were reviewed by Collard and Mackill (2008).

Genetic mapping of the RKN resistance gene(s) in pearl millet would facilitate the understanding of the genetics of the resistance and the use of the resistance gene(s) in future breeding programs. To identify RKN resistance genes or QTLs and tightly linked molecular markers for marker-assisted selection, we developed a genetic map of pearl millet using SSR and AFLP markers, and identified markers that are tightly linked to resistance genes or QTLs utilizing a recombinant inbred line (RIL) mapping population based on the cross Tift 454 × Tift 99B.

Materials and Methods

Plant Materials

Inbred lines Tift 454 and Tift 99B, which were co-developed and released by the USDA-ARS and the University of Georgia at Tifton, GA (Hanna et al., 2005a; Hanna et al., 2005b), were used to develop a RIL population with 180 lines. Inbred Tift 454 is highly resistant to RKN (M. incognita) (Hanna et al. 2005a), while Tift 99B is susceptible. The F1 plants were assessed for RKN resistance in greenhouse (Timper, unpublished data). A single, highly-resistant F1 plant was allowed to self-pollinate to establish an F2 population. The population was further advanced to F7 by the single-seed-descent method in the greenhouse.

RKN Resistance Screening was conducted in Greenhouse in 2009. The set of 180 RILs along with both inbred parents and their F_1 hybrid were planted in 10 cm diameter pots in greenhouse in a randomized complete block design with three replications. Replication one and replication two were placed in one greenhouse bay, and replication three was placed in another greenhouse bay because of the space limitation in greenhouse. The susceptible parent Tift 99B was planted in three pots per replication while the other lines were planted one pot per replication. The soil sterilizing method and greenhouse management were the same as described

by Timper and Wilson (2006). Ten days after planting, plants were thinned to one vigorous plant per pot and subsequently each pot was inoculated with 8000 eggs (4000 on one day and 4000 more two days later) of *M. incognita* race 3, which was cultured on eggplant *Solanum melongena* cv. Florida Market.

Plants were removed from pots eight weeks after inoculation and examined for egg masses on roots for replication one and two. Roots were rinsed in water and stained in a 0.05% phloxine B solution for 3-5 minutes during which egg masses are stained bright red. Egg mass was estimated on a 0 – 5 scale (Holbrook et al., 1983) as follows: 0 = no egg mass, 1 = 1 – 2 egg mass, 2 = 3 – 10 egg masses, 3 = 11 – 30 egg masses, 4 = 31 – 100 egg masses, and 5 = greater than 100 egg masses. After rating replication one and two, we found that the egg masses were very small, so we waited two more weeks to let them get bigger in replication 3.

SAS 9.2 [SAS Institute, Inc. (2008)] GLM Procedure was used for the analysis of variance (one way ANOVA with a 5% level of significance). Fisher's LSD test was done to determine differences among treatments and replications with a 5% level of significance. Microsoft Excel software was used for histograms of the frequency distribution of the progeny mean values.

DNA Extraction and DNA Marker Analyses

DNA extraction for SSR analysis followed the SDS-potassium-acetate method (Li et al., 1995) and AFLP analysis used the Qiagen's DNeasy Plant DNA Extraction Mini Kit (QIAGEN, Valencia, CA). DNA quantification used Fluorocount (GMI Inc., Ramsey, MN).

The principles of AFLP analysis were described by Vos et al. (1995). DNA (~100 ng) was first digested with *Eco*RI and *Mse*I, and then restriction site-specific adaptors were ligated to

both sides of the DNA fragments. Second, pre-amplification was carried out using single selective base primers carrying adaptor-specific sequences. Third, the preamplification products were segregated on 2% agarose gel to check the effect of enzyme digestion and preamplificaton. Fourth, 20 ~ 40-fold dilutions of preamplification products were used for the two-base-selective amplifications with one primer labeled with IRdye 700 (LI-COR Inc., Lincoln, NE). Last, the amplification products were separated on 6% polyacrylamide gel and visualized using LI-COR 4300 (LI-COR Inc., Lincoln, NE) following the manufacturer's instructions. Overall, 64 primer combinations for AFLP analysis were used to screen the parents and F_1, and the 37 primer combinations that produced the most polymorphic bands were chosen to genotype the mapping population. Polymorphic bands were scored manually, and fragment lengths were estimated according to a 50-700 bp sizing standard (LI-COR Inc., Lincoln, NE).

The SSR primers used come from the following resources: 103 pairs of PSMP primers from Qi et al. (2004); 19 pairs of CTM primers from Budak et al. (2003); 55 pairs of PSMP primers from Tracy Money at John Innes Centre, Norwich, UK. (personal communication); 112 pairs of ICMP primers from Senthilvel et al. (2008; 2004); 19 pairs of CUMP primers from Yadav et al. (2007); 102 pairs of PSMS primers from Bertin et al. (2005); 96 pairs of SRSC primers from Feltus et al. (2006); 19 pairs of PCISP primers from Dr. CT Hash, International Crops Research Institute for the Semi-Arid Tropics, Andhra Pradesh, India (personal communication); and 169 pairs of UGTP primers developed in this study.

PCR amplifications were carried out on a PTC-200 Thermo Cycler (MJ Research Inc., Waltham, MA) or MyCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). Each 10 µl reaction mixture contained 10 – 15 ng of genomic DNA, 50 pM of each primer, 1 mM $MgCl_2$, 0.1 mM of each dNTP, 1 × reaction buffer, and 0.2 U *Taq* polymerases. The PCR cycles began

with 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 54 °C for 45 s, 72 °C for 60 s, ended at 72 °C for 10 min, and store at 4 °C. PCR products were separated on a 10% polyacrylamide gel. Electrophoresis instrument, running conditions, and silver staining methods are the same as described by Zhang et al. (2002).

Genetic Mapping and QTL Analysis

Mapping software JoinMap 3 (Van Ooijen and Voorrips, 2001) was used to construct the linkage maps. A LOD score of 3.0 and recombination frequency of 0.4 were used to determine linkage. Kosambi map function (Kosambi, 1944) was used to convert recombination frequencies to map distances. The Chi-squared test was performed on all markers to test for segregation distortion. The names of linkage groups and the orientation of the chromosome arms were inferred by comparing the location of anchor SSR markers on previously published maps (Qi et al., 2004; Senthilvel et al., 2008). The graphs of the linkage groups were drawn using MapChart 2.2 (Voorrips, 2002).

QTL mapping analyses were conducted using MapQTL 5.0 (Van Ooijen, 2004) with mapping step size of 1 cM and five neighboring markers. The LOD threshold 3.2, which was determined by permutation test in MapQTL 5.0 with a genome-wide significance level of $P<0.05$ and n=1,000, was used to declare a significant QTL. Additive effects are defined with respect to the alleles of Tift 99B. Thus, positive genetic effects indicate the alleles of Tift 99B increase the nematode egg mass rating.

Results

Phenotypic Data Analyses

A single factor ANOVA with three replications showed that the nematode egg mass ratings were significantly different ($P < 0.0001$) among genotypes. We noticed that the nematode

ratings were similar for the 1st and 2nd replications, but the 3rd replication had greater egg masses than the other two replications. To investigate the effect of replications on the analysis, a Fisher's LSD test was done. The Fisher's LSD test showed that the difference between the 1st and 2nd replications was not significant at a 5% level of significance, but the 3rd replication was significantly different from the 1st and the 2nd replications. So, the average of the 1st and the 2nd replication was analyzed separately from the 3rd replication.

The egg mass rating of the F_1 was intermediate between the two parents (Fig. 4.1). The frequency distribution patterns of egg mass ratings of the RILs were similar for analyses based on either the mean of the 1st and 2nd replication or the 3rd replication. One peak was near Tift 454 and another was around Tift 99B.

Identification of QTL Associated with RKN Resistance

A genetic map, containing 468 loci (107 SSR loci, 361 AFLP loci) in 7 main linkage groups (LGs) and 2 small LGs each with only 2 loci, was used to conduct QTL analysis. Linkage group names were defined according to anchor markers previously mapped by Qi et al. (2004) and Senthivel et al. (2008). The detailed map information is presented in Liu et al. (2012).

The results of the QTL analysis were consistent between the average of the 1st and the 2nd replication and the 3rd replication. Only one region that was above the 3.2 LOD threshold was detected in LG2 in either data sets. Both LOD value peaks were located at 96.9 cM on LG2 in the interval between marker ICMP 3029 (ori) and marker AFLP 177 (Table 4.1, Fig. 4.2). The LOD thresholds were essentially the same, 13.96 and 13.62 for average of the 1st and 2nd replication and the 3rd replication, respectively (Table 4.1). The phenotypic variances explained by the QTLs in each data set were 32.4% and 31.9% for the average of the 1st and 2nd replications and of the 3rd replication, respectively.

The peak of the QTL located between AFLP177 and EST-SSR marker ICMP 3029 (ori). The LOD scores (based on average of replications 1 and 2) for AFLP177, QTL peak and ICMP 3029 (ori) are 12, 13.6, 13.1, respectively. So according to the 2 LOD principle (LOD score decrease by one LOD at both sides of the peak) to define a QTL interval, the QTL interval for RKN should extend out of our map region. We showed this extra region (unknown length) with a line (Table 4.1, Fig. 4.3). We named this locus *QMi-LG2* by following the conventional nomenclature for QTLs in which the acronym of the scientific name of the pathogen causing the disease in lowercase is followed by the chromosome name.

The resistant parent Tift 454 contributed the allele that significantly decreased the nematode rating by 0.62 and 0.83 for the average of the 1st and 2nd replication and for the 3rd replication, respectively, for this QTL.

Table 4.2 shows some of the most RKN-resistant RILs and most susceptible RILs and the genotypes at the marker loci in the QTL regions in LG2. Generally speaking, the RKN-resistant RILs carried the favorable allele while the susceptible RILs carried the non-favorable susceptible allele. The nearest EST-SSR marker ICMP 3029 (ori), 0.3 cM from the QTL peak, is a co-dominant marker (Senthilvel et al., 2004) and is confirmed by the segregation patterns of the parents, F_1 and the RILs of this population (Fig. 4.3). Given that ICMP3029 (ori) was used in the homozygous condition as the selection marker on the quality trait data set based on the average of the three replications, 85% (61 /72) of the RILs would have been resistant to RKN.

Discussion

Nematode Resistance Gene

Nematode resistance in plants can be controlled by a single dominant, semi-dominant gene (Ballvora et al., 1995; Lorieux et al., 2003; Messeguer, 1991; Pineda, 1993; Salentijn et al.,

1992; Webb et al., 1995; Williams et al., 1994) or by gene that are recessive (Wang and Goldman, 1996). It has also been shown to be quantitatively inherited (Faghihi et al., 1995; Kreike, 1993; Trudgill, 1991; Wang and Goldman, 1996). Nematode resistance genes have not previously been mapped in pearl millet, but two dominant genes were thought to control the nematode resistance in the pearl millet land race "Zongo" (Timper and Wilson, 2006). There are also other pearl millet germplasm sources with higher levels of RKN resistance than "Zongo", "SoSAt-C88" for an example that probably will have additional genes involved in RKN resistance (Timper and Wilson, 2006).

The 3rd replication had a bigger egg masses and was easier to differentiate resistance from susceptible genotypes. With the 1st and the 2nd replication, there was a greater likelihood of rating a susceptible plant resistant because of the small egg masses. The 3rd replication had higher egg mass ratings than the other two replications, so we analyzed the average of the 1st and 2nd replication separately from the 3rd replication. However, even though the two data sets were used separately to conduct the QTL analyses, the LOD values, the QTL positions, and the phenotypic variances explained were almost the same (Table 4.1). In the three replications, the LOD value was around 14, and the phenotypic variance explained the QTL was about 32.0%, and the position of the gene was located at 96.9 cM in the LG2. Thus, both results showed that RKN- resistance in pearl millet appears to be located at the far distal region of LG2 around the EST-SSR marker ICMP3029 (ori).

Out of all the nematode resistance QTLs identified in monocots, only two QTLs that were resistant to wheat root-lesion nematodes (*Pratylenchyus thornei* and *P. neglectus*) were mapped on the short arm of wheat chromosome 6D (Zwart et al., 2010). This chromosome

segment in wheat has a homologous relationship with the pearl millet LG2 distal end (Devos, 2005) where the pearl millet RKN resistance QTL (gene) was located. This information may benefit future marker development for use in breeding pearl millet with nematode resistance.

The RKN resistance gene from Tift 454 is incompletely dominant or semidominant because the parent Tift 454 is almost immune, the F_1 is resistant but still has egg masses, and the parent Tift 99B is susceptible. The ancestors of Tift 454 include Tift $23D_2A_1$ and napier grass (*P. purpureum* Schumacher) (Hanna et al., 2005a). No direct RKN resistance tests were conducted on the parental line Tift $23D_2A_1$, but the RKN resistance tests have been done in green house on the hybrid (Tift 23A × 1258) and Gahi 1 (a synthetic pearl millet that contains Tift 23). Both were susceptible to RKN (Johnson et al., 1977) and this means that the parental line Tift $23D_2A_1$ should not contain the semi-dominant RKN resistance gene. Therefore, it is possible that the gene may have come from the napier grass. However, RKN resistance genes exist in pearl millet because nematode resistance hybrids and open pollinated varieties have been identified in pearl millet (Jonson et al., 1977; Timper et al., 2006). Two dominant genes supposedly control the resistance in the pearl millet cultivar Zongo from West Africa (Timper et al., 2006), but neither of them has yet been mapped. Consequently, the allele relation between our mapped gene and the genes in other pearl millet varieties is unknown.

Molecular Marker Future Uses in Breeding

Toward its deployment in breeding, validation of resistance from donor parent Tift 454 is important to confirm the function of the QTL in other genetic background. This is a major QTL and the likelihood of this QTL being valuable in marker-assisted selection in a future breeding program is high.

Nematode resistance screening in the greenhouse or field is tedious, expensive (Glover et al., 2004), and destructive. Timing is another concern for the screening method. If one cannot get the screening results before flowering; no targeted individual plant can be identified before backcrossing. Furthermore, nematode resistance is confounded with other diseases on the same plants because nematode infection can increase susceptibility to other diseases (Deberdt et al., 1999), so it is inconvenient in a classical disease pyramiding breeding program. Marker-assisted selection is of great interest in breeding pearl millet for nematode resistance. The basic principle of marker-assisted selection is the application of linkage disequilibrium between markers and a QTL or gene of interest and using direct selection of markers to indirectly select a linked QTL or gene (Hospital, 2009).

The codominant EST-SSR marker ICMP3029 (ori) is the most tightly linked genetic marker to the nematode resistance. In our mapping population, 85% (61/72) of the RILs that carried the homologous ICMP3029 (ori) were resistant to RKN. These results suggest that the EST-SSR marker ICMP3029 (ori) will be helpful in marker-assisted selection to develop nematode resistant populations derived from Tift 454, assuming that this marker shows polymorphism between the parents of other populations. With the use of this marker as a selection tool, the breeders could ensure that the resistance gene was not lost during cultivar development.

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Table 4.1. QTL analysis results in LG2

Data	Interval	Peak position (cM)	LOD	Additive†	% Var‡
Mean of 1st and 2nd replication	Xaflp177-Xicmp3029	96.9	13.96	0.62	32.4
Only the 3rd replication	Xaflp177-Xicmp3029	96.9	13.62	0.83	31.9

† A positive additive effect indicates that the allele of Tift 99B is associated with an increased egg mass rating

‡ Percent phenotypic variance explained by the QTL

Table 4.2. Selected RKN resistance and susceptible RILs from the mapping population and their genotypes at the marker loci in the QTL region on LG2

	Marker			Egg mass rating		
RIL	PSMP708	ICMP3029†	AFLP177†	Rep1	Rep2	Rep3
RIL-31	B	A	A	3	2	3
RIL-56	A	A	A	3	2	4
RIL-108	B	A	A	3	2	4
RIL-162	B	A	A	3	3	3
RIL-175	A	A	A	3	3	3
RIL-183	A	A	A	2	3	4
RIL-17	B	B	B	0	0	0
RIL-44	A	B	B	0	0	0
RIL-81	B	B	B	0	0	0
RIL-100	B	B	B	0	0	0
RIL-109	A	B	B	0	0	0
RIL-131	A	B	B	0	0	0

Alleles from susceptible parent Tift 99B that are associated with increased egg mass rating are indicated as A. Alleles from resistant parent Tift 454 that are associated with decreased egg mass rating are indicated as B.

† indicates the markers were significantly ($P < 0.0001$) associated with RKN resistance in the QTL analysis

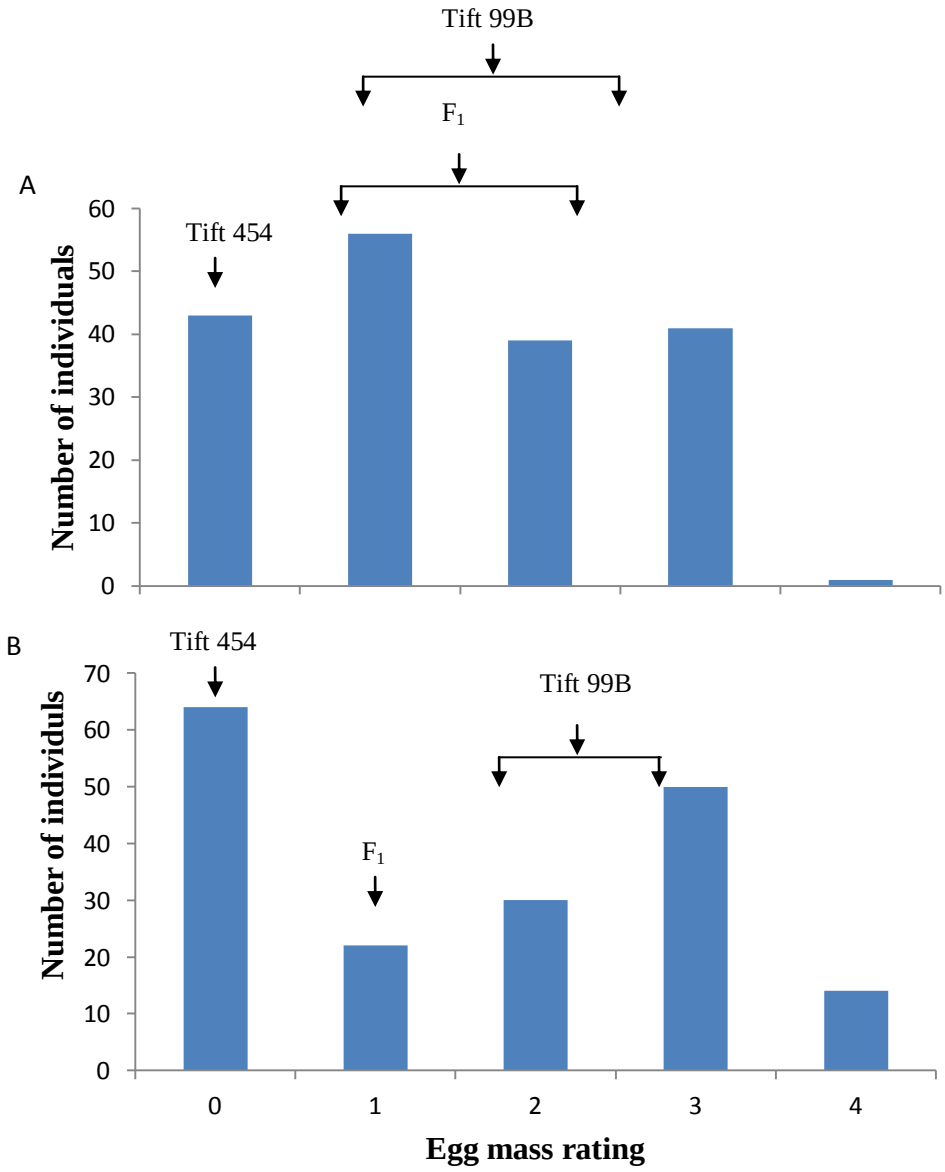

Figure 4.1. Frequency distribution of nematode egg mass rating for the RIL mapping population derived from Tift99B (susceptible) / Tift454 (resistant). A: show the frequency distribution of average of replication 1 and replication 2 in greenhouse bay 1. B: show frequency distribution of replication 3 in greenhouse bay 2. The arrows show average ± standard deviation

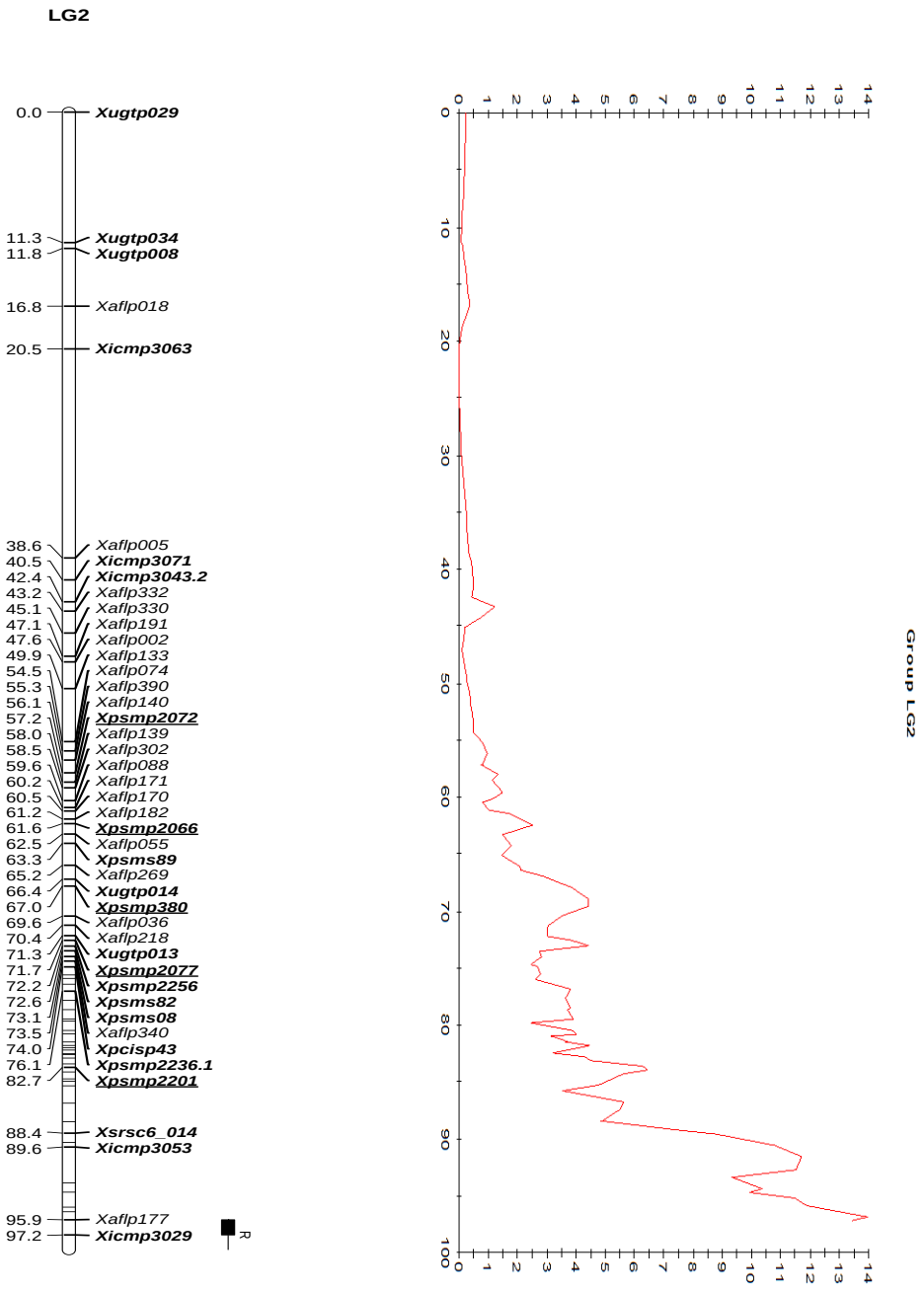

Figure 4.2. LOD scan for interval mapping on pearl millet LG2 for root-knot nematode resistance based on average of the 1st and 2nd replication. The black bar R shows the 1.5-LOD likelihood interval for the presumed QTL. The line connect to the black bar shows the QTL interval extends out of current map. Non-AFLP markers are in bold and the linkage group anchor markers are in bold and underlined.

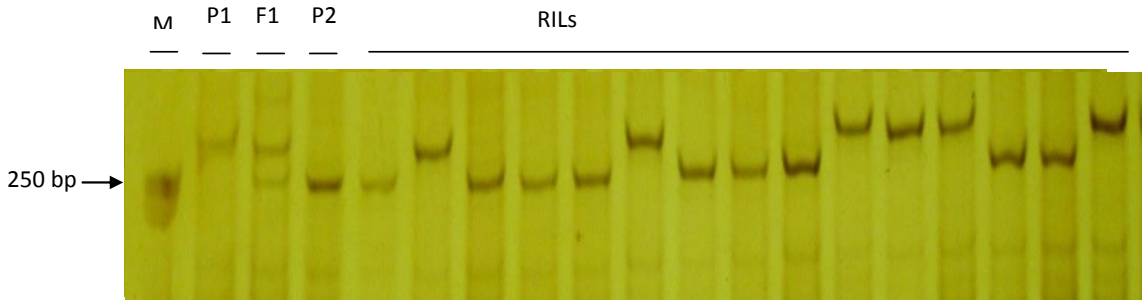

Figure 4.3. Segregation pattern of EST-SSR marker ICMP3029 (ori) on 10% polyacrylamide gel in intervarietal RIL mapping population derived from 'Tift 99B' × 'Tift 454'. M: molecular ladder. P_1: Tift 99B. P_2: Tift 454. RILs: recombinant inbred lines.

CHAPTER 5

CONCLUSION

Pearl millet is a valuable crop and can tolerate drought, acidic soil, low soil fertility, and it has a higher and more reliable yield than other land crops such as sorghum or maize (Andrews et al., 1993) under dry conditions. Nematodes are constraints to pearl millet production (Lee et al., 2004) and cause grain yield loss in the southeastern United States (Timper et al., 2002). A larger concern is the increase in nematode populations on pearl millet and their effect on susceptible rotation crops. Nematode resistant pearl millet is an effective rotation crop with peanut that reduces nematode populations in soil and increases peanut yield (Timper et al., 2007).

Nematode resistance screening in greenhouse or field to develop nematode resistance is tedious, expensive (Glover et al., 2004), and crop destructive. Timing is another concern, if one can get the screening results before flowering, targeted individual plant can be identified for effective backcrossing. Marker-assisted selection is of great interest in breeding pearl millet for nematode resistance because it can eliminate many of the difficulties in breeding for pest resistance.

A linkage map with a dense set of markers in needed as a foundation to be successful in molecular breeding. Genetic mapping plays an important role in marker-assisted selection in breeding as well as for many other topics (Nagamura et al., 1997). The previous pearl millet genetic maps were either incomplete due to lack of markers (Liu et al., 1994; Pedraza-Garcia et al., 2010; Supriya et al., 2011) or contain numerous big gaps due to marker clustering on the central regions of Linkage Groups (LGs) (Qi et al., 2004). In this study, we developed a detailed

genetic map of pearl millet to facilitate the development of pearl millet genetic materials needed in the current market place, particularly RKN resistance.

To perform genetic mapping more efficiently, we developed addition AFLP and SSR markers as well as using many of those previously developed for the construction of earlier pearl millet genetic maps. Besides mapping a QTL for RKN resistance, we further demonstrate the utility of this map by mapping a number of QTLs for agronomic traits. To develop added efficiency to this task, we used a RIL population because genotyping only needs to be performed one time while phenotyping can be done multiple times to reduce environmental and experimental errors (Broman, 2005). Three hundred sixty-one markers were mapped onto 180 RILs for a total map length of 735 cM that putatively covered all of the seven chromosomes found in pearl millet.

The foremost outcome was that the QTL *QMi-LG2* that was mapped at distal region of LG2 explained 32.0% of the phenotypic variance and should be considered as a strong RKN-resistance genetic resource. One putative QTL each for five agronomic traits of plant height, hundred seed weight, heading date, panicle length, and panicle width were detected and prove the utility of this map and its constitutive markers.

This new pearl millet genetic linkage map provided the location of a number of QTLs along with new insights into the pearl millet genome structure as well as giving more SSR and AFLP markers for QTL analysis and gene mapping, map-based gene cloning, and molecular breeding.

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