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Mapping of QTLs underlying flowering time in sorghum [*Sorghum bicolor* (L.) Moench]

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Due to its critical importance in crop yield, the photoperiodic regulation of flowering time is considered an important trait in sorghum breeding programs. In this study, quantitative trait loci for flowering time were detected using an F_2 population derived from a cross between Kikuchi Zairai, a late-flowering cultivar originating from Japan and SC112, an early-flowering cultivar originating from Ethiopia. F_2 plants were grown with their parents under a natural day length and a 12 h day length. Two linkage maps were constructed using 213 simple sequence repeats markers. Nine quantitative trait loci controlling flowering time were identified in F_2 plants grown under a natural day length, whereas 7 QTLs were identified under a 12 h day length. Five QTLs controlling flowering time were shared under both of the day length conditions.

Key Words: sorghum, flowering time, day length, quantitative trait loci, mapping.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important food source for the world's poorest people who inhabit food-insecure regions worldwide, and sorghum has unique properties that make it well suited for food use. Some sorghum varieties are rich in antioxidants and all sorghum varieties are gluten-free and attractive alternatives for those who suffer from wheat allergy (Harris *et al.* 2007). Farrel *et al.* (2006) suggested that sorghum will be of increasing importance to feed the world's expanding populations. Sorghum is also an important animal feed used in many countries, such as the US, Mexico, South America, Australia and Japan (Mekbib 2007). Moreover, sorghum is a biofuel crop of growing importance and is currently the second source of grain-based ethanol in the US after maize. As much as 12% of sorghum production is used to produce ethanol and its various co-products (Scheinost *et al.* 2001, Wagoner 1990).

Sorghum was first domesticated in Ethiopia and is distributed widely throughout tropical, subtropical and temperate environments (Teshome *et al.* 2007). The adaptation in sorghum to a broad range of growing conditions is mainly in response to the photoperiod (Chanterau *et al.* 2001). Flowering time is an important adaptive character that impacts the yield and quality of crop plants. Furthermore, flowering is a crucial event in the life cycle of seed-propagated plants because of its key role in the adaptation of a plant to its environment by tailoring the vegetative and reproductive growth phases to the local climate (Buckler *et al.* 2009).

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Childs *et al.* (1997) reported that a series of six maturity genes was found to alter flowering time in sorghum: Ma_1, Ma_2, Ma_3, Ma_4, Ma_5 and Ma_6. The four maturity genes Ma_1–Ma_4 inhibit flowering under long-day conditions, whereas they promote flowering under short-day conditions. Mutations in Ma_1 cause the greatest reduction in the sensitivity to long days and mutations in Ma_2, Ma_3 and Ma_4 generally have small effects on the sensitivity to long days (Quinby 1967); however, even sorghum varieties with recessive ma_1, ma_2 and ma_3 alleles flower later under long-day condition than under short days (Pao and Morgan 1986). The genes Ma_5 and Ma_6 represent a special case because they very strongly inhibit floral initiation, regardless of the day length, only when both are present in the dominant form (Childs *et al.* 1997).

Childs *et al.* (1997) reported that Ma_3 is one of six genes that regulate the photoperiodic sensitivity of flowering in sorghum. To provide evidence that Ma_3 is synonymous with PHYB, these authors mapped PHYA-, PHYB-, PHYC- and Ma_3-linked molecular markers and then sequenced the three phytochrome genes, demonstrating that ma_3^R in sorghum contains a mutation in PHYB. As a result, Childs *et al.* (1997) observed that Ma_3 encodes the apoprotein of phytochrome B. Recently, Murphy *et al*. (2011) identified that Ma_1 is the pseudoresponse regulator protein 37 (PRR37) through the positional cloning and analysis of SbPRR37 alleles that modulate flowering time in sorghum produced for grain and biofuel. Many QTLs controlling flowering time were identified in previous studies. Chanterau *et al.* (2001) investigated the genetic control of flowering time in sorghum using an RIL population derived from a cross between IS2807, a slightly photoperiod-sensitive tropical caudatum landrace and TS7680, a highly photoperiod-

sensitive tropical guinea landrace. Emphasis was placed on identifying the most relevant trait to account for the basic vegetative phase and photoperiod sensitivity.

Using an RIL population of sorghum derived from the cross between 296B and IS18551, Srinivas *et al.* (2009) detected nine QTLs controlling flowering time: two QTLs on SBI-01, two QTLs on SBI-02 and one QTL each on SBI-03, SBI-05, SBI-06, SBI-07 and SBI-08. The phenotypic variation explained by each QTL ranged from 6.1 to 13.5%. The QTL detected on SBI-01 (*qDan-sbi01-2*) explained 13.5% of the phenotypic variation and was considered to be a major QTL controlling flowering time in sorghum.

Despite extensive analysis of the day length control of flowering in sorghum (Chanterau *et al.* 2001, Srinivas *et al.* 2009), little is known with regard to the effect of variations in the photoperiod or day length on the sorghum flowering time (Menz *et al.* 2002). Such effects are a major concern to breeders because understanding the genetics of flowering is essential to adapt the life cycle of sorghum to the agro-environments in which it is grown. Although genetic studies are inconclusive as to the number of genes and the type of gene action involved in determining the flowering time and sensitivity to photoperiod, a series of studies based on different types of populations have identified quantitative trait loci (QTLs) that are associated with flowering time in sorghum. In contrast, the QTLs controlling the sensitivity to photoperiod changes have not been described in detail or under a wide range of photoperiodic conditions.

SSR markers are effective at detecting the genotypic variation caused by a high degree of polymorphism (Yonemaru *et al.* 2009) and SSR markers with a high degree of polymorphism contribute to the genetic dissection of agriculturally important traits in sorghum. Furthermore, the construction of linkage maps is fundamental to identify the chromosomal location of genes controlling flowering time in sorghum, and genetic studies of flowering time culminated in the identification of genes that influence flowering time in sorghum. To gain a better understanding of the genetic control of flowering time in sorghum, we conducted QTL analysis of the flowering time using SSR markers.

Materials and Methods

Mapping population

A set of 144 F_2 plants were developed from the cross between Kikuchi Zairai (Japan, late-flowering cultivar) and SC112 (Ethiopia, early-flowering cultivar) selected from a diversity research set of sorghum germplasm (Shehzad *et al.* 2009). The F_2 plants and their parental cultivars were sown in early May 2008 in the experimental field of Tsukuba University under a natural day length, with a planting density of 1.5 m × 20 cm. During the growing season, the day length was 14.25 h in May, 14.40 h in June, July and August and 13 h in September, decreasing to 12.5 h after September. The F_2 plants and their parents were also grown during the growing season (May–November) in a controlled 12 h day length facility in 2008. Standard agronomic practices were applied from sowing to harvest. The days to flowering were scored as the number of days from sowing to the time when 50% of the panicle had flowered.

Genomic DNA isolation

The leaves of 40-day-old plants were sampled and used for genomic DNA isolation. Extraction of DNA from leaf tissues was based on the CTAB method described by Murray and Thomson (1980), with modifications. The extraction buffer was composed of 2% CTAB, 50 mM Tris-HCl (pH 8), 10 mM EDTA, 0.7 M NaCl, 0.1% SDS, 0.1 mg/ml proteinase K, 2% insoluble PVP and 2% 2-mercaptoethanol. To remove the cellular debris and proteins, we used chloroform-isoamyl alcohol (24 : 1 v/v) extraction. The DNA was then precipitated by adding 2-propanol and the precipitate was rinsed with 70% and then 95.5% ethanol. The final precipitate was dissolved in 50 µl 1/10 TE and stored at 4°C.

Screening for SSR markers

Microsatellite primers were selected from the genome-wide simple sequence repeats markers developed by Yonemaru *et al.* (2009) using whole-genome shotgun sequences of sorghum. A total of 580 genome-wide SSR markers were randomly selected from all ten of the sorghum chromosomes and screened for the detection of polymorphisms between the parental cultivars of the F_2 mapping population. Overall, 213 markers were polymorphic and were used for constructing linkage maps.

PCR conditions and electrophoresis

PCR amplifications of the sorghum SSRs were performed in a 10 µl reaction mixture containing 10 ng DNA template, 10 × PCR buffer (Mg^{2+} concentration: 20 mM), 2 mM dNTP, 25 ng each primer and 0.02 U Blend Taq Plus polymerase enzyme using Applied Biosystems 9700 and 2700 thermal cyclers. The annealing temperature was determined for all of the markers using the mean of the Eppendorf MasterCycler ep gradient S. The thermal cycler protocol consisted of denaturation at 94°C for 5 min, 35 cycles of 94°C, 55 to 65°C and 72°C, followed by 7 min at 72°C and cooling at 10°C. The PCR products were analyzed on 30% acrylamide gels (10 cm in size) using a constant voltage of 200 V and current of 500 mA for 75 to 110 min, depending on the size of the PCR product. TBE buffer (10×) was used for casting the gel and 1× TBE buffer was used during electrophoresis; the gel was stained with ethidium bromide solution for 5 to 10 min and photographed using a Kodak Digital Science EDAS 290 ver. 3.6 with Kodak ID Image analysis software ver. 3.5. Different bands for the same SSR primer were grouped according to their respective size by comparison with a 50 bp ladder DNA size marker.

Construction of genetic linkage map and mapping of QTLs controlling flowering time

Two linkage maps were constructed for F_2 plants grown

under natural day length and under controlled day length using the computer software MAPMAKER version 3.0 (Lander *et al.* 1987). MAPMAKER performs full multipoint linkage analyses (simultaneous estimation of all of the recombination fractions from the primary data). The linkage groups identified were considered not to be linked if the distance between the flanking markers was greater than 35 cM. The map distances (in centimorgans) were calculated using the Kosambi mapping function. QTL analysis was performed with the composite interval mapping (CIM) method of Windows QTL cartographer (WinQTL) version 2.5 (Wang *et al.* 2004). The LOD threshold for declaring the presence of a QTL for the trait-environment combination was defined by the 1000 permutation test at ≥2.5. The position at which the logarithm of the odds (LOD) score curve reached its maximum was used as the estimate of the QTL location. The value of the additive effect of each QTL peak LOD score position was computed. The percentage of the phenotypic variance explained by a QTL was estimated as the coefficient of determination (R^2) using single-factor analysis from a general linear model procedure (Wang *et al.* 2004). QTLs detected for the different day length environments were considered to be the same if the estimated map position of their peaks fell within 20 cM of each other.

Results

Phenotypic data analysis

The days to flowering varied widely among the parental accessions and F_2 plants grown under a natural day length, whereas the male parent, SC112, and the female parent, Kikuchi Zairai, flowered 67 and 132 days after sowing, respectively. The frequency distribution for the flowering time in F_2 plants ranged from 68 to 135 days and was almost within the variation of their parents (Fig. 1). The analysis indicated considerable differences between the parental cultivars and their F_2 plants with regard to the variation in flowering time. Under 12 h day length, the number of days to flowering was 58 days for SC112 and 102 days for Kikuchi Zairai, and the variation in the flowering time among F_2 plants ranged from 56 to 71 days (Fig. 2).

Linkage mapping and identification of QTLs controlling flowering time

Of the 580 markers screened using the parental cultivars Kikuchi Zairai and SC112, 213 SSR primers generated polymorphic bands and showed a clear and polymorphic banding pattern between the parental cultivars. The polymorphic SSR markers were used for the construction of linkage maps and the mapping of the QTLs controlling flowering time in the F_2 population under natural day length and 12 h day length.

The final map constructed using F_2 plants grown under natural day length included 178 SSR markers that were distributed throughout 17 linkage groups, spanning a length of 2468 cM (Fig. 3). The linkage groups were assigned to the

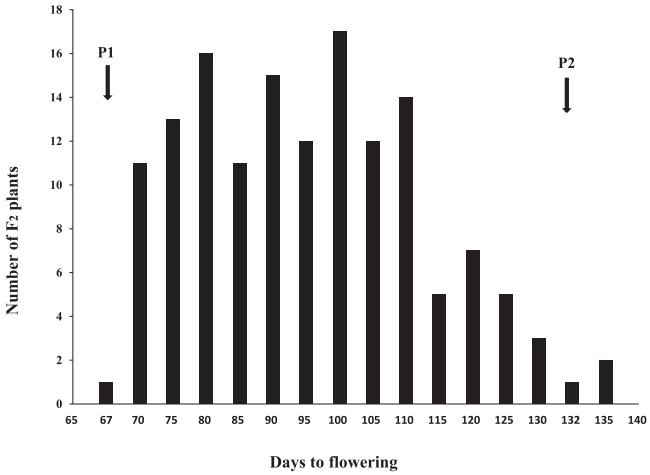

Fig. 1. Variation in flowering time in F_2 plants and their parents grown under natural day length (P1: SC 112, P2: Kikuchi Zairai)

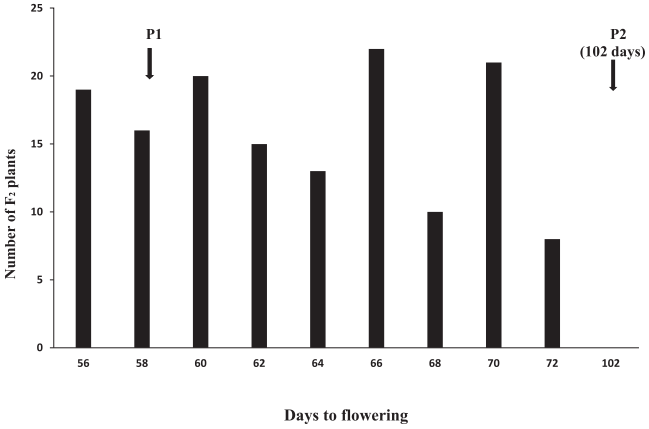

Fig. 2. Variation in flowering time in F_2 plants and their parents grown under 12 h day length (P1: SC 112, P2: Kikuchi Zairai)

ten chromosomes based on the positioning of the mapped SSRs described by Yonemaru *et al.* (2009) and the linkage group nomenclature followed the chromosome naming suggested by Kim *et al.* (2005). The coverage of the SSR markers was relatively equal across all of the chromosomes. The number of markers represented per individual chromosome ranged from 6 on chr 6b to 18 on chr 1. The average number of markers mapped to each chromosome was 10. The distance between the markers ordered at a LOD score ≥2.5 ranged from 2.8 to 33.1 cM, with an average distance of 14 cM between the markers. The distance covered by the markers ranged from 55.9 cM on chr 1b to 225.6 cM on chr 1.

The second linkage map was constructed in a similar fashion using F_2 plants grown under 12 h day length and included 175 SSR markers, covering a total genetic distance estimated at 2340 cM (Fig. 4). The coverage of the SSR markers was relatively equal across all of the chromosomes. The linkage groups ranged from 42.3 cM on chr 1b to 225.7 cM on chr 1 and were assigned to the ten chromosomes. The number of markers represented per individual

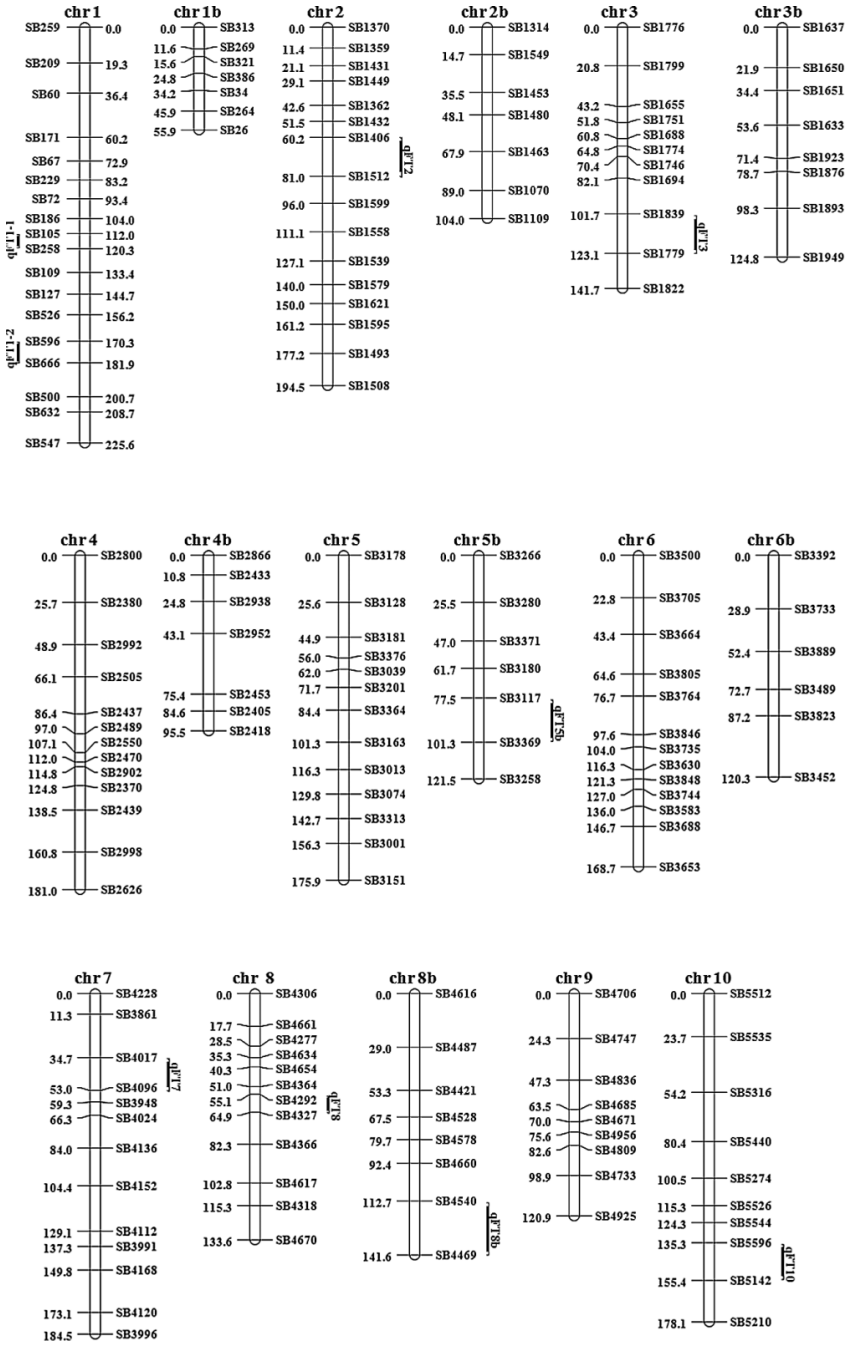

Fig. 3. Location of QTLs for flowering time measured in this study on a genetic linkage map based on F_2 mappinjg population grown under natural day length. QTLs are represented by bars (1-Lod interval) and extended lines (2-LOD interval).

chromosome ranged from 6 on chr 1b and chr 6 to 18 on chr 1. The average number of markers mapped to each chromosome was 10. The distance between the markers ordered at LOD score ≥2.5 ranged from 5 to 31 cM, with an average distance of 13 cM between the markers.

Using CIM analysis with a 1000 permutation test, 9 QTLs controlling flowering time were identified in F_2 plants grown under natural day length (Table 1): *qFT1-1* and *qFT1-2* on chr 1, *qFT2* on chr 2, *qFT3* on chr 3, *qFT5b* on chr 5b, *qFT7* on chr 7, *qFT8* on chr 8, *qFT8b* on chr 8b and *qFT10* on chr 10. These QTLs were mapped with an additive effect that ranged from (3.5) for *qFT1-1* to (6.4) for *qFT5b* and a dominance effect that ranged from (−7.5) for *qFT8* to (9.7) for *qFT2*. The phenotypic variation explained by each QTL ranged from (3.4%) for *qFT1-2* to (9.2%) for *qFT2*, as shown in Table 1. The 9 QTLs identified under the natural day length explained 60% of the total phenotypic variation and were mapped with a LOD score ranging from 2.6 for *qFT1-1* to 6 for *qFT2*.

Under 12 h day length, 7 QTLs controlling flowering

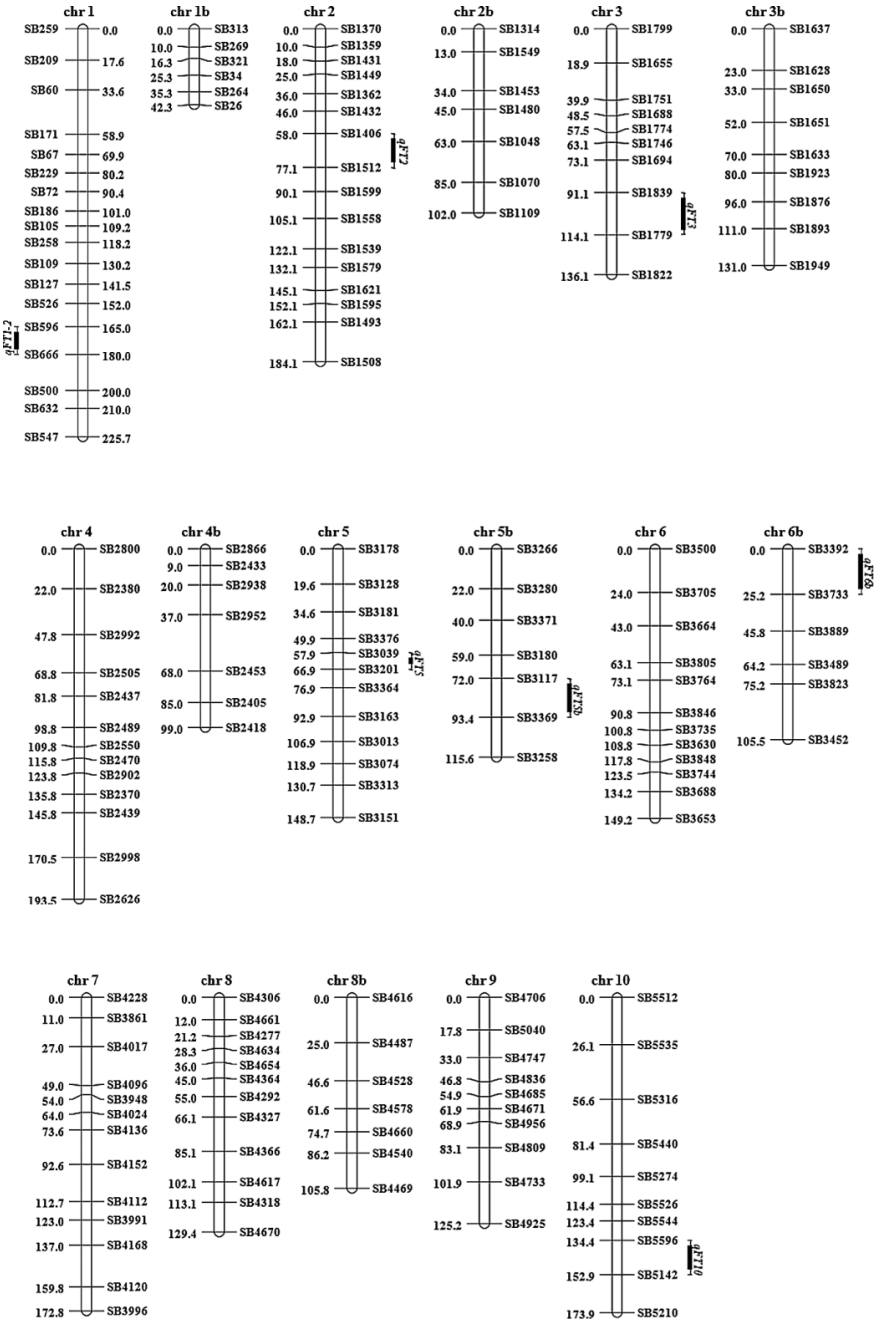

Fig. 4. Location of QTLs for flowering time measured in this study on a genetic linkage map based on F_2 mappinjg population grown under 12 h day length. QTLs are represented by bars (1-Lod interval) and extended lines (2-LOD interval).

time were identified (Table 2). Among these QTLs, *qFT1-2* on chr 1, *qFT2* on chr 2, *qFT3* on chr 3, *qFT5b* on chr 5b and *qFT10* on chr 10 were similarly identified under natural day length. Nevertheless, *qFT5* on chr 5 and *qFT6b* on chr 6b were mapped only under 12 h day length. The 7 QTLs determined under 12 h day length explained the additive effect that ranged from (1.2) for *qFT6b* on chr 6b to (4.43) for *qFT5b* on chr 5b and the dominance effect that ranged from (–10.2) for *qFT2* on chr 2 to (–4.2) for *qFT3* on chr 3. The phenotypic variation explained by each QTL ranged from (4.1%) for *qFT1-2* on chr 1 to (8.8%) for *qFT10* on chr 10 (Table 2). The 7 QTLs identified under 12 h day length explained 46.6% of the total phenotypic variation.

Accordingly, five QTLs were mapped under natural and 12 h day lengths; however, *qFT5* on chr 5 and *qFT6b* on chr 6b were identified only under 12 h day length and explained only 11.2% of the phenotypic variation. Furthermore, *qFT1-1* on chr 1, *qFT7* on chr 7, *qFT8* on chr 8 and *qFT8b* on chr 8b were identified only under natural day length and explained 27.1% of the phenotypic variation.

Table 1. QTLs identified under natural day length

QTL	Chr	Interval	Map position (cM)	LOD	Additive effect[a]	Dominance	Var. Exp[b]
qFT1-1	Chr 1	SB105 SB258	112.0 120.3	2.6	3.5	−5.1	5.3
qFT1-2	Chr 1	SB596 SB666	170.3 181.9	4.2	3.7	−4.0	3.4
qFT2	Chr 2	SB1406 SB1512	60.2 81.0	6	5.7	9.7	9.2
qFT3	Chr 3	SB1839 SB1779	101.7 123.1	5.6	5.1	−2.4	6.3
qFT5b	Chr 5b	SB3117 SB3369	77.5 101.3	6.5	6.4	−7.1	6.5
qFT7	Chr 7	SB4017 SB4096	34.7 53.0	5.0	3.6	−6.0	7.3
qFT8	Chr 8	SB4292 SB4327	55.1 64.9	2.7	5.2	−7.5	6.8
qFT8b	Chr 8b	SB4660 SB4540	112.7 141.6	4.8	3.6	−6.2	7.7
qFT10	Chr 10	SB5596 SB5142	135.3 155.4	4.3	6.0	−5.4	7.5

[a] CS112 alle decreased the number of days to flowering.
[b] Phenotypic variation explained by each QTL.

Table 2. QTLs identified under 12 h day length

QTL	Chr	Interval	Map position (cM)	LOD	Additive effect[a]	Dominance	Var. Exp[b]
qFT1-2	Chr 1	SB596 SB666	165.0 180.0	3.2	4.1	−7.3	4.1
qFT2	Chr 2	SB1406 SB1512	58.0 77.1	4.8	2.2	−10.2	8.3
qFT3	Chr 3	SB1839 SB1779	91.1 114.1	6.1	4.4	−4.2	7.0
qFT5	Chr 5	SB3039 SB3201	57.9 66.9	5.7	2.3	−5.1	6.2
qFT5b	Chr 5b	SB3117 SB3369	72.0 93.4	6.2	4.4	−7.2	7.2
qFT6b	Chr 6b	SB3392 SB3733	0.0 25.2	2.8	1.2	−8.2	5.0
qFt10	Chr 10	SB5596 SB5142	134.4 152.9	4.9	5.5	−6.3	8.8

[a] CS112 alle decreased the number of days to flowering.
[b] Phenotypic variation explained by each QTL.

Table 3. List of QTLs controlling flowering time in sorghum detected in previous studies and the present study

Chr	Marker	Mapping population	Authors
1	SB596	Kikuchi Zairai × SC 112	Present study (qFT1-2)
1	Dsenhsbm 13	RIL (296B × IS18551)	Srinivas *et al.* 2009
2	Xtxp298	RIL (296B × IS18551)	Srinivas *et al.* 2009
2	SB1406	Kikuchi Zairai × SC 112	Present study (qFT2)
3	Dsenhsbm 87	RIL (269B × IS18551)	Srinivas *et al.* 2009
3	SB1839	Kikuchi Zairai × SC 112	Present study (qFT3)
5	SB3125	Kikuchi Zairai × SC 112	Present study (qFT5)
5	Xtxp23	RIL (296B × IS18551)	Srinivas *et al.* 2009
5	SB3117	Kikuchi Zairai × SC 112	Present study (qFT5b)
6	SB3392	Kikuchi Zairai × SC 112	Present study (qFT6b)
6	GlumeT	RIL (296B × IS18551)	Srinivas *et al.* 2009
7*	SB4096	Kikuchi Zairai × SC 112	Present study (qFT7)
7	Xtxp312	RIL (296B × IS18551)	Srinivas *et al.* 2009
8*	SB4292	Kikuchi Zairai × SC 112	Present study (qFT8)
8	Xtxp292	RIL (296B × IS18551)	Srinivas *et al.* 2009
8	SB4660	Kikuchi Zairai × SC 112	Present study (qFT8b)
10*	SB5596	Kikuchi Zairai × SC 112	Present study (qFT10)
10	UMC 113	RIL (IS2807 × 249)	Dufour 1996
10	UMC 113	RIL (IS2807 × IS7680)	Chanterau *et al.* 2001

* New QTLs (identified in the present study).

Discussion

In the present study, we analyzed the QTLs underlying flowering time in the sorghum cultivars Kikuchi Zairai and SC112 and their F_2 plants grown under conditions of both natural and 12 h day lengths. A wide variation in flowering time was noted among the parental cultivars and their F_2 plants under natural day length. The F_2 population demonstrated transgressive segregation for flowering time, which can be caused by both of the parental cultivars contributing favorable or unfavorable alleles for flowering time or a breakage of the linkage between favorable and unfavorable alleles, in addition to the failure to declare small QTLs statistically. The normal distribution signified the continuous genetic variation that exists between F_2 plants.

Although a smaller range of variation in flowering time under 12 h day length was found for F_2 plants, all of the plants flowered earlier under 12 h day length than when grown under natural day length. The decrease in days to flowering under 12 h day length suggested that sorghum is a short-day plant and flowers most rapidly when illuminated for fewer hours per day (Craufurd *et al.* 1999). These results were also reported previously by Garner and Allard (1923) who showed that flowering in sorghum was accelerated by a daily reduction of the day length. In the present study, flowering in a larger number of F_2 plants was accelerated under 12 h day length when compared to the flowering time of the early-flowering Ethiopian cultivar. Accordingly, the Japanese cultivar allele appeared to delay the flowering time under natural day length, whereas the Ethiopian cultivar allele suppressed the delayed effect on flowering by the Japanese cultivar allele and accelerated flowering under 12 h day length. Under 12 h day length, the Ethiopian cultivar flowered nine days earlier than under natural day length and the Japanese cultivar flowered 30 days earlier.

The linkage maps constructed in this study are most likely among the rare sorghum genetic linkage maps constructed entirely of SSR markers. In contrast, the available sorghum genetic linkage maps are based mainly on RFLPs or a combination of different markers types, especially RFLPs with other marker types, such as SSRs (Chanterau *et al.* 2001, Menz *et al.* 2002), AFLPs, RAPDs (Haussmann *et al.* 2002) and DArTs (Mace *et al.* 2009). However, under both of the day length conditions, the total map length was larger than the range previously reported: the distances between the adjacent markers are larger in our map than in previously published maps. This result may be due to the segregation pattern of the genotypic data and the type of SSR markers used in this study; most of the markers were highly distorted and skewed. The SSR markers used were more affected by distortion than the other markers used in previous studies. Most of the markers showed 3 : 1 segregation ratio, and markers with unclear polymorphism were excluded to minimize scoring errors; however, the physical distance between the selected markers was relatively large compared with previous maps.

Five QTLs controlling flowering time were detected under both natural and 12 h day lengths, whereas *qFT1-1* on chr 1, *qFT7* on chr 7, *qFT8* on chr 8 and *qFT8b* on chr 8b were detected only under the natural day length. These four QTLs were considered to be sensitive to the photoperiod due to the response to the change in day length. These QTLs explained 27.1% of the total phenotypic variation and controlled the photoperiodic sensitivity, as a discrepancy in the day length or photoperiod was required for their expression. Conversely, *qFT5* on chr 5 and *qFT6b* on chr 6b were identified only under 12 h day length and were expressed under a fixed day length, suggesting that their expression was not affected by the change in day length and that they were insensitive to the photoperiod.

The 9 QTLs identified under natural day length explained 60% of the variation of the flowering time. The 7 QTLs identified under 12 h day length explained 46.6% of the variation of the flowering time, which explains the complex genetic nature of the flowering time in sorghum and the possibility of environmental influences on this trait.

In this study, positive additive effects suggested that the alleles of SC112 contributed to the earliness of the flowering time of F_2 plants. Furthermore, the small additive effects of individual QTLs indicated the complexity of the genetic control of flowering time in sorghum.

These results are similar to the finding of a study conducted by Srinivas *et al.* (2009) in which nine QTLs controlling flowering time were identified in sorghum, with very small additive effects ranging from 1.24 to 1.96. These results are also similar to the finding of Mace *et al.* (2012) who described that small additive effects of QTLs controlling morphological traits can be explained by the smaller heritability of flowering time.

Similarly, Buckler *et al.* (2009) studied the variation in flowering time with a set of 5000 recombinant inbred lines (maize Nested Association Mapping population, NAM) and explained that one million plants were assayed in eight environments but showed no evidence of any single large-effect QTLs. Indeed, the authors identified 36 QTLs that explained 89% of the total variance of the flowering time in maize. Buckler *et al.* (2009) identified evidence of numerous small-effect QTLs shared among families; however, allelic effects differ across founder lines. In their study, no major QTLs were identified at which allelic effects were determined by the geographic origin or large effects of epistasis or environmental interactions. On the basis of these results, Buckler *et al.* (2009) suggested that in outcrossing species maize, the genetic architecture of flowering time is dominated by small, additive QTLs, concluding that a simple additive model accurately predicts flowering time in maize, in contrast to the genetic architecture observed in rice and *Arabidopsis*.

These findings in maize described by Buckler *et al.* (2009) strongly support the results of the present study because Buckler *et al.* (2009) concluded that there were two different types of genetic architecture of flowering time in plants: one based on numerous small-effect QTLs controlling flowering time in outcrossing species, (maize) and another type based on a single large-effect QTL in rice and *Arabidopsis*.

Numerous QTLs controlling flowering time in sorghum have been identified in previous studies (Crasta *et al.* 1999, Dufour 1996, Feltus *et al.* 2006, Hart *et al.* 2001, Lin *et al.* 1995, Paterson *et al.* 1995, Srinivas *et al.* 2009); however, no QTL controlling flowering time or sensitivity to photoperiodic changes with a major effect was identified in previous studies of sorghum. Moreover, it is expected that new recombinations will help to identify new QTLs; therefore, we compared our results with previous studies on flowering time and photoperiodic responses in sorghum to account for possible new QTLs in addition to the QTLs previously identified (Table 3).

qFT2 on chr 2 was mapped to a position adjacent to that mapped by Srinivas *et al.* (2009), as shown in Table 3 and *qFT3* (101.7–123.1 cM) was mapped to a position adjacent to the QTL mapped on chr 3 by Srinivas *et al.* (2009). The QTLs identified on chr 5 in this study (*qFT5* and *qFT5b*) were located at the same physical positions as the QTLs reported by Srinivas *et al.* (2009).

However, no QTLs were mapped to the same genomic regions as *qFT7* (34.7–53.0 cM) and *qFT10* (134.4–152.9 cM) in previous studies. In addition, no QTL controlling flowering time in sorghum was reported in previous studies on chr 8 at the same position as qFT_8 delimited by SB4292 and SB4327 on chr 8 in this study. Therefore, *qFT7* q*FT*8 and *qFT10* mapped in the present study on chr 7, chr 8 and chr 10, respectively, are considered to have been newly mapped, as they were not reported in previous studies (Table 3). In addition, *qFT8b* was previously mapped by Srinivas *et al.* (2009). The map location of genes involved in the photoperiodic response in sorghum will be discussed in comparison with rice genes involved in photoperiodic responses. The

region on chr 8 of sorghum, which carries a photoperiod QTL, aligns with a region on chr 6 of rice between SSR marker locus RZ144 and isozyme *pgi*-2, which is linked to *Se*-1, a major photoperiod sensitivity gene in rice (Yano *et al.* 1997).

Recently, Murphy *et al.* (2011) reported that Ma_1 has the largest impact on flowering time in sorghum. Thus, we can suggest that the Ethiopian cultivar might promote flowering time via the effect of Ma_1 or its homologs. In addition, Lin *et al.* (1995) mapped the QTL (FlrAvgD1 = QMa1.ugaD) linked to SBI06 (31–59 cM) and suggested that this QTL corresponded to Ma_1. Using genotypes known to segregate for Ma_1, Klein *et al.* (2008) showed that Ma_1 mapped to an adjacent region on SBI-06 (approx. 11–21 cM). In the present study, *qFT6b* was mapped in the region delimited by SB3392 and SB3733 (0.0–25.2 cM) on chr 6b under 12 h day length and could correspond to the Ma_1 allele because it was mapped to a region adjacent to SBI-06 (Klein *et al.* 2008, Lin *et al.* 1995).

Childs *et al.* (1997) mapped the Ma_3 maturity gene to SBI-01 (115.5–125.7 cM) and determined that the ma_3^R mutation of this gene causes a phenotype similar to plants known to lack phytochrome B. In the present study, *qFT1-1* was mapped to the region delimited by SB105 and SB258 (112.0–120.3 cM) on chr 1 under natural condition, corresponding to the region adjacent to the Ma_3 allele as reported by Child *et al.* (1997). Consequently, *qFT1-1* could correspond to the Ma_3 allele, as it was mapped on a region adjacent to SBI-01.

As the data in Lin *et al.* (1995) were inconsistent with the assigned map location of QMa1.ugaD in Feltus *et al.* (2006), further studies are suggested to confirm these results. Furthermore, the correspondence between the QTLs that modulate flowering time identified in genetic studies and Ma_1-Ma_6 is not entirely clear because the location of Ma_2 and Ma_4 on the linkage map is not known.

The present study indicated that the flowering time in sorghum was controlled by a large number of QTLs with small effects, suggesting that the genetic architecture of the flowering time in sorghum was similar to maize. This study represents a preliminary and basic study of the QTLs controlling flowering time in sorghum, and the results of this study emphasize the investigation of the genetic architecture of flowering time in sorghum, comprising the scope of our future research. Finally, the interaction of the QTLs controlling flowering time in sorghum with the photoperiod appears to be fundamental to the improvement of this crop and to feed the world's expanding populations, especially because sorghum is particularly adapted at low levels of input and is suited to hot and dry agro-ecologies in which it is difficult to grow other food crops.

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