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Association analysis of genes controlling variation of flowering time in West and Central African sorghum

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Abbreviations

CG	Candidate gene
DFL50%	Days to 50% flowering
EST	Expressed sequence tag
GA	Gibberellic acid
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IFM	Indirect (statistical) functional markers
LD	Linkage disequilibrium
MAS	Marker-assisted selection
PIFs	Phytochrome interacting factors
PRI	Photoperiod response index
QTL	Quantitative trait locus
RFLP	Restriction fragment length polymorphism
SSR	Simple sequence repeat
STATs	Signal transducers and activators of transcription
WCA	West and Central Africa

Chapter 1
General Introduction

Cereals are the most important calorie contributors to the human diet with 2.4 trillion tonnes produced in 2009 (FAOSTAT, 2011). Sorghum [*Sorghum bicolor* (L.) Moench], in particular, constitutes a major nutrition source for millions of people especially in Africa and Asia. It is a staple food for more than 300 million people and the world's fifth most important cereal in terms of production (FAOSTAT, 2007). Sorghum is an annual, predominantly autogamous cereal (Ollitrault et al., 1997) and was domesticated in northeastern Africa (Doggett, 1988). African sorghum production is centered in the Savannah zone of West and Central Africa (WCA), where the grain of this crop is a major component of the diet for millions of people (Purseglove, 1985). Sorghum stover is also used as building material, cooking fuel and feed for cattle in many regions of WCA (de Vries and Toenniessen, 2001). In 2009, an estimated 16.5 million tonnes of sorghum was produced in WCA (FAOSTAT, 2011). Sorghum production is a cornerstone to achieve food security and economic growth in semi-arid zones of WCA.

Africa is considered as particularly vulnerable to climate change, primarily due to its low adaptive capacity and its sensitivity to many predicted changes (IPCC, 2001; Callaway, 2004). Climate change in combination with acute problems such as poverty, dwindling natural resources, food insecurity (FAO, 2006), drought and land degradation (Thomas et al., 2008) will put additional pressure on agriculture. Sorghum, being a major crop in Africa, will be sensitive to climate change too. Therefore, there is an immediate need to search for novel and effective solutions to this problem such as the development of best growing varieties as well as the implementation of the best possible cultivation practices. An important challenge for plant breeders is to develop crop varieties that are adaptive to the changing environment and ensure stable yield returns. Understanding the genetic mechanisms underlying important agronomical traits will provide a foundation for effective and sustained use of genetic resources and for maximizing productivity and adaptation in

climatically variable environments.

1.1 Flowering and photoperiodism in plants

Knowledge of the genetic basis of variation of flowering is very crucial to plant breeders, because it helps in selecting crop varieties adapted to regions with differing climatic and daylength conditions to ensure stable growth and yield performance. Flowering is an extremely important trait in a plant's life as it assures that the plant flowers at the optimum time for pollination, seed development and dispersal (Waser, 1978). Plants normally associate the onset of flowering with suitable environmental conditions. Photoperiod or day length is one of the most important environmental signals which enable the plants to adapt to seasonal changes in their environment (Jordan, 2006). The ability of plants to respond to the change in the length of the day is called photoperiodism (Garner and Allard, 1920). Plants which induce flowering when the day length is decreased are called short day plants, whereas long day plants accelerate induction of flowering when length of the day is increased (Thomas and Vince-Prue, 1997). Characteristically, photoperiod sensitive plants respond in their growth and reproductive constitution to the length of the day, for instance by growing faster when the day length shortens or fail to flower until a particular day length is met (Conklin and Stilwell, 2007).

1.2 Challenges in sorghum production and photoperiod sensitive flowering in WCA

Sorghum is usually grown in regions characterized by hot and dry climates typical of WCA, where it is difficult to grow most other food grains. Sorghum originated within a zone between the equator and 15˚ north latitude in Eastern Africa (Mann et al., 1983; Quinby, 1967). In this zone, day lengths would vary during the year – from 11 to 13 hours (Pao and Morgan, 1986). In WCA, the typical growing period of sorghum is from May to November. Crop growth usually takes place under decreasing daylength

conditions. Sorghum is a short day photoperiod sensitive crop, i.e., flowering occurs when daylength falls below a certain number of hours. Most of the West African sorghum cultivars grown under rainfed conditions are highly photoperiod sensitive (Grenier et al., 2001).

The life cycle of sorghum crops varies greatly between sowing dates (Clerget et al., 2004). The duration of its growing cycle shortens when sowing is late due to delayed rains (Folliard et al., 2004). A serious problem for sorghum production in WCA is that the start of the rainy season is extremely variable among years. For instance, the start of the rainy season in Ségou, Mali, can range from the 10th of May to the 15th of July (Kouressey et al., 2004). Farmers are forced to adjust their individual sowing dates according to the start of the rains (Niangado, 2001). In contrast, the timing of the end of the rainy season is generally less variable from year to year for a particular location. Another problem for sorghum production in WCA is that farmers are unable to sow their entire crop area at once due to labor limitations and other cultivations issues. Hence, sowing dates are commonly spread over a month after the onset of the rainfall season. Therefore, farmers require varieties that flower at the end of the rainy season regardless of sowing date (Clerget et al., 2004).

Photoperiodic sensitivity of local landraces is the key adaptation trait of sorghum in WCA, because it assures flowering at the end of the rainy season, independent of the date of planting (Vaksmann et al., 1996). Furthermore, photoperiod sensitivity of the local landraces is useful to minimize grain mold and insect and bird damage typical in early maturing varieties. It also helps in avoiding incomplete grain filling, a problem typical to late maturing varieties due to soil water shortage which is usually common at the end of the season (Cocheme´ and Franquin, 1967; Curtis, 1968a, 1968b; Kassam and Andrews, 1975; Vaksmann et al., 1996). Therefore, in WCA cultivars with photoperiod sensitivity have the potential to increase yield and improve yield stability (Haussmann et al., 2007).

Inclusion of photoperiod sensitivity in breeding strategies for WCA is relatively recent. In the past, the main objective of breeding strategies was to allow sorghum to produce

in the longer daylengths in temperate areas (Miller, 1982) which caused the removal of the photoperiod sensitivity characteristic (Doggett, 1986; Kouressy et al., 1998; Major and Kiniry, 1991). This led to the removal of photoperiod sensitivity from much of the breeding material (Niangado, 2001). Present day sorghum cultivars are photoperiod insensitive. The introduction of photoperiod sensitivity is further constrained by the limited understanding of the role of photoperiod sensitivity by extension services in cultivar adaptation and lack of simple screening methods available to select cultivars with an appropriate response to the photoperiod (Folliard et al., 2004).

1.3 Molecular genetics of flowering in sorghum

Understanding the genetic basis of photoperiodic flowering time in sorghum has been the topic of some studies in the past. A series of six maturity quantitative trait loci (QTLs) has been found to affect flowering time in sorghum: Ma_1, Ma_2, Ma_3, Ma_4, Ma_5, and Ma_6 (Quinby, 1967; F.R. Miller, unpublished data as cited by Childs et al., 1997). The first four maturity QTLs inhibit flowering under long days but allow early flowering under short days. Of these first four QTLs, mutations at Ma_1 cause the greatest reduction in sensitivity to long days. Mutations at Ma_2, Ma_3, and Ma_4 generally have a more modest effect on sensitivity to long days (Quinby, 1967). Several other studies followed in the 1990s, investigating photoperiodic flowering of sorghum (Childs et al., 1992; Lin et al., 1995; Paterson et al., 1995). These studies highlighted the role of *PHYTOCHROMES* (*PHYA-C*) as an important gene family in flowering but surprisingly no flowering QTLs were associated with *PHYTOCHROMES* in sorghum (Paterson et al., 1995). Fine scale mapping of the ma_3R allele in sorghum indicated that the Ma_3 maturity gene encodes *PHYB* and truncation of the *PHYB* message in the *ma3R* allele corresponds to reduced photoperiod sensitivity (Childs et al., 1997). To provide evidence that Ma_3 is synonymous with *PHYB*, Childs et al. (1997) carried out mapping of *PHYA*, *PHYB*, *PHYC*, and Ma_3-linked molecular markers. A sequence analysis of the three *PHY* genes demonstrated that ma_3R contains a mutation in *PHYB*. It is interesting that mutations in sorghum Ma_3 reduce sensitivity to non-inductive day-lengths (Pao and Morgan, 1986; Childs et al., 1995). A similar

effect was found in *Arabidopsis* (*Arabidopsis thaliana) PHYB*. But to our knowledge, there has been no study analyzing the effect of candidate genes (CGs) involved in the photoperiod pathway of flowering time in sorghum.

1.4 Basis of molecular genetics of flowering time research: from *Arabidopsis* to important food crops

Our current knowledge on regulation of flowering time has been facilitated by decade-long research using molecular-genetic approaches on the model plant *Arabidopsis* (Roux et al., 2006; Bernier and Perilleux, 2005; Putterill et al., 2004; Simpson and Dean, 2002). Understanding molecular mechanisms of flowering time in species such as wheat (*Triticum aestivum L.*) and barley (*Hordeum vulgare L.*), with large, genome size and few genomic resources, has largely been the result of comparative use of floral pathways from *Arabidopsis* (Henderson and Dean, 2004; Bäurle and Dean, 2006). Similarly, recent studies (Hayama and Coupland, 2004; Izawa, 2007a, 2007b; Yano et al., 2001) on control of flowering time in rice (*Oryza sativa L.*) have extended our knowledge of flowering processes in a model grass species with short day response. However, different growth strategies compromise the relevance of rice as a model for cereals: rice is a short-day plant with no vernalization requirement, while wheat and barley (as well as *Arabidopsis*) are long-day plants which use vernalization as a control. Despite these differing responses to environmental signals, and the ancient divergence of the monocots and their magnolid relatives from the eudicot lineage, orthologous genes have been found to be involved in similar flowering response (Yano et al., 2001; Kojima et al., 2002; Hayama et al., 2003). Therefore, these studies serve as the basis for the flowering time research in important cultivated cereals like sorghum.

In *Arabidopsis,* the flowering time genes are mainly integrated into four closely interacting pathways: vernalization, autonomous, gibberellic acid (GA), and photoperiod. The vernalization pathway is comprised of *VERNALIZATION* 1 and 2 [*VRN* 1 and *VRN* 2, (Chandler et al., 1996)] which ensure flowering after a long period of cold. To achieve flowering in the autonomous pathway, internal developmental

signals are required instead of environmental factors. Both pathways regulate a strong repressor of the flowering gene *FLOWERING LOCUS C* (*FLC*) (Bernier and Perilleux, 2005; Putterill et al., 2004). Genes included in the GA pathway such as *GIBBERELLIC ACID INSENSITIVE* (*GAI*), *REPRESSOR OF GA1-3* (*RGA*), and *RGA-LIKE1-3* (*RGL*1-3) act as constitutive growth repressors, whose growth-repressing activity is opposed by GA in modulating floral development of *Arabidopsis* (Cheng et al., 2004; Tyler et al., 2004). The photoperiod pathway involves: genes encoding; the photoreceptors; the circadian clock (an endogenous oscillator which regulates the rhythm in a period of approximately 24 hours); the clock-associated genes, such as *GIGANTEA* (*GI*); and the downstream gene *CONSTANS* (*CO*) (Bernier and Perilleux, 2005). A crucial feature of the flowering regulatory network is that all four pathways ultimately regulate a common set of key integrator genes such as *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*) and *FLOWERING LOCUS T* (*FT*), which act on the floral meristem identity genes *APETALA 1* (*AP1*) and *LEAFY* (*LFY*) to initiate flowering (Boss et al., 2004; Henderson and Dean, 2004; Roux et al., 2006).

Light affects the timing mechanism in plants that sets the phase of the photoperiodic response rhythm through photoreceptors (light absorbing pigments) such as phytochromes, and cryptochromes. Plants use their circadian clock to process the light signal received from photoreceptors to sense the light conditions and regulate flowering (Delvin, 2002). Important genes involved in the circadian clock are *CIRCADIAN CLOCK ASSOCIATED (CCA1)* and *LATE ELONGATED HYPOCOTYL* (*LHY). CCA1* and *LHY* encode highly conserved single-MYB transcription factors which, when expressed at high and constitutive levels, disrupt the normal functioning of the clock (Schaffer et al., 1998; Wang et al., 1998). The circadian clock acts to establish a rhythm of the *CO* gene expression, at least partially mediated by the flowering time gene *GI* (Mizoguchi et al., 2005). The regulation of *FT* takes place in leaves from which *FT* mRNA travels to the apex to interact with transcription factor *FD* and initiate floral development (Abe et al., 2005; Wigge et al., 2005).

1.5 Association studies on flowering in plants

Linkage disequilibrium (LD) based association studies, i.e., studies using the non-random associations of alleles at different loci (Flint-Garcia et al., 2003), have been performed to determine marker-trait associations in plants. Two LD based association study strategies are in use, CG association and genome-wide association. In the CG approach, one tests the hypothesis of a correlation between DNA polymorphisms in a gene and the trait of interest. Genome scan involves testing most of the segments of the genome for association by genotyping densely distributed genetic marker loci covering all chromosomes. One can consider the CG approach as a subset of genome-wide approach (Rafalski, 2010). A series of flowering time genes identified through molecular developmental genetics in *Arabidopsis* makes flowering time a particularly attractive trait for CG association studies (Mouradov et al., 2002; Komeda, 2004; Bäurle and Dean, 2006). There have been attempts to use CG approaches to identify flowering time quantitative genes in *Arabidopsis* (e.g., Caicedo et al., 2004; Olsen et al., 2004). Several other studies in important crops such as maize, rice, barley, and pearl millet (Thornsberry et al., 2001; Hayama et al., 2003; Stracke et al., 2009, Saïdou et al., 2009) have also followed. One important aspect that needs to be considered while performing these marker-trait association studies is population structure.

1.6 Effect of population structure on association studies

Population structure, known as the physical arrangement of related and unrelated individuals within a population, can lead to spurious association in association studies. Spurious association is an association between a phenotype and a marker that is not linked to any causative locus (Lander and Schork, 1994). Such associations occur because population subdivision causes marker-allele frequencies to vary among segments of the population, as the result of genetic drift or founder effects (Slatkin, 1991). Any marker allele that is in high frequency in the overrepresented subpopulations will then be associated with the phenotype (Ewens and Spielman, 1995; Pritchard and Rosenberg, 1999). Beer et al. (1997) analyzed 13 quantitative traits on 64

North American oat varieties and landraces grouped according to RFLP genotype at 48 loci. Significant associations between RFLP fragments and group means occurred for 11.2% of fragments indicating many more associations found than initially expected. However, an observed marker-trait association does not necessarily imply that markers showing a significant effect on the phenotype are linked to QTL. Rather, the marker-trait disequilibrium may exist in the absence of linkage, and instead may have arisen simply as a consequence of population structure. Therefore, knowledge of population structure and kinship in association studies is critical (Yu and Buckler, 2006). Several methods have been described to correct the results for population structure (Pritchard et al., 2000; Yu and Buckler, 2006; Stich et al., 2008). STRUCTURE (Pritchard et al., 2000) is the most widely used clustering software applied to detect population genetic structure. Thornsberry et al. (2001) adapted Pritchard's approach for use with quantitative variation and then successfully applied it to the evaluation of maize flowering time using the *DWARF8* gene.

1.7 Objectives of the study

The important role of photoperiod sensitivity in crop adaptation highlights the need to incorporate this trait for variety acclimation in WCA. However, it is challenging and time consuming to select cultivars with photoperiod sensitivity (i.e., cultivars having daylength requirement to induce flowering at a desirable time) because of lack of efficient selection methods. Techniques such as marker-assisted selection (MAS) are one possible solution. Employment of functional, allele-specific markers would greatly enhance the selection efficiency for this major adaptation trait.

The main goal of this study was to apply an association analysis approach to investigate the association between CG polymorphisms with photoperiod sensitive flowering in an inbred panel of sorghum accessions from WCA. For this purpose, we conducted a CG-based association study on six important genes assumed to be involved in the variation of flowering time. Five of the six genes were associated with photoperiod pathway of flowering time (*CRY1, CRY2, LHY, GI,* and *HD6*) and one gene was from the GA pathway, which was characterized in a pilot study (*SbD8*). A

panel of 219 mostly inbred sorghum accessions representative of WCA and exhibiting a wide range of photoperiodic responses was compiled following a pilot study on a subset of sorghum and pearl millet (*Pennisetum glaucum*) inbred accessions for genes (sorghum: *SbD8* and pearl millet*:PgD8*) homologous to *D8* (*DWARF8*) in maize.

The objectives of our study were to:

i. Investigate in a diverse subset of sorghum and pearl millet genotypes: (a) the presence, (b) the expression and (c) the molecular diversity of genes homologous to *D8.* Chapter 2 describes a pilot study which was conducted on a diverse set of sorghum and pearl millet genotypes to determine the presence, the expression and the molecular diversity of the genes homologous to *D8* in maize.
ii. Evaluate the flowering time of West and Central African sorghum accessions under field conditions. Chapter 4 describes the phenotyping of the sorghum accessions of our study.
iii. Assess the population structure in sorghum accessions based on SSR markers and investigate the association between flowering time variation, and candidate gene polymorphisms in partially amplified genes assumed to be related to the variation in flowering time in sorghum [*CRY1, CRY2, LHY, GI, HD6,* and *SbD8*]. Chapter 3 describes the population structure analysis and Chapter 4 describes the association analysis that was conducted on the CGs selected.

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Chapter 2

Genetic diversity and linkage disequilibrium of two homologous genes to maize *D8*: sorghum *SbD8* and pearl millet *PgD8*

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Abstract. Yield and yield stability of sorghum [*Sorghum bicolor* (L.) Moench] and pearl millet [*Pennisetum glaucum* (L.) R.Br.] are highly influenced by flowering time and photoperiodic sensitivity in the arid to semi-arid regions of West and Central Africa. Photoperiodic sensitivity is the key adaptation trait of local landraces because it allows flowering at the end of the rainy season, independent of variable sowing dates. Flowering time genes are mainly integrated into four pathways with close interaction among each other: vernalization, autonomous, GA (gibberellic acid), and photoperiod. In the GA pathway, maize *D8*, wheat *RHT1*, and rice *SLR* have been identified as homologous genes to the Arabidopsis *GAI*, which is a negative regulator of GA response. We have identified two homologous genes to *D8*: Sorghum *SbD8* and pearl millet *PgD8*. The expression of these genes was confirmed in the root and leaves of sorghum and pearl millet as revealed by EST database search and reverse

transcription PCR, respectively. The genetic diversity of *SbD8* was considerably lower than that of *PgD8*. The extent of linkage disequilibrium in *PgD8* is lower than that of maize *D8*. *SbD8* and *PgD8* polymorphisms might be appropriate for dissection of photoperiod sensitivity using association mapping approaches.

Chapter 3

Population structure in sorghum accessions from West Africa differing in race and maturity class

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Abstract. Accounting for population structure to minimize spurious associations is extremely important in association analyses. With sorghum genomic sequence information being available, there is a growing interest in conducting such association studies for several important agronomic traits using a candidate gene approach. The objectives of the study were to conduct a systematic survey of molecular genetic diversity and analyze the population structure in cultivated sorghum [*Sorghum bicolor* (L.) Moench] accessions from West Africa. Our analysis included 219 West African cultivated sorghum accessions with differing maturity intended for a marker-trait association study. A total of 27 simple sequence repeats (SSRs) were used, which resulted in detection of 513 alleles. Genetic diversity estimates for the accessions were found to be high. The accessions were assigned to two subgroups using a model-based approach. Our findings partly agree with previous studies in that the guinea race accessions could be distinguished clearly from other accessions included in the

analysis. Race and geographical origin of the accessions may be responsible for the structure we observed in this study. The extent of linkage disequilibrium for all combinations of SSRs was in agreement with expectations based on the mating system.

Chapter 4

Association analysis of photoperiodic flowering time genes in West and Central African sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract. Photoperiod-sensitive flowering is a key adaptive trait for sorghum (*Sorghum bicolor*) in West and Central Africa. In this study we carried out an association analysis to investigate the effect of polymorphisms within the genes putatively related to variation in flowering time on photoperiod-sensitive flowering in sorghum. A panel of 219 genetically characterized sorghum accessions from West and Central Africa was evaluated for their photoperiod response index (PRI) based on two sowing dates under field conditions. Sorghum accessions used in our study were genotyped for single nucleotide polymorphisms (SNPs) in six genes assumed to be

involved in the photoperiodic control of flowering time. Applying a mixed model approach and previously-determined population structure parameters to these candidate genes, we found significant associations between several SNPs with PRI for the genes *CRYPTOCHROME* 1 (*CRY1-b1*) and *GIGANTEA* (*GI*). The negative values of Tajima's D, found for the genes of our study, suggested that purifying selection has acted on genes involved in photoperiodic control of flowering time in sorghum. The SNP markers of our study which showed significant associations with PRI can be used to create functional markers to serve as an important tool for marker assisted selection of photoperiod-sensitive cultivars in sorghum.

Chapter 5
General Discussion

In WCA, MAS for appropriate photoperiod response of the varieties has the potential to greatly increase the efficiency in developing improved sorghum cultivars adaptable to different ecological zones. To our knowledge, there have been no formal attempts to conduct an association analysis to investigate the effect of genes involved in variation of flowering time in sorghum using a mixed model approach. This study contributes to filling this research gap. As an initial step towards developing molecular markers for the genes regulating photoperiodic flowering in sorghum, a pilot study was performed involving the important flowering time gene *D8* in a subset of sorghum and pearl millet.

5.1 Presence, expression, and molecular diversity of *SbD8* and *PgD8*

Fragments of genes in sorghum (*SbD8*) and pearl millet (*PgD8*) homologous to the maize *D8* gene were successfully amplified. The result suggests that genes that are homologous to *D8* are also present in sorghum and pearl millet. Based on the deduced amino acid sequence alignment of *D8, SbD8,* and *PgD8*, seven conserved regions were found. In *PgD8* in the region VIII (Figure 1 in Li et al., 2010), which was only conserved between maize and pearl millet, we found one 3 bp insertion or deletion (InDel). In maize, a 6 bp deletion flanking the SH2-like domain of *D8* was significantly associated with flowering (Thornsberry et al., 2001). Cluster analysis performed for the *D8* gene also showed that maize is more closely related to pearl millet than sorghum. These findings suggest that, similar to maize, SH2 might play a role in controlling flowering time in pearl millet. A blast search on *SbD8* against the sorghum EST database in NCBI showed a very high coverage, confirming that *SbD8* is expressed in sorghum. In the case of pearl millet, no EST was found with significant homology to *PgD8* in the pearl millet EST database in NCBI. This difference can be attributed to the rapid progress that took place in the past in sequencing sorghum as opposed to pearl millet. We performed RT-PCR to investigate the expression of *PgD8* (Figure 3 in

Li et al., 2010). Pearl millet mRNA was successfully amplified by RT-PCR by desired primer combinations, which confirmed that *PgD8* is expressed in pearl millet.

Sequence analysis showed that *PgD8* had higher nucleotide diversity than *SbD8* which can be due (1) the lower inbreeding generation (S4 vs. S6 in our study material) of pearl millet compared with sorghum and (2) the higher rate of polymorphism in allogamous species such as pearl millet compared to autogamous species such as sorghum (Rafalski, 2002). For sorghum as well as pearl millet, the nucleotide diversity for non-synonymous polymorphic sites was higher than for synonymous polymorphic sites. Based on the Tajima's D test value of *SbD8,* we conclude that, for sorghum, polymorphisms are selectively neutral, whereas Tajima's D test value for *PgD8* suggested that it has been a target of selection.

The study showed that homologs of maize *D8* are present and expressed in sorghum (*SbD8*) and pearl millet (*PgD8*). In addition to a high degree of conservation between cultivated cereals such as maize sorghum and pearl millet, the study highlighted the potential role of the flowering time gene *Dwarf8* in the regulation of flowering time in sorghum and pearl millet. Based on the findings, gene *SbD8* was included in the association study to check its potential involvement in control of flowering time in sorghum.

After successful characterization of *SbD8* and *PgD8* in sorghum and pearl millet, we compiled a set of 219 sorghum accessions from WCA for a CG-based association study of the genes putatively involved in the photoperiod pathway of flowering time. The results are discussed below.

5.2 Association analysis of the photoperiodic flowering time genes in sorghum

Association studies can serve as a powerful tool for understanding the genetic basis of quantitative variation, and can even succeed in identifying candidate genes involved in its determination (see Hirschhorn et al., 2002). Our study investigated the effects of

polymorphisms within six genes involved in the determination of flowering time using a CG-based association analysis. The association analysis was performed with the following steps: i) phenotyping - the panel of accessions of sorghum was sown twice at two different dates to characterize their flowering response to the photoperiod in the field; ii) genotyping - genetic characterization of the sorghum accessions was done with SSR markers and CG primers; and iii) marker–trait association analysis was carried out to determine the effect of candidate gene polymorphisms on the phenotypic values using a mixed model approach.

5.2.1 Photoperiod sensitivity of flowering in sorghum accessions

Phenotyping of sorghum accessions for their flowering response was carried out in the growing season of 2007 (June-October) at ICRISAT sub-station, Samanko in Mali with two sowing dates. DFL50% of the accessions of two sowing dates was used to calculate PRI for each accession. DFL50% was used as a simple, non-destructive and indirect trait to determine the end of the vegetative phase and start of reproductive stage marked by the initiation of flowering in response to the photoperiod. These accessions based on their PRI values showed a wide range of response to the photoperiod. The PRI ranged from close to zero up to values close to 30 (the difference between first and second sowing dates) or even higher for non-photoperiod-sensitive flowering (showing a stable vegetative period) indicating highly photoperiod-sensitive flowering (sharp shortening of the vegetative period with late sowing). The significant difference between the mean DFL50% for June and July sowing indicates that sorghums from WCA show a reduction in the vegetative phase when they are sown late due to the delayed start of the rainy season. This reduction in mean DFL50% had a negative effect on overall vegetative growth, since the mean plant height of the accessions of July sowing was significantly lower than the mean plant height of the June sowing. A similar observation on the reduction of vegetative growth was made by Folliard et al. (2004) on a guinea sorghum cultivar resulting from decreasing day-length conditions where the total number of leaves was reduced to half when sown at four different dates. Clerget et al. (2008) also found that the plant height and number of total leaves initiated were higher in the varieties sown in

June compared to the ones sown in July. An explanation for these results could be that the apical growth is terminated by the start of the flowering. The earlier the flowering occurs the shorter is the duration of the vegetative phase. July sown accessions flowered earlier than the accessions sown in June, which may have resulted in reduced plant height in accessions sown in July.

5.2.2 Inference of population structure and association analysis

The population structure, the target genomic region, and the number of polymorphic sites can all have a major impact on the outcome of association analysis (Akey et al., 2003; Ke et al., 2004; Pritchard et al., 2000a). The genotypic analysis of the sorghum accessions was carried out using 27 SSR markers (for details see materials and methods section in Bhosale et al., 2011). We used the STRUCTURE approach to assign individuals to the subgroups. STRUCTURE uses a model-based Bayesian clustering approach to assign individuals to subgroups. Furthermore, with STRUCTURE, it is possible to include additional information such as the geographic sampling locations of individuals (Pritchard et al., 2000b). After employing STRUCTURE analysis and Evanno's method to estimate the number of subgroups, our sorghum accessions were divided into two subgroups (Figure 2 in Bhosale et al., 2011). Subgroup one consisted of 64 and subgroup two of 109 accessions. Forty-six accessions had both subgroups membership probabilities of less than 0.80, and thus were assigned to a mixed group. After coding the sorghum accessions from each STRUCTURE subgroup by their race and maturity class (Figure 2 in Bhosale et al., 2011), subgroup one was dominated by the race guinea primarily belonging to the early to intermediate maturity classes. The result of Fisher's exact test indicated that there were significantly ($p = 0.06$) more early genotypes in subgroup one than in subgroup two. In contrast, subgroup two was rather mixed, involving all the major accessions of sorghum belonging to the intermediate to late maturity classes. The first subgroup included mainly race guinea (83%) originating from western West Africa such as Mali and Burkina Faso and the second subgroup included accessions mainly from Nigeria and Niger and also accessions originating from other countries and other major races (see Bhosale et al., 2011 for details). The race guinea could clearly be distinguished from the other races

and this observation was in compliance with previous studies. Therefore, the study suggests that race, geographical origin, and maturity of the accessions are the most likely forces behind the observed structuring pattern of the accessions.

Overall, we found a high level of genetic diversity among the sorghum races which was comparable to previous studies. Race guinea was the most diverse and race kaura was the least diverse among the sorghum races. However, direct comparison between our results and previous studies is probably not valid since the estimates of gene diversity can vary (Deu et al., 2008) depending on the sampling schemes (single plant or bulked DNA), number and type of SSRs used, numbers of repeats of these SSRs, and their location in the genome (coding or non-coding DNA regions). We found higher LD estimates compared to the previous study by Hamblin et al. (2004). Possible reasons may be, firstly, that we used SSR (higher LD) markers as opposed to the RFLP (lower LD) and, secondly, we surveyed a larger number of accessions than Hamblin et al. (2004). Our study also showed that selection of early-intermediate maturing sorghum accessions over late maturing ones led to higher LD values in subgroup one than subgroup two.

5.2.3 Marker-phenotype association of the flowering time genes in sorghum

Subsequent to the phenotyping and the genotyping of the sorghum inbred accession panel, as a final step association analysis was conducted using a QK (Yu et al., 2006) approach that takes population structure as well as kinship information into account. This model performs better in association studies compared to models not considering the above mentioned factors (Yu et al., 2006; Stich et al., 2008). The association analysis for all the polymorphism found within the six CGs was carried out using values of PRI for each accession. The data on CG polymorphisms were obtained as described by Bhosale et al. (2012) in methods section.

From the six genes studied, we found in the fragments of genes *CRY1-b1* and *GI* several polymorphic sites which were significantly ($p < 0.005$) associated with PRI variation in the sorghum panel. *CRYPTOCHROMES* (*CRY1* and *CRY2*) and

phototropins are the two types of blue light/UV-A receptors important for plant photomorphogenesis. In *Arabidopsis* and rice, *CRYPTOCHROMES* exhibit similarities of function in regulating photoperiodic flowering. In rice, *OsCRY1* is a regulator of photomorphogenesis. Similar to *AtCRY1* and like *AtCRY2*, *OsCRY2* is also involved in the promotion of flowering time in rice (Lin et al., 1996; Guo et al., 1998; Hirose et al., 2006). Several polymorphisms in the *CRY1-b1* gene were significantly associated with PRI, where the most important polymorphisms showed an effect on PRI value of up to -4.2 days. This SNP at position 722 in *CRY1-b1* was located in the FAD binding domain at the N-terminal domain of *SbCRY1*. Hence, this domain appears to be important in photomorphogenesis in sorghum.

Several polymorphic sites in the *GI* gene homolog were also found to be significantly associated with PRI, with polymorphism (SNP888) having the largest effect on PRI of about 8 days. Previous studies in rice show that over-expression of the *GIGANTEA* gene (*OsGI*) inhibits flowering (Hayama et al., 2003) and over-expression of *AtGI* in transgenic *Arabidopsis* plants promotes flowering (Fowler et al., 1999) under long days. Similar to the observations in rice (Hayama et al., 2002, 2003), the positive allele effect on PRI observed in this study (Table 5 in Bhosale et al., 2012) indicates that *SbGI* enhances photoperiodic response to SD conditions in sorghum, i.e., *SbGI* shortens the time to sorghum flowering in the later July sowing which is more exposed to SD conditions, while in the June sowing (initially more exposed to LD conditions), *SbGI* delays sorghum flowering. These findings suggest that, to determine the exact mode of action of the *GI* gene homolog in sorghum, a detailed investigation of *GI* by comparison of sorghum accessions grown under short day and long day conditions is necessary.

In the case of gene *SbD8*, we did not find any significant association between the polymorphism found in the SH2 domain and PRI. Hence, our results showed that *SbD8* does not play a role in controlling flowering time in sorghum.

It is important to mention that the field experiment in our study was conducted during one year (2007) and at one location. Significant genotype × year interactions for

measures of photoperiod-sensitive flowering response might occur in multi-location trials over years. The sorghum accessions of our study were observed previously for their photoperiodic behavior. Therefore, a difference in genotype ranks was expected, with limited impact on an association analysis.

For the CGs studied, Tajima's D values were negative including the gene *SbD8* (Table 4 in Bhosale et al., 2012). Possible causes for the negative Tajima's D values may be, firstly, that the sorghum accessions originated from different geographical locations in WCA and thus had little common history. Secondly, population structure existing among the ancestral populations as a result of multiple domestications and introgressions from wild relatives could give rise to negative Tajima's D values (see Hamblin et al., 2006). Thirdly, the negative Tajima's D values might indicate that the gene may have been subjected to adaptive selection as variation in flowering time may confer adaptive advantages in sorghum (see Tenaillon et al., 2001). The contradictory Tajima's D values of *SbD8* in Chapter 4 and of the pilot study described in Chapter 2 may have been due to different fragments of gene *SbD8* studied.

The sequence analyses of the sorghum inbred line dataset of the study shows evidence of purifying selection for photoperiodic flowering time genes. This conclusion, though, needs to be considered with some degree of caution. To effectively capture the signature of selection on the photoperiodic flowering time network, it will be worthwhile to include other important genes, because the number of genes studied was relatively low. It is necessary to characterize the entire gene network in the photoperiod pathway to know how selection has shaped the photoperiod pathway of flowering time that enabled sorghum to adapt to climatic zones with different day length conditions.

5.3 Conclusions and outlook

In our study, we found significant association between the CG polymorphisms in fragments of genes *CRYPTOCHROME 1* and *GIGANTEA* involved in the photoperiod pathway of flowering time with PRI in sorghum. These results suggested that *CRI1*

and *GI* might be the preferential targets of selection for flowering time in sorghum. Characterization of genes upstream or downstream of these two genes may reveal, why variation in sorghum and other crops occur in the same set of genes associated with flowering time. To utilize these polymorphisms in breeding, molecular markers could be developed by designing cleaved amplified polymorphic sequence (CAPS). Functional markers could also be created directly from the significant SNPs. These markers can serve as powerful tools in MAS for development of improved photoperiod sensitive sorghum varieties. Additionally, molecular selection signatures on the flowering time gene network could be detected by correlating genome wide scans (e.g. Stinchcombe & Hoekstra, 2008; Linnen et al., 2009; Mariac et al., 2011) with the phenotypic variation and potential environmental causes to obtain a clearer picture of the evolutionary processes underlying the adaptation and spread of sorghum in WCA.

A high degree of structural similarity is found between the maize *D8* gene and *PgD8*, including the existence of an Indel flanking SH2 domain involved in maize flowering time variation. Association study of *PgD8* might reveal details of the potential role of *PgD8* in flowering time control in pearl millet.

This study also sheds light on the existence and possible causes of the population structure in WCA sorghum accessions. The findings revealed a high level of genetic diversity and linkage disequilibrium which can contribute to better understand the sorghum germplasm for effective sorghum breeding in WCA.

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Chapter 6
Summary

Sorghum is extremely important for the food security in the arid to semi-arid regions of West and Central Africa (WCA). A serious constraint to the sorghum production in WCA is the scattered beginning but relatively fixed end of the rainy season among years, forcing farmers to adjust their individual sowing dates according to the start of the rains. Owing to the delayed sowing and fixed end of the rainy season, farmers require varieties that flower at the end of the rainy season, regardless of the sowing date. Photoperiod sensitivity of sorghum accessions is an important adaptation trait that allows flowering or synchronized flowering of the accessions at the end of the rainy season. This is also particularly important in avoiding grain mold, insect and bird damages for early maturing varieties, and incomplete grain filling due to soil water shortage occurring at the end of the season in late maturing varieties. Cultivars with photoperiod sensitivity may have the potential to increase yield and yield stability. Unfortunately, in WCA most of the present day cultivars are photoperiod insensitive. Furthermore, unavailability of simple screening methods in selecting photoperiod sensitive cultivars complicates the situation. Breeding techniques such as marker assisted selection (MAS) by employment of molecular markers would greatly enhance the selection efficiency for this major adaptation trait. Candidate-gene (CG) based association studies can assist in investigating the effect of polymorphisms in flowering time genes on phenotypic variation. Allele-specific molecular markers can be developed after a significant marker-phenotype association is identified. These markers can effectively be used in MAS of photoperiod sensitive sorghum cultivars.

In this study we carried out a CG based association analysis to investigate the association between variation for photoperiodic sensitivity of flowering time in sorghum and polymorphisms in six partially amplified genes putatively related to variation in flowering time. Five out of six CGs were known to be involved in photoperiod pathway of flowering time [*CRYPTOCHROME* 1 (*CRY1*-b1), *CRYPTOCHROME* 2 (*CRY2*), *LATE ELONGATED HYPOCOTYL* (*LHY*), *GIGANTEA*

(*GI*), *HEADING DATE* 6 (*HD6*)], and the gene *SbD8* was involved in the gibberellic acid (GA) pathway of flowering time.

In the first part of the study we determined the presence, the expression and the molecular diversity of genes homologous to the important flowering time gene *D8* in maize on a set of 26 sorghum and 20 pearl millet accessions. Homologs of *D8* were successfully amplified and tested for their expression in sorghum (*SbD8*) and pearl millet (*PgD8*). Pearl millet, because of its autogamous nature, showed higher nucleotide diversity than sorghum, which is an allogamous species. In maize, a 6 bp deletion flanking the SH2-like domain of *D8* was found to be significantly associated with flowering by Thornsberry et al. (2001). We found in the *PgD8* gene a 3 bp insertion or deletion (Indel) flanking the SH2 domain in the region, which was only conserved between *D8* and *PgD8*. Cluster analysis performed for the *D8, SbD8,* and *PgD8* indicated that maize is more closely related to pearl millet than sorghum. These findings suggest that, similar to maize, the indel in *PgD8* flanking the SH2 domain might play an important role in determination of flowering. It is advisable to carry out an association study to reveal the potential role of *PgD8* in flowering time control in pearl millet.

After successfully amplifying and confirming the expression of *SbD8* and *PgD8,* we carried out the association analysis on the selected CGs. A panel of 219 mostly inbred accessions of sorghum from major sorghum growing areas in WCA was complied. In the second part of the study the association analysis panel of accessions was phenotyped for their flowering response in the field in 2007 in Mali. The entire panel was sown twice (June and July), photoperiod response index (PRI) was estimated as the difference between DFL50% of the two sowing dates of the accessions. The PRI of the accessions showed a wide range from close to zero (photoperiod-insensitive) up to values close to 30 or above (highly-photoperiod sensitive). This result confirmed that the range of response based on the choice of the accessions was appropriate for an association analysis. The plant height reduction observed in accessions sown in July compared to the once sown in June was in accordance with previous studies performed in West African sorghum varieties.

The sorghum accessions were genotyped using 27 simple sequence repeat markers. Population structure analysis using software STRUCTURE was carried out to control the false positives in the association analysis. The results showed existence of two subgroups in our sorghum accessions. The first subgroup included mainly race guinea (83%) originating from western West African countries such as Mali and Bukina Faso and the second subgroup included accessions mainly from Nigeria and Niger and also accessions originating from other countries and other major races. The race guinea could clearly be distinguished from the other races. Fisher's exact test for the presence of earliness among subgroups showed that there are significantly ($p = 0.06$) more early maturing accessions in subgroup one than subgroup two. But there was an absence of a clear structuring pattern. The study suggests that the race, the geographical origin, and maturity of the accessions are the most likely forces behind the observed structuring pattern of the accessions. We found a high level of genetic diversity among the sorghum accessions. Race guinea was found to be the most diverse and race kaura was the least diverse. In general, the estimates of the gene diversity were comparable to previous studies. The results showed that clustering of early-intermediate maturing guinea varieties may have increased the linkage disequilibrium (LD) in subgroup one compared to subgroup two. The differences in the extent of LD between our study and those in the previous studies can be due to the differences in the molecular markers used as well as differences in the racial composition of the accessions studied.

In the final part of the study the association analysis was carried out using a mixed-model method. This method takes both population structure and kinship information into account. The candidate genes polymorphism data were obtained by amplifying and sequencing of the chosen genes. The association analysis for the polymorphism found within the CGs was carried out using values of PRI for each accession. From the six genes studied, genes *CRY1-b1* and *GI* had several polymorphic sites which were significantly ($p < 0.005$) associated with PRI variation in the sorghum panel. The most important polymorphism in the gene *CRY1-b1* showed an effect on PRI value of up to -4.2 days. This single nucleotide polymorphism (SNP) at position 722 in *CRY1-b1* was located in the flavin adenine

dinucleotide binding domain (N-terminal domain) of *SbCRY1*; hence, this domain appears to be important in photomorphogenesis in sorghum. In the case of the *GI* gene homolog, SNP888 had the largest effect on PRI of about +8 days. Similar to the studies in rice, the *GI* gene delayed flowering under June sowing (long-day conditons) and shortened the time to flower in sorghum under July sowing (short-day conditons). Therefore, the action of the *GI* gene homolog in sorghum might be revealed by a detailed investigation of *GI* by comparison of sorghum accessions grown under short-day and long-day conditions. In the case of gene *SbD8*, no significant association with PRI could be found; hence, the potential involvement of this gene in flowering time control of sorghum was not confirmed. Negative Tajima's D values, of CGs indicated that the genes may have been subjected to adaptive selection as variation in flowering time may confer adaptive advantages in sorghum.

The results showed that CG-based association analysis using a mixed model approach can be successfully applied to unravel the genetic variation related to phenotypic variation in flowering time. The polymorphisms significantly associated with PRI can be used to develop cleaved amplified polymorphic sequence markers. Functional markers could also be created directly from the significant SNPs. These molecular markers can serve as powerful tools in MAS for sorghum to identify cultivars sensitive to photoperiod.

Chapter 7
Zusammenfassung

Sorghum ist äußerst wichtig für die Nahrungsmittelsicherheit in den ariden und semi-ariden Regionen West- und Zentralafrikas (WCA). Ein ernstes Hemmnis für den Sorghumanbau in WCA ist der uneinheitliche Beginn und das relativ fixe Ende der Regenzeit von Jahr zu Jahr. Dies zwingt die Bauern dazu den Aussaattermin an den Beginn des Regens anzupassen. Aufgrund zeitverzögerter Aussaat und wegen des fixen Endes der Regenzeit benötigen die Bauern Sorten, die unabhängig vom Aussaattermin am Ende der Regenzeit blühen. Die photoperiodische Empfindlichkeit von Sorghum Akzessionen ist ein wichtiges Adaptationsmerkmal, das zur Blüte oder zur einheitlichen Blüte von Akzessionen am Ende der Regenzeit führt. Dies ist besonders wichtig, um Körnerfäulnis, Beschädigungen aufgrund von Insekten und Vögeln bei frühreifen Sorten und unvollständige Kornfüllung aufgrund von Wasserknappheit am Ende der Regenzeit bei spätreifen Sorten zu vermeiden. Photoperiodisch empfindliche Sorten könnten in der Lage sein die Erträge sowie die Ertragsstabilität zu erhöhen. Allerdings sind in WCA die meisten aktuellen Sorten photoperiodisch unempfindlich. Darüber hinaus wird die Situation durch das Fehlen einfacher Prüfmethoden zur Selektion photoperiodisch empfindlicher Sorten erschwert. Züchtungsmethoden wie die Marker-gestützte Selektion (MAS) würden durch den Einsatz molekularer Marker die Selektionseffizienz für dieses wichtige Merkmal überaus erleichtern. Assoziationsstudien basierend auf Kandidatengenen (CG) können dazu beitragen, den Effekt von Polymorphismen in Blühzeitgenen auf die phänotypische Variation zu untersuchen. Wenn eine signifikante Marker-Phänotyp-Assoziation identifiziert worden ist, können Allel-spezifische molekulare Marker entwickelt werden. Diese Marker können für die MAS photoperiodisch empfindlicher Sorghumsorten effektiv genutzt werden.

In der vorliegenden Studie führten wir eine CG-basierte Assoziationsanalyse durch, um die Assoziation zwischen der Variation für photoperiodische Empfindlichkeit von Sorghum für Blühzeit und Polymorphismen in sechs partiell amplifizierten Genen, die

vermutlich mit der Variation für Blühzeit in Verbindung stehen, zu untersuchen. Für fünf der sechs CG war bereits bekannt, dass sie in den photoperiodischen Signalweg für Blühzeit eingebunden sind [*CRYPTOCHROME* 1 (*CRY1-b1*), *CRYPTOCHROME* 2 (*CRY2*), *LATE ELONGATED HYPOCOTYL* (*LHY*), *GIGANTEA* (*GI*), *HEADING DATE* 6 (*HD6*)]. Das Gen *SbD8* war eingebunden in den Gibberellinsäure-(GA)-Signalweg für Blühzeit.

Im ersten Teil der Studie bestimmten wir anhand eines Satzes von 26 Sorghum- und 20 Perlhirseakzessionen die Präsenz, die Expression und die molekulare Diversität von Genen, die homolog zu dem wichtigen Blühzeitgen *D8* in Mais sind. Homologien von *D8* wurden erfolgreich amplifiziert und ihre Expression in Sorghum (*SbD8*) und Perlhirse (*PgD8*) getestet. Die Perlhirse zeigte aufgrund ihrer autogamen Natur größere Nukleotiddiversität als Sorghum, welches eine allogame Art ist. In Mais wurde von Thornsberry et al. (2001) eine 6 bp lange Deletion gefunden, welche die SH2-ähnliche Domäne von *D8* flankierte und signifikant mit der Blüte assoziiert war. Wir fanden im *PgD8*-Gen eine 3 bp lange Insertion oder Deletion (Indel), welche die SH2-Domäne in der Region flankierte, die nur zwischen *D8* und *PgD8* konserviert war. Eine Cluster-Analyse für *D8*, *SbD8* und *PgD8* zeigte, dass Mais näher mit Perlhirse als mit Sorghum verwandt ist. Diese Ergebnisse legen den Schluss nahe, dass, ähnlich wie bei Mais, das Indel in *PgD8*, welches die SH2-Domäne flankierte, eine wichtige Rolle für die Festlegung der Blüte spielen könnte. Es ist ratsam eine Assoziationsstudie durchzuführen, um die potentielle Bedeutung von *PgD8* für die Kontrolle der Blühzeit in Perlhirse zu entschlüsseln.

Nachdem *SbD8* und *PgD8* erfolgreich amplifiziert und deren Expression bestätigt worden waren, führten wir eine Assoziationsanalyse für die selektierten CG durch. Es wurde ein Satz 219 größtenteils ingezüchteter Sorghumakzessionen aus wichtigen Anbaugebieten für Sorghum in WCA zusammengestellt. Im zweiten Teil der Studie wurden die Akzessionen für die Assoziationsanalyse für ihr Blühverhalten in Mali im Jahr 2007 phänotypisiert. Der gesamte Satz wurde zweimal ausgesät (Juni und Juli). Der photoperiodische Response-Index (PRI) wurde als Differenz zwischen DFL50% von den zwei Aussaatterminen geschätzt. Der PRI der Akzessionen zeigte eine weite

Spanne von fast Null (photoperiodisch unempfindlich) bis zu Werten nahe 30 oder darüber (hochgradig photoperiodisch empfindlich). Dieses Ergebnis bestätigte, dass die Spanne basierend auf der Auswahl der Akzessionen geeignet für eine Assoziationsanalyse war. Der Rückgang in der Pflanzenhöhe, der für die im Juli gesäten Akzessionen beobachtet wurde, im Vergleich zu den im Juni gesäten Akzessionen war in Übereinstimmung mit früheren Studien, die mit Westafrikanischen Sorghumsorten durchgeführt worden waren.

Die Sorghumakzessionen wurden mit 27 sog. s*imple-sequence-repeat-Markern* genotypisiert. Eine Populationsstrukturanalyse wurde mit der Software STRUCTURE durchgeführt, um falsch-positive Genotypen in der Assoziationsanalyse unter Korntrolle zu halten. Die Ergebnisse zeigten die Existenz von zwei Untergruppen in unseren Sorghumakzessionen. Die erste Untergruppe beinhaltete vor allem die Rasse Guinea (83%) aus dem westlichen Westafrika wie Mali und Burkina Faso. Die zweite Untergruppe beinhaltete vor allem Akzessionen aus Nigeria und Niger sowie Akzessionen aus anderen Ländern und andere wichtige Rassen. Die Rasse Guinea konnte klar von den anderen Rassen unterschieden werden. Fishers exakter Test auf Präsenz von Frühreife in den Untergruppen zeigte, dass es signifikant ($p = 0,06$) mehr frühreife Akzessionen in der ersten Untergruppe gab als in der zweiten. Allerdings war ein klares Strukturierungsmuster nicht vorhanden. Die Studie legt den Schluss nahe, dass die Rasse, die geographische Herkunft und die Reifezeit der Akzessionen die wahrscheinlichsten Kräfte hinter dem beobachteten Strukturierungsmuster der Akzessionen sind. Wir fanden ein hohes Maß an genetischer Diversität zwischen den Sorghumakzessionen. Die Rasse Guinea zeigte die höchste Diversität und die Rasse Kaura die geringste. Grundsätzlich waren die Schätzungen für die Gendiversität vergleichbar mit denen aus früheren Studien. Die Ergebnisse zeigten, dass verglichen mit der zweiten Untergruppe eine Häufung von Guineasorten mit früher und mittlerer Reifezeit das Kopplungsungleichgewicht (LD) in der ersten Unterguppe erhöht haben könnte. Die Unterschiede im Ausmaß des LD zwischen unsere Studie und denen in früheren Studien können durch Unterschiede in den verwendeten molekularen Marker sowie durch Unterschiede in der Rassenzusammensetzung der untersuchten Akzessionen zustande gekommen sein.

Im letzten Teil der Studie wurde eine Assoziationsanalyse unter Verwendung eines gemischten Modelles durchgeführt. Diese Methode berücksichtigt sowohl die Populationsstruktur als auch Abstammungsinformation. Die Daten der Kandidatengen-Polymorphismen wurden durch Amplifizierung und Sequenzierung der ausgewählten Gene erhalten. Die Assoziationsanalyse für den Polymorphismus, der innerhalb der CG gefunden wurde, wurde mit den Werten des PRI für jede Akzession durchgeführt. Von den sechs untersuchten Genen hatten *CRY1-b1* und *GI* einige polymorphe Stellen, die signifikant ($p < 0,005$) mit der Variation für PRI im Sorghumsatz assoziiert waren. Der wichtigste Polymorphismus im Gen *CRY-b1* zeigte einen Effekt auf den PRI-Wert von bis zu -4,2 Tagen. Der singuläre Nukleotidpolymorphismus (SNP) bei Position 722 in *CRY1-b1* war in der Flavin-Adenin-Dinukleotid-Bindungsdomäne (N-terminale Domäne) von *SbCRY1* lokalisiert; damit scheint diese Domäne wichtig für die Photomorphogenese in Sorghum zu sein. Im Falle der *GI*-Gen-Homologie hatte SNP888 den größten Effekt auf PRI von etwa +8 Tagen. Ähnlich wie in Studien mit Reis verzögerte das *GI*-Gen in Sorghum die Blüte nach Aussaat im Juni (Langtagsbedingungen) und verkürzte die Zeit bis zur Blüte nach Aussaat im Juli (Kurztagsbedingungen). Die Wirkungsweise der *GI*-Gen-Homologie in Sorghum könnte mittels einer detaillierten Untersuchung von *GI* durch einen Vergleich von Sorghumakzessionen unter Kurztags- und unter Langtagsbedingungen entschlüsselt werden. Im Falle des Gens *SbD8* konnten keine signifikanten Assoziationen mit PRI gefunden werden; damit wurde die mögliche Beteiligung dieses Gens in der Kontrolle der Blühzeit von Sorghum nicht bestätigt. Negative Tajima's D-Werte der CG zeigten, dass die Gene adaptiver Selektion ausgesetzt gewesen sein könnten, da Variation in der Blühzeit zu adaptiven Vorteilen in Sorghum beitragen kann.

Die Ergebnisse zeigten, dass die CG-basierte Assoziationsanalyse mit einem gemischten Model erfolgreich eingesetzt werden kann, um die genetische Variation in Beziehung zur phänotypischen Variation für Blühzeit zu erklären. Die Polymorphismen, die signifikant mit PRI assoziiert waren, können verwendet werden, um sog. *cleaved-amplified-polymorphic-sequence-Marker* zu entwickeln. Ebenso könnten funktionelle Marker direkt aus den signifikanten SNPs entwickelt werden.

Diese molekularen Marker können als leistungsfähige Werkzeuge in der MAS für Sorghum dienen, um Sorten zu identifizieren, die photoperiodisch empfindlich sind.

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Stuttgart, im August 2011

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