

# Variation in flowering time in sorghum core collection and mapping of QTLs controlling flowering time by association analysis

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**Abstract** A wide range of variation in flowering time was observed within a diversity research set of 107 sorghum accessions ranging from 56 to 133 days. Accessions were classified into early medium and late flowering groups. 45 accessions were grown under three different environments of photoperiod (11, 12 and 15 h). Sorghum accessions gradually responded to the decreasing of day-length. The 12 h of photoperiod could be considered as a threshold above which day-length delays the flowering time in sorghum. Association analysis was performed to identify the QTLs controlling flowering time and photoperiod sensitivity using 107 accessions of sorghum grown under natural condition and 45 accessions grown under controlled conditions. Four QTLs controlling flowering time were detected under natural condition of day-length at threshold 2.5 using K model. A total of seven flowering time loci were detected under controlled conditions of day-length. One QTL controlling photoperiod sensitivity was detected on chromosome 1 and one QTL controlling

photoperiod insensitivity was detected on chromosome 4.

**Keywords** Flowering · LD · Photoperiod sensitivity · QTL · Sorghum

## Introduction

Flowering time is one of the essential traits determining adaptation during crop domestication. In sorghum (*Sorghum bicolor* (L.) Moench) flowering is considered as a crucial event because of its key role in the adaptation and geographical distribution of this crop. Flowering time is affected by environmental stimuli where photoperiod is one of the major determinant factors for this trait (Kikuchi and Handa 2009). Most of plant species exhibit some degree of photoperiodism, which is a control of the time or the date of flowering by the photoperiod. Whereas the effects of photoperiod on flowering time in sorghum are essential for the crop domestication, these effects are not well understood (Michael et al. 2008). Despite extensive analysis of the day-length control of flowering in sorghum, little is known regarding effect of variation in photoperiod or day-length on flowering time of sorghum (Menz et al. 2002). Since sorghum was recognized as short-day species by Garner and Allard (1923), photoperiod sensitivity in this species has been systematically eliminated by breeders to enlarge the range of adaptability and to extend its cropping area to

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temperate environment (Chanterau et al. 2001). A better understanding of response and sensitivity of flowering time in sorghum to the photoperiod will facilitate the control of flowering time. In recent years, gene mapping using linkage disequilibrium (LD) has become one of the most active areas of research in plant genetics. LD is a powerful strategy for identifying genes underlying quantitative traits in plants (Casa et al. 2008). Thus the objectives of the current study were to analyze the variation in flowering time in a diverse core set of sorghum, to explore the sensitivity of flowering time to the variation in photoperiod and to identify QTLs controlling flowering time using association analysis.

## Materials and methods

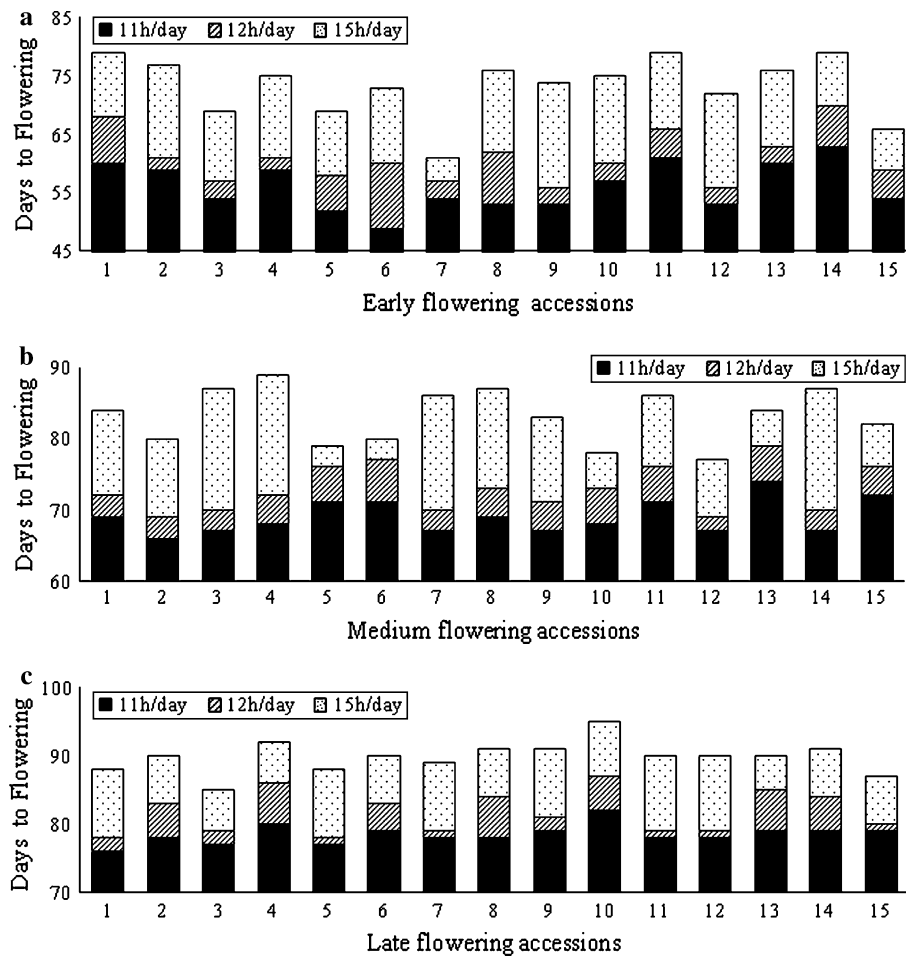
A diversity research set of 107 sorghum accessions developed by Shehzad et al. (2009a) representing African and Asian countries was used in this study (Supplementary Table 1). According to their flowering time, accessions were divided into early, medium and late flowering groups. Fifteen accessions were randomly selected from each group. A total of 45 accessions were grown as replicated sets in three identical cabinets (at the experimental field of Tsukuba University). The day-length was set to 11, 12 and 15 h, respectively. The main effect of the photoperiod treatments was defined for each accession by counting number of days from sowing to flowering.

Furthermore association analyses were conducted using the core collection grown under natural condition of day-length and the 45 accessions grown under controlled conditions to identify QTLs associated with flowering time and photoperiod sensitivity. A total of 98 markers previously described in Shehzad et al. (2009b) were used for association analyses. Population structure was performed using the program STRUCTURE version 2.2 (Pritchard et al. 2000). Bayesian clustering analyses with the admixture models were used where number of populations ( $J$ ) ranged from 2 to 9. Markov chain Monte Carlo (MCMC) sampling was repeated  $1 \times 10^6$  times after  $1 \times 10^4$  cycles of a burn-in period. The posterior probability of  $J = 2$  was the largest among other values and was selected after two times repetition. The  $\mathbf{Q}$  matrix, whose  $(I, j)$ -th element was  $q_{ij}$ , was

further incorporated into the association mapping models where the effect of population structure was considered. A kinship matrix,  $\mathbf{K}$ , was calculated as allele sharing rates of the 98 SSR markers as suggested by Zhao et al. (2007) and used in the models with  $\mathbf{K}$  effect. LD between SSR markers were estimated by  $D'$  and  $r^2$ , where  $D'$  is the standardized disequilibrium coefficient and  $r^2$  represents the correlation between alleles at two loci. A statistical software TASSEL (Trait Analysis by Association, Evolution and Linkage) ver.2.0.1 (Bradbury et al. 2007) was used to obtain  $P$  values representing the significance of LD. To identify QTLs significantly associated with flowering time general linear model (GLM) as well as mixed linear model (MLM) were applied for analysis in TASSEL software. In GLM two different models were used (1) naïve model where there is no control of population structure and kinship (2) Q model based on population structure (Yu et al. 2006). In MLM we used two models: (1) the model which accounted for kinship ( $\mathbf{K}$ ), (2) the model that takes into account both the population structure and the kinship ( $\mathbf{Q} + \mathbf{K}$ ).

## Results

A wide range of variation in flowering time was observed within the panel of 107 sorghum accessions (Supplementary Table 1) ranging from 56 to 133 days. On the basis of number of days to flowering the 107 sorghum accessions were classified into three groups: early flowering group with less than 75 days, medium flowering from 75 to 95 days and late flowering group with more than 95 days from sowing to flowering. Under controlled conditions day-length varied substantially across experiments resulting in considerable variation in flowering time for most of the accessions from different flowering groups (Fig. 1). A photoperiod of 11 and 12 h accelerated flowering for the majority of early, medium and late accessions compared with 15 h of photoperiod. Above 12 h of photoperiod the increase of day-length generated a delay in flowering for accessions of all flowering groups. Difference in number of days to flowering between 12 and 15 h of photoperiod ranged from 4 to 18 days for the early flowering group, from 3 to 17 days in medium group and from 5 to 11 days for the late group. There was

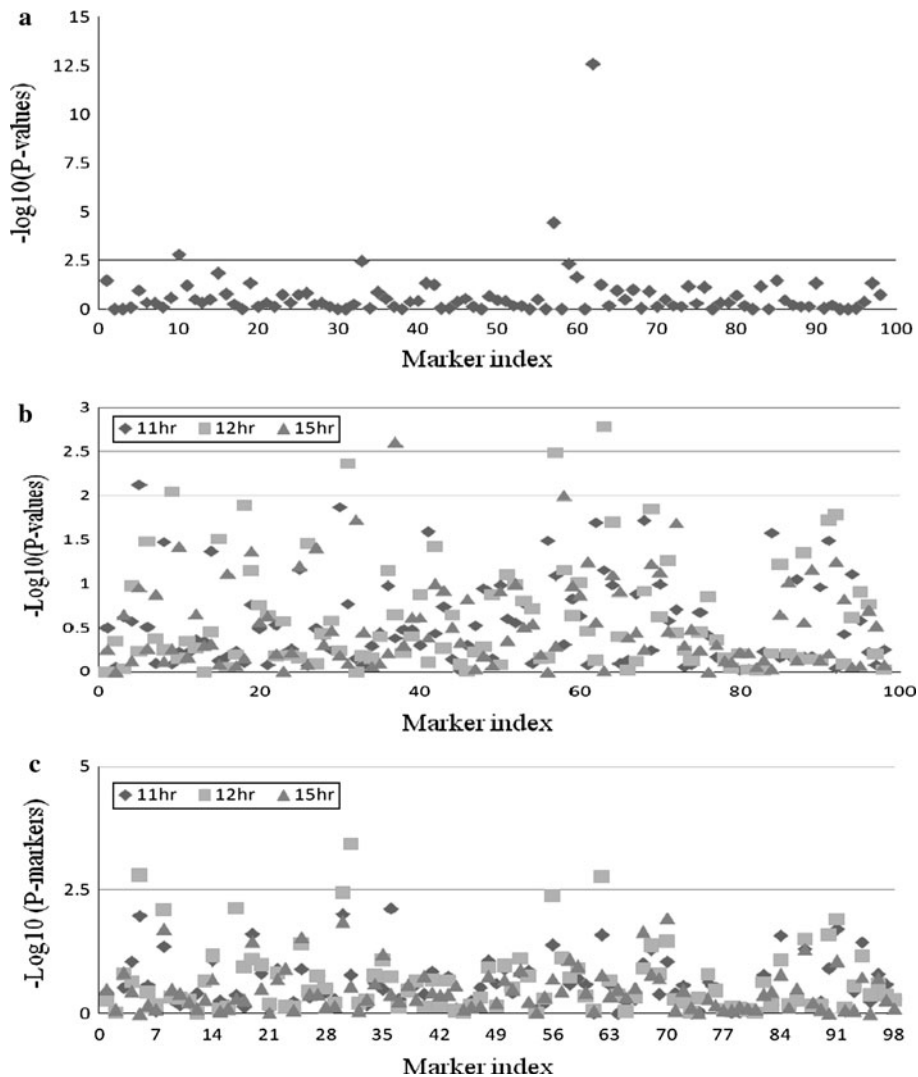


**Fig. 1** Variation in flowering time in 45 sorghum accessions grown under controlled conditions of photoperiod (**a** early flowering accessions; **b** medium flowering accessions; **c** late flowering accessions)

no gradual response of flowering time to photoperiod between 12 and 15 h, and variation of number of days to flowering was strongly affected by the increase of the day-length. The 12 h of photoperiod per day could be considered as a threshold above which day-length delay the flowering time in sorghum.

Using association analysis no QTL controlling flowering time was identified under natural condition by GLM models. Using K model, four SSR loci were identified to be associated with flowering time under natural condition at a threshold of 2.5 and one locus at 2.4. Xtxp159 on chromosome 5 and Xtxp51 on chromosome 4 showed a strong association ( $P < 0.0001$ ) with flowering time (Fig. 2a). Using naïve model three markers suggesting associations with flowering time were detected at a threshold 2. Xtxp10, was identified on chromosome 6; Xtxp159 on chromosome 5 and

Xtxp297 on chromosome 2 under 11, 12 and 15 h of photoperiod, respectively. Xtxp159 was detected under natural condition of day-length and short-day condition. In Q model Xtxp13 was weakly associated with flowering time. It was detected on chromosome 2 under short day conditions only. Using K model (Fig. 2b) four loci were identified to be associated with flowering time at a threshold 2.5. Xtxp298, Xtxp51 and Xtxp312 were detected on chromosome 2, chromosome 4 and chromosome 5 respectively, under 12 h of photoperiod. Xtxp100 was detected on chromosome 2 under long-day condition. Three loci were detected using K model at a threshold 2 under 11, 12 and 15 h of day-length respectively (Table 1). For Q + K model (Fig 2c), the number of associated markers was the largest among all models. A total of eight markers were associated with flowering time under short-day



**Fig. 2** **a** Association analysis of 98 SSR markers and flowering time using K model for 107 sorghum accessions under natural condition of day-length. **b** Association analysis of 98 SSR markers and flowering time using K model for 45

sorghum accessions under controlled conditions of day-length. **c** Association analysis of 98 SSR markers and flowering time using (Q + K) model for 45 sorghum accessions under controlled conditions of day-length

condition. Five loci were significantly associated with flowering time at threshold 2.5. Xtxp298, Xtxp61 and Xtxp312 on chromosome 2, chromosome 1 and chromosome 5 respectively, were found to be the most strongly associated with flowering time under 12 h of photoperiod. A range of LD was observed in the 45 accessions grown under controlled conditions of photoperiod. The triangle plot for pairwise LD between marker sites in a hypothetical genome fragment, where pairwise LD values of polymorphic sites were plotted on both X and Y axis; above the

diagonal displays  $r^2$  values and below the diagonal displays  $P$  values from rapid 1,000 shuffle permutation test (Fig. 3). Each cell represents the relationship between two markers with the color codes indicating the significance of LD.

## Discussion

The results obtained in this research suggested that sorghum accessions gradually responded to the

**Table 1** A total of loci associated with flowering time identified using 98 SSR markers

Photoperiod (h)	Marker	Chromosome	<i>P</i> values
11	Xtxp61	1	2.12317
	Xtxp13	2	2.05941
	Xtxp315	2	2.11415
	Xtxp10	6	2.11991
12	Xtxp61	1	2.78197
	Xtxp75	1	2.04416
	Xtxp279	1	2.08766
	Xtxp302	1	2.11431
	Xtxp13	2	2.43607
	Xtxp13	2	2.26574
	Xtxp298	2	3.42966
	Xtxp298	2	2.36285
	Xtxp51	4	2.49049
	Xtxp212	4	2.39630
	Xtxp159	5	2.12045
	Xtxp159	5	2.75682
	Xtxp312	5	2.78336
15	Xtxp297	1	2.02636
	Xtxp100	2	2.60788
	Xtxp27	4	2.00007
Natural condition	Xtxp58	1	2.79588
	Xtxp56	2	2.48148
	Xtxp51	4	4.43995
	Xtxp59	4	2.35654
	Xtxp159	5	12.59246

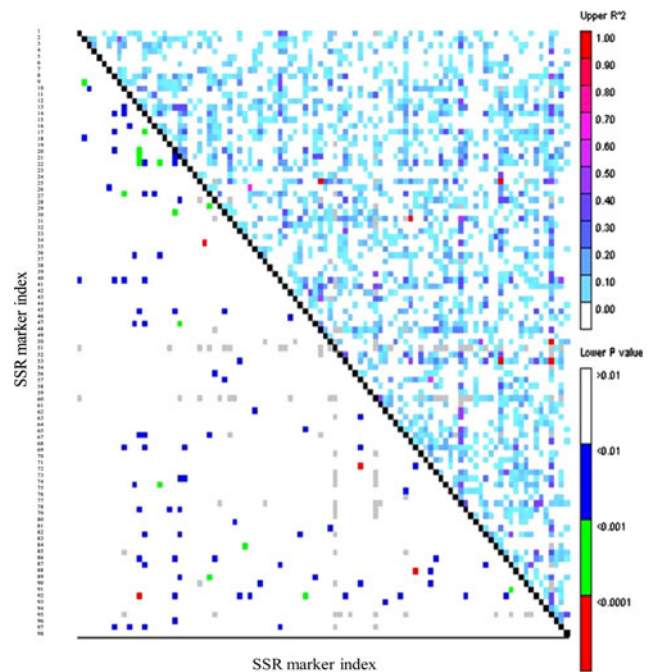
decreasing of day-length. Long-day conditions delay the flowering time by increasing the number of days to flowering. These results were confirmed previously by Garner and Allard (1923) and by Folliard et al. (2004) who proved that for sorghum crop, progress towards flowering is accelerated when day-length decreases. On the basis of these outcomes we suggested that the exacted photoperiod compulsory for sorghum flowering belongs to the interval of 11–12 h of photoperiod. Variation in response to photoperiod and sensitivity to it fluctuated within accessions. Some accessions seemed to be weakly affected by change in photoperiod. Other accessions appeared to be strongly affected by changes in day-length and subsequently severely sensitive to photoperiod. Kassam and Andrews (1975) suggested that there were two major mechanisms controlling

flowering time and adaptation in sorghums; firstly mechanism in which genotypes are sensitive or insensitive to photoperiod and secondly mechanism in which genotypes are inherently early/late flowering.

The association analysis was performed to identify QTLs controlling flowering time of the genotyping data of 98 SSR markers. Using core collection grown under natural condition of day-length Xtxp51 and Xtxp159 were found to be significantly associated with flowering time by K model. Same loci were identified under 12 h of photoperiod. These loci were detectable under varying photoperiod indicating that their expression is photoperiod insensitive. Two loci controlling flowering time were located—Xtxp61 on chromosome 1 and Xtxp13 on chromosome 2 were expressed exclusively in short-day conditions suggesting that their expression was relatively sensitive to photoperiod. These two loci accelerated flowering under short photoperiod. We also detected two photoperiod sensitive QTLs on chromosome 2 and chromosome 6 since they were only detectable under 11 h of photoperiod suggesting that there is a minimum photoperiod necessary for their expression. These loci are sensitive to photoperiod of some degree. Three loci were detectable exclusively under long-day condition, suggesting that there is a maximum photoperiod necessary for their expression (Table 1).

We have compared our results with other studies of the photoperiod response in sorghum to account for possible orthologies. The QTL detected on chromosome 6 in this study has a close map position to the one detected by Lin et al. (1995) and Chanterau et al. (2001). This QTL was detected under 11 h of photoperiod and appeared to be responsible for the control of the photoperiod sensitivity in sorghum. In same way QTL detected on chromosome 5 has the same position as previously reported by Srinivas et al. (2009) and can be considered as QTL controlling photoperiod sensitivity in sorghum. The QTL detected on chromosome 1 only under short day-length appears to be newly mapped in sorghum. No previous study reported QTL controlling flowering time and sensitivity to photoperiod located in same position as the one detected on chromosome 1 in this study. This QTL can be considered as a QTL controlling sensitivity to photoperiod in sorghum. Moreover the QTL detected on chromosome 4 under

**Fig. 3** LD plot generated by 98 SSR markers. Each cell represents the relationship between two markers with the color codes for the presence of significant LD. *Colored bar* codes for the significant threshold levels



12 h as well as under natural condition of photoperiod indicating a photoperiod insensitive expression, seems to be newly detected and was not reported in previous studies.

There was no a strong degree of LD between markers (Fig. 3) that might be because of low number of genotypes used under controlled conditions and also low number of SSR loci. Four markers mentioned a significant association with the flowering time ( $P < 0.0001$ ). The success of association mapping depends on the possibility of detecting LD between DNA marker alleles and alleles affecting phenotypic expression (Stich et al. 2005). These results can be explained by the small number of germplasm and the number of markers used for this association. Many QTL might be missed because of the low density of markers associated with flowering time in this panel (Shehzad et al. 2009b).

## Conclusion

In this study a large number of markers was identified to be associated with flowering time with different levels of significance with all models. Two QTLs controlling photoperiod sensitivity (Xtxp61) and photoperiod insensitivity (Xtxp51) in sorghum were

detected in this study. Further studies are required to elucidate the expression of these QTLs and investigate their effects on flowering time in sorghum.

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