# Molecular Mapping and Analysis of Flowering Time in Sorghum

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# CHAPTER 1

## General introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop after wheat, rice, maize and barley. It is a self-pollinated crop grown on over 44 million hectares (USDA 2004) in both temperate and tropical regions. Sorghum is mainly grown as a rainfed crop by subsistence farmers in the semiarid tropical regions of Africa and Asia as well as by other farmers in the USA and the Latin America. It is a suitable crop for drought and heat-stressed environments and can be grown from sea level to elevations in excess of 300 m, in high rainfall areas, in semiarid regions, and in different seasons (Singh and Lohithaswa 2006).

### 1. Center of origin of sorghum

The origin of sorghum and its diversification into five major races (Harlan and de Wet 1972) and thousands of different genotypes began in the distant human past and is only partially known. The work of botanist, plant breeders, archaeologists and geographers has uncovered the probable evolutionary pathway in the domestication of sorghum and the probable spatial dynamics of the evolution under cultural control. A great deal has been learned in the last few about the origins of cereal and the people responsible for the domestication of sorghum races years.

Harlan and De Wet (1971) suggested that sorghum is an African grass originated and domesticated in the Sub-Saharan region of Africa and spread to India and China. Sub-Saharan and North East region of Africa were the primary centers of origin and diversity of sorghum. Many authors reported that Ethiopia is the centre of origin and diversity for sorghum (Mann *et al.* 1983; Doggett 1988;

Teshome *et al.* 2007) as it is rich in the number of snowdenian species and also contains several varieties of the durra type, which represents the highly evolved varieties among the cultivated races. Sorghum is the most important staple crop in Ethiopia. It is grown on 1,468,070 ha with a total production of 2,173,598 Mt (Mekbib 2007). It accounts 14.2% and 13.6% of the crop area and production respectively. Numerous varieties of sorghum were created though a disruptive selection for more than one level of a particular character within a population. This results from a balance of selection for cultivated traits by farmers and natural selection for wild characteristics, generating both improved sorghum types, wild types and intermediate types (Doggett 1970).

Sorghum was taken from Ethiopia to West Africa across the Sudan, from where it was first grown among the Mande people of the upper Niger. Sorghum was also taken from Ethiopia to East Africa, from where it was distributed among the Nilotic and Bantu people. It spread to India during the first millennium and was taken from there to China (Doggett 1976). Sorghum races in India are closely related to those in Northeast Africa. From West Africa sorghum was distributed to the USA and other parts of the world during the late 1800s to early 1900s.

## 2. Domestication of sorghum

The diversity of new sorghum types, varieties and races created though the movement of people, disruptive selection, geographic isolation and recombination of these types in different environments would have been large (Wright 1931; Doggett 1970). Sorghum has been carried to many new habitats to become the staple grain for millions of people. It has diversified into a sugar source, a construction material, a raw material for household implements and a raw material for industry (Singh and Lohithaswa 2006). Cultivated races of sorghum originated by disruptive selection and domestication in east central Africa from the wild snowdenian species, *Sorghum arundinaceum.*

Human selection for cultivated characters (non-shattering heads, large seeds, easy thresh-ability and suitable height and maturity) and natural selection for wild type characters resulted in divergence into polymorphic populations in the presence of considerable gene flow between the wild relatives and cultivars types. Sorghum is adapted to a wide range of environmental conditions and particularly adapted to drought. It has a number of morphological and physiological characteristics that contribute to its adaptation to dry conditions, including an extensive root system and waxy bloom on the leaves that reduce water loss (Singh and Lohithaswa 2006). This characteristic represents an interesting trait for

areas that receive small quantities of precipitations. It implies increasing use of marginal farmland in addition to the tolerance to the global climatic trends (Mekbib 2007). Sorghum is also tolerant to water logging and can be grown in high rainfall areas. It is a crop of hot and semiarid tropical environments with 400 to 600 mm rainfall that are too dry for maize. Sorghum is also grown in temperate regions and at latitudes of up to 2,300 m in the topics (Singh and Lohithaswa 2006).

## 3. Taxonomy of sorghum

Sorghum was firstly described by Linnaeus in 1773 under the name of *Holcus*. The classification of Sorghum genus was attempted by Brotero (1804), Roxburghii (1820), Steudel (1854), Chiovenda (1912), Piper (1915) and Stapf (1917) as cited in by Mekbib (2007). The most detailed classification was made by Snowden in 1935 (Mekbib 2007). As mentioned by Mekbib (2007), Snowden (1935) described 31 cultivated species and 17 related wild species and gave 48 different types well defined by a number of distinct characters. After decades of bio-systematic research, Harlan and de Wet (1972) have developed a simplified classification useful to plant scientists. The genetic diversity within *S. bicolor* (L.) Moench raised from the basis of the thousands of years natural and farmer selection and sorghum breeding programs that have occurred internationally during the last century (Mekbib 2007).

The cultivated taxa were first grouped into 28 species by Snowden in 1936 (Mekbib 2007). All classification schemes since then have been based on this historic work. A simplified classification design of cultivated sorghum was proposed by Harlan and de Wet (1972) based on morphological characteristics that most of breeders have come to recognize and utilize. The International Plant Genetic Resources Institute Advisory Committee on sorghum and millet germplasm has recommended this classification to be used in describing sorghum germplasm (Singh and Lohithaswa 2006). The system of classification of cultivated races into five basic races and 10 intermediate races and those of wild races into six spontaneous races is presented below:

**Basic races:** bicolor; guinea; caudate m; kafir; durra

**Intermediate races:** guinea-bicolor; caudatum-bicolor; kafir-bicolor; durra-bicolor; guinea-caudatum; guinea-kafir; guinea-durra; kafir-caudatum; durra-caudatum; kafir-durra.

**Spontaneous races**: arundinaceum; aethiopicum; virgatum; propinquum; shattercane; verticilliflorum.

Sorghum was named by Moench in 1974. All commercial groups of sorghum such as grain sorghum, fodder sorghum, broomcorn and sorgo are classified under a single botanical species *Sorghum bicolor* (L.) Moench. The genus sorghum belongs to one of the 16 subtribes of the tribe Andropogonaeae of the subfamily Panicoidae of the family Poaceae (Singh and Lohithaswa 2006). Garber (1950) suggested that the genus *Sorghum* comprises six sub-genera including the species *Sorghum bicolor* (L.)

**Kingdom** Plantae – Plants.

**Subkingdom** Tracheobionta – Vascular plants

**Superdivision** Spermatophyta – Seed Plants

**Division** Magnoliophyta - Flowering plants

**Class** Liliopsida – Manocotyledons

**Subclass** Commelinidae

**Order** Cyperales

**Family** Poaceae – Grass family

**Genus** Sorghum Moench – sorghum

**Species** *Sorghum bicolor* (2n=20)

**Subspecies** *sorghum bicolor* ssp. *arundinaceum* (common wild sorghum)

**Subspecies** *sorghum bicolor* ssp. *bicolor* (grain sorghum)

**Subspecies** *sorghum bicolor* ssp. *drummondii* (Soudan grass)

**Species** *Sorghum almum* (2n=40) Columbus grass

**Species** *Sorghum halepense* (2n=40) Columbus grass

**Species** *Sorghum propinquum* (2n=40) Columbus grass

## 4. Morphology of sorghum

Sorghum is a vigorous grass that varies between 0.5 m and 5.0 m in height. It is an annual crop. It produces one or many tillers, which emerge initially from the base and later from the stem nodes. The root system consists of fibrous adventitious roots that emerge from the lowest nodes of the stem, below and immediately above ground level. Roots are normally concentrated in the top 0.9 m of soil but may extend to twice that depth and can extend to 1.5 m in lateral spread. The stem is solid, usually erect and can be dry or juicy, insipid or sweet to taste. The center of the stem can become pithy with spaces. Leaves vary in number from 7 to 24, depending on the cultivar. They are born alternately in two ranks. Leaf sheaths vary in length from 15 to 35 cm and encircle the stem with their margins overlapping. The leaf sheath has often a waxy bloom. Leaves are from 30 to 135 cm long and 1.5 to 13 cm wide, with flat or wavy margins. Midribs are white or yellow in dry pithy cultivars or green in juicy cultivars (Singh and Lohithaswa 2006).

The flower of sorghum is a panicle, usually erect, but sometimes recurved to form a gooseneck. The panicle has a central rachis, with long or short primary, secondary, and sometimes tertiary branches, which bear groups of spikelet. The length and closeness of the panicle branches determine panicle shape, which varies from densely packed conical or oval to spreading and lax. Grain is usually partially covered by glumes. The seed is rounded and bluntly pointed, from 4 to 8 mm in diameter and varying in size, shape and color with cultivar (Singh and Lohithaswa 2006).

## 5. Usage of sorghum

Sorghum represents one of the main food crop for the world's poorest and most food insecure people. Sorghum has been used in food product and various food items in many parts of the world. It has unique properties that make it well suited for food uses. Some sorghum varieties are rich in antioxidants and all sorghum varieties are gluten-free, an attractive alternative for wheat allergy suffers (Harris *et al.* 2007). Farrel *et al.* (2006) suggested that sorghum will be of growing importance to feed the world's expanding populations. Developing countries account for roughly 90% of the world's sorghum area and 77% of the total output. In developing countries, the crop is grown by small scale farming households operating at the margins of subsistence. Sorghum is also an important animal feed used in many countries like the U.S., Mexico, South America, Australia and Japan. It is one of the most important summer annual forage crops next to maize (corn) in mainly Southern part of Japan. Good-quality

sorghum is available with a nutritional feeding value that is equivalent or lower than the nutritional feeding value of corn. Sorghum grain can be processed to further improve its feed value and techniques such as grinding, crushing, steaming, steam flaking, popping and extruding have all been used to enhance the grain for feeding. The products are then fed to dairy cattle, laying hens and poultry and pigs, and are used in pet foods (Mekbib 2007).

Moreover, sorghum is a biofuel crop of growing importance. It is currently the second source of grain-based ethanol in the US after maize. As much as 12% of domestic sorghum production goes to produce ethanol and its various co-products. With demand for renewable fuel sources increasing, demand for co-products like sorghum-DDGS (distiller's dried grains with soluble) will increase as well due to sorghum's favorable nutrition profile. The generally lower water demands and market price for sorghum than maize, versus their equal per-bushel ethanol yields, suggests that sorghum will be of growing importance in meeting grain-based biofuels need (Wagoner 1990; Scheinost *et al.* 2001).

## 6. Breeding objectives

Sorghum is grown in a wide range of physical conditions in locations ranging from equator to over 50° N and 30° S. It is therefore subjected to a wide variety of temperature, daylength and moisture regimes. Improved sorghum cultivar for a particular environment always involves breeding for adaptation to the specific climate conditions found there. This adaptation of a crop is usually indicated by the appropriate crop duration for that environment and by acceptable and stable yield levels and appropriate grain qualities (Singh and Lohithaswa 2006).

The type of cultivar required for a target location influences the objectives of the plant breeder. For example many landraces and early varieties were photoperiod sensitive, with a critical photoperiod of 12 h: once the day length is shorter than 12 h, the sorghum plant changes from vegetative to reproductive stage of development. Growing these photoperiod-sensitive landraces/lines as a summer crop in temperate zones of America and Australia where the day length is longer than 13 h was difficult, especially as many growth-related characteristics are poorly expressed under these long-day conditions. This made breeding improved varieties in semi-arid temperate and subtropical climates difficult (Reddy *et al.* 2006).

Improved cultivars for specific location must possess resistance to the major constraints to production encountered and grain-stover-quality factors appropriate for sorghum. These constraints include biotic stress such as disease, insects, and parasitic weeds and abiotic stress, the requirements for which are usually quite different from one location to another. Some of the major pests include midge (*Stenodiplosis sorghicola* Coquillett), greenbug (*Schizaphis graminum* Rondani), various aphids, shootfly (*Atherigona soccata* Rondani) and stem borer (*Chilo partellus* Swinhoe) (Sharma 1993). Major diseases include downy mildew (*Sclerophthora macrospora* (Sacc.) Thirum), anthacnose (*Colletotrichum gramnicola* (Ces.) Wils), sorghum rust (*Puccinia sorghi svhwein.*), leaf blight (Which (P)), ergot (*Claviceps sorghicola*) and head and kernel smut caused by Sporisorium *relianum* and *Sporisorium sorgi* respectively (House 1985).

Success in breeding for insect resistance in sorghum varieties has been varied. Resistance to some pests is quantitatively inherited and therefore difficult to transfer into high-yielding cultivars (Tao *et al.* 2003). Development of disease-resistant sorghum varieties has relied on identifying sorghum varieties/landraces with natural genetic resistance to the particular disease. Resistance to these constraints is deliberately bred into cultivars by crossing resistant type with cultivars possessing other desirable traits and selecting plants with both resistance and desirable trait. Commercial sorghum varieties have been developed with resistance to grain moulds (caused by several unspecialized fungal pathogens and saprophytes) and anthracnose (Reddy *et al.* 2006 and Thakur *et al.* 2008). Increasing yield and improvement of quality are the main concerns of sorghum breeding programs.

## 7. Classical breeding

With the release of the first commercial sorghum hybrid in 1964, sorghum became the second crop after maize in developing high-yielding hybrids using cytoplasmic-genic male sterility system. Since the first commercial sorghum hybrid, a total of eighteen more hybrids have been released. The hybrids played a major role in raising productivity and production. Beside hybrids, fifteen high-yielding varieties have also been released. A major advantage of varieties over hybrids is their relatively better grain quality and multiple resistance or tolerance against major pests and diseases (Singh and Lohithaswa 2006).

Plant breeding efforts over the past six decades have contributed tremendously to the genetic improvement of cereals in terms of yield and quality. However, traditional approaches to crop improvement have several limitations, and increase in yield and productivity cannot be sustained

indefinitely (Vasil 1994). Most of sorghum breeding programs have focused on agronomic performance to insure food security. However, grain quality is also an essential requirement for the development of improved cultivars. Moreover, improving drought tolerance is an important objective in a sorghum breeding program.

Early breeding for host plant resistance to sorghum midge, shoot fly, and stem borers brought about worthwhile resistance in sorghum. However, fast evolution races require incorporation of multiple resistance genes which has not been possible though classical breeding efforts. Therefore, genomics-based mapping, DNA markers, molecular linkage map and expression profiles gene sequences, have been adopted from the crop improvement perspective to address limitation of classical breeding efforts. It will accelerate identification and incorporation of use full genes into cultivars, facilitate positional cloning of candidate genes, provide new opportunities for assessing and expanding the gene pool in sorghum though comparative mapping of related and unrelated taxa, and contribute to the understanding of the biological basis of complex traits and phenomena important to crop improvement and in the development of transgenic (Singh and Lohithaswa 2006).

## 8. Genetic importance of sorghum

Small genome of sorghum has long been an attractive model for advancing understanding of the structure, function, and evolution of cereal genomes (Price *et al.* 2005). Sorghum is representative of tropical grasses in that it has "C4" photosynthesis, using complex biochemical and morphological specializations to improve carbon assimilation at high temperatures and light intensity. Its lower level of gene duplication than many other tropical cereals makes sorghum, like rice, an attractive model for functional genomics. Sorghum is more closely related to many major cereal crops with complex genomes and high levels of gene duplication than rice. Sorghum genome contains ca. 750 Mb of DNA, which is slightly larger than that of rice (430 Mb) but 3- to 4-fold smaller than that of maize (2400 Mb) (Arumuganathan and Earle 1991).

A rich history of genome analysis, culminating in the recent complete sequencing of the genome of a leading inbred, provides a foundation for invigorating progress toward relating sorghum genes to their functions. The diverse sorghum germplasm collection of > 40.000 accessions has been used to generate populations for mapping important traits loci. These studies have identified sorghum loci regulating plant morphology, disease resistance, environmental stress tolerance and other traits (Islam-

Faridi *et al.* 2002). Based on its importance as one of the world's leading cereal crops, a biofuel crop of high and growing importance, and a botanical model for many tropical grasses with complex genomes (Andrew *et al.* 2008), sorghum is considered a subject of plant genomics research. It is considered as an important target for plant genomics due to its adaptation to harsh environments, diverse germplasm collection, and relatively small genome size (Harris *et al.* 2007).

Sorghum is the closest cultivated relative of sugarcane. Sugarcane has a large genome that has duplicated at least twice since it diverged from sorghum, around 5 million years ago (Al-Janbi *et al.* 1997). The extensive similarity in the gene order between these two genomes, where intercrosses are still possible, makes sorghum the best model crop for the Androponeae tribe (Ming *et al.* 1998; Price *et al.* 2005) with the aim of understanding the extensive gene rearrangements and assisting the development of genetic maps in sugarcane. Sequencing of *Sorghum* provides another model genome within the grasses, which particularly when utilized in conjunction with rice, will stimulate evolutionary understanding of the entire Poaceae. Sequencing will stimulate gene and allele discovery and crop improvement in *Sorghum* as it did in rice. Sugarcane genomics will be supported by the *Sorghum* sequence data (Al-Janbi *et al.* 1997).

Genetic resources for sorghum and sugarcane improvement have been enhanced by the application of genomic tools to analysis of wild relatives in the *Sorghum* and *Saccharum* genera. Mutant populations (including TILLING populations) of sorghum expand the options for gene discovery and genetic manipulation. Protocols for EcoTILLING (Cordeiro *et al.* 2006) and quantitative SNP analysis in the complex sugarcane genome should be valuable tools for gene mapping, gene discovery and association genetics in sugarcane. The availability of a *Sorghum* genome sequence will further accelerate the potential to apply these techniques in both *Sorghum* and sugarcane. Gene discovery in this germplasm will also be supported by application of advances in expression profiling tools as has been applied to other crop species in the Poaceae (McIntosh *et al.* 2007).

## 9. Progress in genome characterization and genetic mapping in sorghum

Determination of the relative positions of genes on chromosomes and of the distance, in linkage units or physical units, between them is critical for marker-assisted-selection, gene cloning and elucidating the functions of these genes, thereby contributing to accelerated crop improvement. Due to their economic and scientific value, cereal genomes have been studied over the last 15 years using

highly advanced technologies. The similarity at the DNA level makes it possible to use comparative genetics to look for particular genes of unknown sequence between the genomes with the aim of using that information to develop new varieties or discovering new genes that could have a potential impact on traits that are of global importance (e.g. food quality, drought resistance, photoperiod sensitivity) (Cockram *et al.* 2007). Sorghum was the first angiosperm for which a bacterial artificial chromosome (BAC) library was published (Woo *et al.* 1994).

Construction of linkage map is the most fundamental step required for a detailed genetic study and marker-assisted breeding approach in any crop (Tanksley *et al.* 1989). Sorghum genome mapping based on DNA markers began in the early 1990s, and since then several genetic maps of sorghum have been constructed. Initially, the genetic maps of sorghum were based largely on DNA probes previously mapped in the maize genome (Pereira *et al.*1994). Later, three more maps were constructed using mainly sorghum genomic DNA probes (Xu *et al.* 1994). Another sorghum map published was based on both maize and sugarcane probes (Dufour *et al.* 1997). All of these maps were developed using RFLP markers, and most of the mapping populations were $F_2$, with the exception of the maps of Dufour *et al.* (1997) and Peng *et al.* (1999).

Dufour *et al.* (1997) used two recombinant inbred line (RIL) populations for the construction of a composite map, which was later extended by Boivin *et al.* (1999) with the addition of a large number of RFLP and AFLP markers to the map of Dufour *et al.* (1997). Tao *et al.* (1998) constructed a sorghum map using an RIL population and variety of probes, including sorghum genomic DNA, maize genomic DNA, sugarcane genomic DNA cereal anchor probes and eight SSR loci.

Genetic mapping in sorghum takes advantage of its straight forward diploid genetics, amenability to inbreeding, high levels of DNA polymorphism between *Sorghum* species, and manageable levels of DNA polymorphism within *S. bicolor*. More than 800 markers mapped in sorghum are derived from other taxa (hence serve as comparative anchors) and additional sorghum markers have been mapped directly in other taxa, or can be plotted based on sequence similarity. Anchoring of the sorghum maps to those of rice (Paterson *et al.*1995; Paterson *et al.* 2004), maize (Bowers *et al.* 2003; Whitkus *et al.* 1992), sugarcane (Dufour *et al.* 1997; Ming *et al.* 1998), millet (Jessup *et al.* 2003), switch grass (Missaoui *et al.* 2005), Bermuda grass (Bethel *et al.* 2006), and others provides for the cross-utilization of results to simultaneously advance knowledge of many important crops.

Quantitative phenotypes have been a major area for genetic studies for over a century because they are a common feature of natural variation in a population. They include commercially important traits in crops plants (Kearsey and Farquhar 1998). The basis of all QTL detection is the identification of association between genetically determined phenotypes and specific genetic markers. The genetic mapping of sorghum has been employed in the mapping of genes for a large number of traits. The interspecific population has been especially useful for characterization of genes related to domestication such as seed size, shattering (Paterson *et al.* 1995), tillering, and rhizomatousness (Paterson *et al.*1995). Plant height and flowering time (Lin *et al.* 1995; Ulanch *et al.* 1996) have been high priorities. Similarly, the importance of hybrid sorghum motivated much research into the genetic control of fertility restoration (Klein *et al.* 2001; Klein *et al.* 2005).

Resistance genes have been tagged for numerous diseases (Tao *et al.* 1998), key insect pests (Kastar *et al.* 2002; Tao *et al.* 2003), and also the parasitic weed, striga (Mutengwa *et al.* 2005; Haussmann *et al.* 2004). Genes and QTLs have been identified that are related to abiotic stresses including post reproductive stage drought tolerance (stay-green); preharvest sprouting, and aluminum tolerance. Additional morphological characteristics have also been mapped in inter-specific and/or intra-specific population (Feltus *et al.* 2006). Much of the value of the sorghum sequence may be realized though better understanding of the levels and patterns of diversity in extant germplasm, which can contribute both to functional analysis of specific sorghum genes and to deterministic improvement of sorghum for specific needs and environments.

**10. Heading and flowering time**

Plant development is not fixed but shows a wide plasticity based on a constant adjustment of developmental regulation to changing environmental conditions. Heading time and the floral transition (or flowering time), are classified among the most plastic developmental decisions in the life cycle of plants. Heading is a phase in the development of cereal plants, characterized by the emergence of a head from the sheath of the upper leaf (from the spike in wheat, rye, barley, and other spiked grains and from the panicle in oat, millet, rice, and other paniculate grains). In corn, heading begins with the tasseling of the male inflorescence, or the panicle, on the apex of the stem. Four or five days later the female inflorescence, or the cob, appears on the axil of the leaf. During heading a plant requires more nutrients and a greater amount of moisture. Proper nourishment, moisture, and light promote good development

of the inflorescences and simultaneous heading. Prolonged heading results in uneven maturation, making harvesting difficult and leading to crop losses.

Flowering is a complex phenotype which is the end result of numerous physiological and biochemical processes within a plant. These processes are regulated by the interaction of many genes within an organism, and are also influenced by environmental stimuli (Murfet 1977). In annual species like *Arabidopsis*, flower initiation, defined as the morphological changes that make meristem to specify flower, is immediately followed by the development of flowers. Therefore flower initiation can be considered the crucial regulatory point on which selection acts to ensure flowering and fruiting on time.

The transition to flowering is one of the major phase changes that a plant makes during its life cycle. The transition must take place at a time that is favorable for fertilization and the formation of seeds, hence ensuring maximal reproductive success. To meet these needs a plant is able to interpret important endogenous and environmental cues such as changes in levels of plant hormones and seasonable temperature and photoperiod changes (Ausín *et al.* 2005). Many perennial and most biennial plants require vernalization to flower.

To achieve reproductive success, plants must select the most favorable season to initiate reproductive development. This selection requires the existence of molecular mechanisms to continuously monitor environmental factors and to properly respond to the adequate conditions. Many environmental factors influence flowering time (Bernier and Perilleux 2005). Those changing in a predictable fashion along the year, such as light and temperature, are the most relevant in terms of the selection of the flowering season. These predictable factors show complex patterns of variation and interaction in different temporal ranges (i.e. diurnal versus annual variation in light and temperature).

However, even less predictable factors such as nutrient or wind can also modulate flowering time, depending on the species. Environmental factors display patterns of variation in the short (i.e. diurnal variation) and long ranges (i.e. seasonal annual fluctuation). Plants are able to perceive all this environmental variation and modulate their growth and development with responses that can be in the short term such as growth response to ambient temperature or in long terms like the flowering response to vernalization. This complexity determines the need for different molecular mechanisms in the perception of environmental variation and the generation of different temporal responses. Diversity is also broad from the side of the plant species (Ausín *et al.* 2005).

The molecular interpretation of these signals is though the transmission of a complex signal known as florigen, which involves a variety of genes, including CONSTANS, FLOWERING LOCUS C and FLOWERING LOCUS T. Florigen is produced in the leaves in reproductively favorable conditions and acts in buds and growing tips to induce a number of different physiological and morphological changes. The first step is the transformation of the vegetative stem primordia into floral primordia. This occurs as biochemical changes take place to change cellular differentiation of leaf, bud and stem tissues into tissue that will grow into the reproductive organs (Turck *et al.*2008).

Growth of the central part of the stem tip stops or flattens out and the sides develop protuberances in a whorled or spiral fashion around the outside of the stem end. These protuberances develop into the sepals, petals, stamens, and carpels. Once this process begins, in most plants, it cannot be reversed and the stems develop flowers, even if the initial start of the flower formation event was dependent of some environmental cue. Once the process begins, even if that cue is removed the stem will continue to develop a flower (Searle *et al.*2006).

**11. Flowering time and photoperiod in sorghum**

Sorghum was firstly domesticated in Ethiopia. It was distributed widely thoughout tropical, subtropical and temperate environments (Teshome *et al.* 2007). The adaptation to a broad range of growing conditions has been mainly due to the evolution of response of flowering to photoperiod (Chanterau *et al.* 2001). Flowering time is an important adaptive character which impacts yield and quality in crop plants. It is a crucial event in life cycle of seed propagated plants because of its key role in the adaptation and geographical distribution of the crops. In fact, flowering time reflects the adaptation of a plant to its environment by tailoring vegetative and reproductive growth phases to local climate (Edward *et al.* 2009).

Flowering is affected by environmental stimuli where photoperiod is considered as the major environmental determinant for flowering. In fact, plants co-ordinate flowering with optimal seasonal conditions to maximize reproductive success. In tropical regions many plants flower during the cooler seasons of the year to avoid the extreme heat of summer. In temperate regions many plants flower during spring to avoid damage to floral organs by freezing winter temperatures.

Many plants growing in the tropics flower as day length decreases, whereas many plants from temperate regions flower in response to increasing day length. Fluctuations in the length of the day

affect developmental processes and behaviors of many crops. These fluctuations, called also photoperiodism, allow detection of seasonal changes and anticipation of environmental conditions such as low temperatures and drought. In fact one mechanism by which plants synchronize flowering with optimal seasonal conditions is by sensing changes in daylength, or photoperiod which represent an important signal that regulates flowering time (Greenup *et al.* 2009).

Photoperiodism was first described in detail by Garner and Allard in 1920 though the demonstration that many plants flower in response to changes in daylength (Garner and Allard 1920). Plants are classified into three major classes according to their daylength response: long-day plants, short-day plants and day-neutral plants. Flowering of long-day plants occurs when the day becomes longer than some crucial length, whereas that of short-day plants arises when the day becomes shorter (Kikuchi and Handa 2009).

Photoperiod sensitivity refers to the fact that some plants will not flower until they are exposed to day lengths that are less than a critical photoperiod (short-day plants) or greater than a critical photoperiod (long-day plants). Long-day and short-day plant designations refer to the daylength required to induce flowering. Facultative long-day or short-day plants are those that show accelerated flowering in long-day or short-day but will eventually flower regardless of photoperiod. Most plants including sorghum must pass though a juvenile stage (lasting .about. 14-21 days for sorghum) before they become sensitive to photoperiod.

There are two subcategories of photoperiod responses that can be displayed by plants: absolute responses (qualitative and obligatory responses) and facultative responses (quantitative responses) (Thomas and Vince-Prue 1997). Photoperiod and sensitivity to it limit the potential for successful exchange of germplasm across different latitudes (Craufurd *et al.* 1999). The photoperiodic control of flowering is one of the main development processes of plants because it is directly related to successful reproduction (Thomas et Vince-Prue. 1997). Photoperiodism offers both opportunities and challenges in agriculture.

According to Morgan *et al.* (2002) opportunities include firstly development of cultivars that flower at the most appropriate time in a given environment or location, secondly development of cultivars that can be brought to flower or delayed in flowering with a treatment, and finally broadening the lines available for use in production of hybrids (Page *et al.* 2002). Beyond the relatively simple

question of when a crop will flower, there are a number of problems in reproductive development including pollen incompatibility in both self and cross species situations, viviparity, dormancy and quality of seeds. Evidence from several species indicates that the progression to flowering is a failsafe condition (Koornneef *et al.* 1998). Photoperiodism delays the genetic tendency to flower by forcing the plant to wait until a specific signal is sensed (Weigel 1995).

Whereas the effects of photoperiod on flowering time in sorghum are essential for the crop adaptation, these effects are not well understood (Michael *et al.* 2008). Despite extensive analysis of the daylength control of flowering in sorghum, little is known regarding effect of variation in photoperiod or daylength on flowering time in sorghum (Menz *et al.* 2002). In fact, since Sorghum was recognized as a short-day species by Garner and Allard (1923), daylength sensitivity in this species has been systematically eliminated by breeders to enlarge the range of adaptability and extend the crop area to temperate environment (Chantereau *et al.* 2001).

Photoperiod sensitivity remains an important characteristic for adaptation of sorghum to different climatic environment. It is a key feature matching flowering time to the length of the rainy season and securing the level and the quality of the crop. Consequently, a better understanding of response and sensitivity of flowering time, in sorghum, to the photoperiod will facilitate the control of flowering time which is one of the major objectives in sorghum breeding programs. While the environmental trigger is undoubtedly photoperiod, the details of the response have not been elucidated: variation in the critical photoperiod (i.e. that photoperiod above which in SPDs longer days delay time to flowering), photoperiod sensitivity, the number of short days after the longest day and photoperiod temperature interactions have all been proposed as possible mechanisms (Curtis 1968; Kassam and Andrews 1975).

The photoperiodic control of flowering has been long studied with many long-day and short-day plants (Thomas and Vince-Prue 1997). During the last decade, molecular-genetic approaches were applied to understanding the control of flowering time, mainly in the long-day plant Arabidopsis, and notable progress has been made in identifying the molecular mechanisms by which Arabidopsis recognizes daylength and promotes flowering specifically under long-days. Also, recent genetic studies in rice enabled the mechanisms of the daylength response in this short-day plant to be compared with those of Arabidopsis.

## 12. The objectives of this research

The control of flowering is central to reproductive success in plants, and has a major impact on grain yield in crop species. Flowering time is a complex trait that shows almost continuous variation in cereals (Cockram *et al.* 2007). Along with temperature, photoperiod is the most important environmental variable that determines when a plant will flower and set seed. In plant evolution, sensitivity to photoperiod can be considered a survival characteristic. Sensitivity to photoperiod is under genetic control and interacts with other temperature and flowering genes to hasten or delay the flowering response (Chang *et al.* 1969).

Though genetic studies are inconclusive as to the number of genes and the type of gene action involved in determining days to flowering and sensitivity to photoperiod, some reports based on different type of populations has identified QTLs associated with flowering time in sorghum but the QTLs controlling the sensitivity to photoperiod changes were not described in detail and with a wide range of photoperiod conditions.

Due to the lack of reports focused on photoperiod sensitivity genes in sorghum we report in the present study the identification of QTLs for flowering time and photoperiod sensitivity in sorghum. This study presents a new view regarding the sensitivity to photoperiod in sorghum. In fact sorghum is considered as a short-day crop for which development being delayed by an increase in photoperiod above a critical value, between 10 and 14 h day depending upon cultivar (Caddell and Weibel, 1971). However, information on the response of progress towards flowering to the photoperiod is limited (ICRISAT, 1989). This may, in part, reflect specific problems unique to this crop.

Therefore understanding the genetics of flowering is essential to adapt the life cycle of sorghum to the agro-environments in which it is grown. This objective represents the major concern for breeders. Moreover, yield and yield stability in sorghum, which are the main concerns for farmers, are highly influenced by flowering time, which is a key adaptation trait for local varieties. Consequently a better understanding of response to photoperiod will facilitate the control of flowering time which is a major objective of this study.

We consider here the effect of different ranges of photoperiod on flowering time in a core collection of sorghum and in an $F_2$ population derived from a cross between two selected cultivars

within this core collection. This study aimed (1) to analyze the variation in flowering time in a core collection of sorghum (2) to investigate the effect of daylength changes on flowering time in sorghum and to demonstrate the threshold for the response of flowering time in sorghum to the changes in photoperiod using different photoperiod conditions (3) to identify QTLs controlling flowering time and photoperiod sensitivity in sorghum using linkage disequilibrium analysis and linkage analysis.

# CHAPTER 2

## Variation in flowering time in a core collection of sorghum and variation in response to photoperiod

### 1. Introduction

Flowering time is one of the essential traits determining adaptation during crop domestication. Flowering time in sorghum is considered as a crucial event because of its key role in the adaptation and geographical distribution of this crop. Sorghum was classified as a short-day plant, and variation in the response to environmental stimuli determines its adaptation to the wide range of different environments in which it is grown (Craufurd *et al.* 1999). Photoperiod is one of the major determinant factors for this trait (Kikuchi and Handa 2009).

Short-day plants within the tropics often show acute sensitivity to photoperiod and the response is very closely adapted to latitude and the normal growing season (Roberts *et al.* 1996). While the environmental trigger is undoubtedly photoperiod, the details of the response have not been elucidated: variation in the critical photoperiod (above which, in short-day plants, longer days delay time to flowering), photoperiod sensitivity, the number of short-days after the longest day and photoperiod x temperature interactions have all been proposed as possible mechanisms (Kassam and Andrews 1975).

Matching phenology to the abiotic and biotic constraints is widely recognized as a prerequisite for good adaptation. Landraces that have evolved over millennia at a particular location should be well adapted to those particular locations or similar agro-ecological environments. Therefore characterizing response of the flowering time in landraces from a wide range of sorghum agro-ecological environments to photoperiod should improve our understanding about the photoperiodic basis of natural adaptation in

sorghum. Furthermore, the study of a wide range of sorghum landraces should provide a more comprehensive description of genetic variation in responsiveness to photoperiod and where to find that variation geographically.

The short-day plant photoperiod response of cultivated grain sorghum has largely been eliminated in elite breeding lines in order to produce varieties with a wide range of adaptability and extend the crop area to temperate environments. Nertherless the photoperiod sensitivity in landraces reamains important for local farmers. It is a key feature adjusting flowering time to the length of the rainy season and securing the level and the quality of harvestes. Thus the improvement of sorghum in tropical areas requires a better understanding of genetic factors implicated in photoperiod response (Trouche *et al.* 1998). Analysis of photoperiod response can be done using different sowing date for evaluation. The varietal photoperiod sensitivity can be directly estimated though the measurment of vegetative phase variations. It can also be modeled by using the basic cooncept introduced by Major (1980) who identified three genetic compenents to describe the photoperiod response: (1) Basic vegetative phase (BVP) defined as the shortest possible time to floral initiation; (2) Minimum optimal photoperiod (MOP) defined as the photoperiod theshold beyond wich the vegetative period is influenced by changes in daylength; and (3) Photoperiod sensitivity slope (PSS) that expresses the varietal linear increase in flowering time as daylength increases.

Molecular markers, genetic mapping and QTL analysis allowed new investigations for understanding genetic control of flowering time. Many QTLs controlling flowering time were identified in previous studies, however the effect of photoperiod change on flowering time and the sensitivity of sorghum to the variation in daylength were not intensely examined. Moreover the range of the variation of photoperiod above wich variation in daylength tremendously affect the flowering time and consequently the crop yield is not entirely investigated in sorghum.

The objectives of this study were to analyze the variation in flowering time in core collection of sorghum and to illuminate the difference among accessions in the response and sensitivity to daylength or photoperiod. We focused on flowering time because it represents a critical stage of development in the life cycle of most of plants and it is one of the most important traits for the adaptation of sorghum to different cultivation areas as explained by Craufurd and Wheeler (2009). Control of flowering time is therefore a major objective in sorghum breeding programs.

Furthermore the effects of photoperiod on flowering time in sorghum are essential for the crop adaptation, but these effects are not well understood (Michael *et al.* 2008) and little is known regarding effect of variation in photoperiod or daylength on flowering time in sorghum (Menz *et al.* 2002). Chantereau *et al.* (2001) reported that since Sorghum was recognized as a short-day species by Garner and Allard (1923), daylength sensitivity in this species has been systematically eliminated by breeders.

On another hand, a core collection is a limited set of accessions representing, with a minimum of repetitiveness the genetic diversity of a crop species and its wild relatives. This definition readily extends to a collection that includes a group of related species or one that is the aggregate of several collections of the same taxa held in network of cooperating gene-banks. The word 'core' suggests the central or innermost part, the heart and the most important part. The core is used as a reference point to an identified set of material, most commonly a collection.

The core collection will provide a focus for evaluation where information on a growing set of variable can be obtained and assessed on a structured and limited set of accessions. In this way studies on the core collection provide an overall view of the properties to be found in the whole collection. Core collections are established to improve the conservation and use of genetic resources. They can help in gene-banks management, in the decision that need to be taken on what should be conserved and in the improved use of material held in gene-banks (Frankel 1984).

## 2. Materials and methods

### 2.1. Plant Material

A diversity research set of 107 sorghum accessions developed by Shehezad *et al.* (2009) representing African and Asian countries was used in this study (Table 2.1). The representative set includes accessions originated from 27 African and Asian countries representing major growing areas of sorghum which are tropical or subtropical lowland environments. In this core collection, 25 accessions are from East Asia (Japan; 11, Korea; 7, Taiwan; 1, China; 6), two from Southeast Asia (Cambodia; 1, Myanmar; 1), 26 are from South Asia (India; 8, Pakistan; 13, Afghanistan; 2, Bangladesh; 1, Nepal; 2) and two accessions are from Southwest Asia (Iran; 1, Israel; 1). The remaining 52 accessions are from African origin including Chad; 2, Congo; 1, Lesotho; 3, Morocco; 5, South Africa; 7, Central Africa; 1, Sudan; 11, Nigeria; 4, Algeria; 1, Uganda; 4, Ethiopia; 5, Kenya; 3, Zimbabwe; 3, and Tanzania; 2.

## 2.2. Variation in flowering time in a core collection of sorghum

The core collection of sorghum was planted in the experimental field of University of Tsukuba during the growing season of 2008 (May-Nov) for the first year, and during the growing season of 2009 (May-Nov) in the second year of our experiment. The same set of accessions was seeded in two different fields at the experimental field of University of Tsukuba during these two years. The two fields used were characterized by different soil quality and characteristics.

For each sorghum accession in the core collection five plants were grown by planting density of 1.5m x 20 cm. Number of days from sowing to heading (DH), number of days to flowering (anthesis) (DF) and plant height (PH), were recorded for all accessions according to NIAS Gene bank descriptors of sorghum. Phenotypic measurements for all traits were recorded from a total of five plants representing each accession. Heading date was recorded when more than 50% of plants per accession exert the top of panicles. Flowering time was recorded when 50% of the panicle (for 50% of plants per accession) flowered. According to their flowering time, accessions were divided into early, medium and late flowering groups.

## 2.3. Variation in flowering time in response to photoperiod changes

According to their flowering time accessions were divided into early, medium and late flowering groups. Fifteen accessions were randomly selected from each group. The total of 45 selected accessions consisted of: 30 accessions from Asia (Japan; 11, Korea; 3, China; 3, Pakistan; 3, India; 3, Israel;1, Iran; 1, Lesotho; 2, Myanmar; 1, Nepal; 1, Bangladesh 1) and 15 accessions from Africa (Ethiopia; 3, Morocco; 2, Uganda; 2, Algeria, Sudan, Tanzania, Nigeria, Central Africa, Kenya, Chad and Zimbabwe; 1 accession respectively).

The 45 accessions were planted in 20 cm of diameter pots and were grown as replicated sets in three identical cabinets or controlled conditions at the experimental field of Tsukuba University during the growing season of 2008 (May-Nov) and during the growing season of 2009. The controlled conditions were represented by three growth chambers with mobile cover or cabinet automatically programmed to open and close at the appropriate time to provide the requested photoperiod starting from the post germination stage. Daylength were set to 11, 12 and 15 h, respectively. This experiment was conducted to study the variation in flowering time in sorghum accessions originated from different regions, in response to the variation in photoperiod or daylength. The main effect of the photoperiod

treatments was defined for each accession by counting number of days from sowing to flowering. Plants were irrigated during the early growth stages to promote good growth. Irrigation was continued until harvesting. Number of days from sowing to heading, number of days to flowering and plant height were accurately calculated for all accessions under three ranges of daylength.

# 3. Results

## 3.1. Variation in flowering time a in core collection of sorghum

During the growing season of 2008 (May-Nov) data related to heading date, flowering date and plant height were registered for all accessions. The difference between number of days to heading and number of days to flowering was not substantial. It ranged from one to four days only for all accessions. For some accessions flowering occurred in the same day as heading occurred. Most of accessions flowered two to three days after heading as shown in Fig 2.1.

On the other hand a wide range of variation in flowering time was observed within the panel of the 107 sorghum accessions (Fig 2.1) ranging from 56 days (MARIANGARIJORA MUDDAHIHAL from India) to 133 days (LAMBAS from Sudan). Number of days to flowering in African accessions ranged from 66 days (MILO PET. 139/51 EX TANGANYIKA, Central Africa) to 133 days (LAMBAS from Sudan) while in Asian accessions it ranged from 56 days (MARIANGARIJORA MUDDAHIHAL from India) to 129 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 group from 75 to 95 days and late flowering group with more than 95 days from sowing to flowering. Moreover, a wide range of plant height was observed in a the set of sorghum accessions ranging from 62.4 cm for RABI YANGAR JORA MITHUGADUR from India, to 427.5 cm for AKLMOI WHITE originated from Kenya. The variation in plant height for the total of 107 accessions of sorghum was shown in Fig 2.2. However, no correlation between the variation in flowering time and plant height was found in the core collection of sorghum used in our study. While no correlation between number of days to flowering and plant height was detected in this study (Fig 2.2).

During the growing season of 2009, most of accessions could not germinate because of the field and seeds conditions (contamination with fangs and immature seeds). Germination test was repeated several times, but most of accessions were not able to accomplish the process of growing till flowering

because of the late sowing and the climate changes during 2009 especially for late flowering accessions that couldn't reach the maturity during the growing season of 2008. For accessions that flowered under these conditions data were not included in our analysis, only the data recorded during the growing season of 2008 used for analysis in our study.

On the basis of number of days to heading and number of days to flowering observed in 2008, the 107 sorghum accessions were classified into three groups: early, medium and late flowering accessions. Regarding this classification early and medium flowering accessions were more often originated from Asia. Within the panel of Asian accessions 30% of accessions were classified as early flowering accessions and 49% as medium flowering accessions. While more than 55% of African accessions belonged to the late flowering group, 36% of the accessions were classified in the medium flowering group and only 8% of African accessions were classified as early flowering accessions. The classification of African and Asian accessions into three flowering groups is shown in Fig 2.3.

### 3.2. Variation in flowering time in response to photoperiod changes

A total of 45 accessions were selected from the core collection of sorghum and were grown under three ranges of daylength (11 hs, 12 h and 15 hs) during the growing season of 2008 and after that during the growing season of 2009. However Only the data recorded in 2009 were exploited in our study. Infact, during the growing season of 2008 some mechanical problems occurred to the controlled conditions used for this experiment. The cabinets stopped operating for several days at different growing stages and data related to our traits were not approved. The experiment was conducted in 2009 after reparing the cabinets.

The current experiment aimed to identify the variation in flowering time in the 45 selected accessions of sorghum grown under the controlled conditions of photoperiod and to explain the effect of variation in temperature and photoperiod on this trait. Unfortunately it was not possible to record the daily temperature inside the controlled conditions durimg the growing season of our crop. Furthermore we disposed only data recorded during 2009 for this experiment therefore it was not possible to investigate the effect of the variation in temperature on the variation in flowering time for all flowering groups using only 2009 data.

Under controlled conditions, daylength varied substantially across experiments resulting in considerable variation in number of days from sowing to heading and therefore variation in flowering

time for most of the accessions from different flowering groups as shown in Table 2.2, Table 2.3 and Table 2.4. The variations in flowering time due to the variation in daylength are illustrated in Fig 2.4 which displayed that accessions belonging to different flowering groups (early, medium and late) are more affected by short day photoperiod.

Short day photoperiod accelerated flowering for most of the accessions grown under controlled conditions of daylength and deriving from different geographical origins. Accessions in early flowering group flowered in an interval of 49 to 63 days under 11 hs of photoperiod while flowering was delayed to 56 to 70 days under 12 hs and, 61 to 79 days under 15 hs of photoperiod as illustrated in Table 2.2. A photoperiod of 11 hs accelerated flowering for the majority of early, medium and late accessions compared with 12 and 15 hs of daylength. This result integrated most of accessions from all flowering groups. Difference in number of days to fowering between 11 hs and 15 hs of photoperiod ranged fom 8 days (accession number 5 from China) to 20 days (accession number 14 from Lesotho) for the medium accessions (Table 2.3) and from 8 days (acession number 3 from Nepal) to 13 days (accession number 8 from Uganda and 10 from Ethiopia) for late accessions (Table 2.4). Furthermore 12 h of daylength accelerated flowering for the majority of early, medium and late flowering groups compared with 15 h of daylength.

Above 12 hs of photoperiod the increase in daylength generated a delay in flowering for accessions in all flowering groups. Difference in number of days to flowering between 12 and 15 hs of photoperiod ranged from 4 to 18 days for the early flowering group, from 3 to 17 days for the medium flowering group and from 5 to 11 days for the late flowering group (Table 2.2, Table 2.3 and Table 2.4; Figure 2.4). There was no a gradual response of flowering time to photoperiod between 12 and 15 hs daylength. The variation in number of days to flowering was strongly affected by the increase in the daylength. The 12 hs of photoperiod could be considered as a theshold above which daylength delay flowering time in sorghum.

To understand the difference in response of flowering time in sorghum to 12 hs and 15 hs of daylength we established the regression analysis between these two treatments for all flowering groups (Fig 2.5). The upshot of this analysis validated the hypothesis of the existence of photoperiod theshold nearby 12 hs. Under 12 hs of photoperiod flowering occurred in advance compared with 15 hs of photoperiod per day for most of the accessions in different flowering groups (Fig 2.4).

Sorghum accessions were classified into three major classes according to their photoperiod sensitivity: insensitive, relatively insensitive and sensitive accessions. Accessions were considered insensitive to change in photoperiod when difference in number of days to flowering between 12 hs and 15 hs daylength is less than five days. They were classified as relatively insensitive if the difference in number of days to flowering is comprised between 5 and 10 days and as sensitive accessions when this difference is superior than 10 days.

## 4. Discussion

### 4.1. Variation in flowering time in a core collection of sorghum

A considerable variation in number of days to flowering ranging from 56 to 133 was perceived in a core collection consisting of 107 accessions of sorghum (Fig 2.1). Most of the Asian accessions flowered earlier than African accessions (Table 2.2, Table 2.3 and Table 2.4; Fig 2.3). The variation in flowering time across geographical origin is resulting from substantial variation in climate conditions in countries of origin.

The results revealed in this study can explain that sorghum was firstly domesticated in Africa and distributed throughout the world. The distribution occurred because of variation in response to photoperiod among accessions originated from different geographical regions as proved by Alagarswamy *et al.* (1997). Quinby and Karper (1945) explained that sorghum adaptation has been mainly facilitated by evolution of the genes controlling response to photoperiod and their interaction with daylength. Sorghum germplasm adjust flowering to the length of the growing season. To better understand these results we contemplate to study the variation in response to photoperiod of sorghum accessions under different daylength treatments and to underlie the variation in sensitivity to photoperiod among different accessions.

### 4.2. Variation in flowering time in response to photoperiod changes

The present study aimed to determine the effect of photoperiod or daylength changes on flowering time in sorghum and to estimate the theshold of photoperiod requested by sorghum to ensure the transition from the vegetative to the reproductive stages and to achieve its normal growth. Although the accessions of sorghum were planted on the same date, the transition from vegetative to reproductive stages was held in different times and flowering time occurred under specific daylength required by each accession to ensure this transition.

The results obtained in this research suggested that the increased photoperiod significantly increased the time requested by sorghum accessions to flower. The increase in flowering time was a linear function of the photoperiod. Short-day conditions (11 hs and 12 hs) accelerated the flowering time for accessions from different geographical origins. Sorghum accessions gradually responded to the decreasing in daylength. Whereas a photoperiod of 15 hs delayed the flowering time by increasing the number of days to flowering. These results were confirmed previously by Garner and Allard (1923) explaining that sorghum is a short-day plant. They were also authenticated by Folliard *et al.* (2004) who proved that for sorghum crop, progress towards flowering is accelerated when daylength decreases.

On the basis of these outcomes we suggested that the accurate photoperiod compulsory for flowering in sorghum belongs to the interval of 11 to 12 hs of photoperiod. We could identify that variation in flowering time in response to photoperiod and sensitivity to it, fluctuated within accessions. Some accessions seemed to be weakly affected by the changes in photoperiod, nevertheless other accessions are strongly affected by daylength changes.

For instance, in early flowering group, for the accession number 7 originated from Nigeria difference in number of days to flowering between 12 hs and 15 hs of photoperiod is equivalent to 4 days while for other accessions this difference ranged from 7 to 16 days. For accessions 5 and 6 from medium flowering group and originated from China and Japan respectively this difference is equivalent to 3 days only while its fluctuated from 5 to 17 days for other accessions. Accession number 13 from late flowering group, flowered 5 days in advance under 12 hs of photoperiod compared with 15 hs of daylength, while this delay is ranging from 7 to 11 days for remainig accessions in the same group (Table 2.2, Table 2.3 and Table 2.4). The enumerated accessions are weakly affected by changes in daylength and than insensitive or relatively insensitive to photoperiod (Fig 2.5). For these accessions variations in number of days to flowering were not associated with variation in daylength.

Conversely some accessions appeared to be strongly affected by changes in daylength and subsequently severely sensitive to photoperiod. For accession number 2 from Korea, accession number 9 from Pakistan and accession number 12 from India included in early flowering group, differences in number of days to flowering between 12 hs and 15 hs of photoperiod were equivalent to 16, 18 and 16 days respectively. In this group 80% of accessions delayed flowering more then 10 days under 15 hs of photoperiod. While for medium and late flowering groups 60% and 13% of accessions respectively delayed their flowering more than 10 days after increasing daylength to 15 hs. Therefore late flowering

accessions appeared to be less sensitive to photoperiod changes and less affected by the increases in daylength than early and medium flowering acessions.

Thomas and Vince-Prue (1997), explained this response to the variation in photoperiod by the existence of two subcategories of photoperiod responses that can be displayed by plants: absolute responses (qualitative and obligatory responses) and facultative responses (quantitative responses). Roberts *et al.* (1996) cited that tropical crops are normally short-day plants but with decreasing photoperiod sensitivity if grown outside the tropics. Short-day plants within the tropics often show acute sensitivity to photoperiod, and the response is very closely adapted to latitude and the normal growing season (Curtis 1968; Kassam and Andrews 1975; Roberts *et al.* 1996).

Furthermore sorghum was considered as short-day plant that flowers most rapidly if illuminated during less than a certain number of hours per day (Thomas and Vince-Prue 1997). This sensitivity of floral induction to day-lengh is an adaptation to regional climate shown by many tropical speacis. However the patterns of response to daylength during the photoperiod sensitive phase vary widely among sorghum genotypes, ranging from qualitative response where floral induction requires that daylength falls below a genotype theshold, to quantitative response ( Dingkuhn *et al.* 2008).

Most crop plants respond to photoperiod. In general short-day and long-day plants respond in similar manner with photoperiods longer or shorter, respectively, than the critical or base photoperiod delaying flowering (e.g. maize, a short-day species). In quantitative types, flowering is delayed but not prevented in the non-inductive photoperiod. In qualitative types, if the photoperiod transgresses a critical threshold flowering will not occur. While qualitative responses have been observed in some crop plant (e.g. pigeonpea, soyabean), the photoperiod in most growing seasons does not transgress the ceiling or maximum photoperiod, or does so only for a short period. Most crop plants are effectively short or long-day plants. One exception to this may be sorghum in parts of West Africa (Dingkuhn *et al.* 2008).

Kassam and Andrews (1975) reported that for particular sorghum landraces grown at locations in south or north of their latitude of origin, flowering occurs earlier or later, respectively, than in location of origin. This analysis suggested that there were two major mechanisms controlling flowering time and adaptation in sorghum. Firstly mechanism in which the genotypes are sensitive or insensitive to daylength or photoperiod, given that photoperiod sensitivity is the most important mechanism governing

adaptation. Secondly mechanism in which the genotypes are inherently early otherwise late flowering genotypes.

Moreover, Quinby (1973) explained that photoperiod sensitivity in sorghum bicolor is controlled by at least four maturity genes *Ma1*, *Ma2*, *Ma3* and *Ma4*. The $ma^3R$ allele was initially isolated because it caused field-grown plant to be photoperiod insensitive (Quinby and Karper 1961). The $ma^3R$ mutation has always been characterized as causing photoperiod insensitivity (Quinby 1973; Pao and Morgan 1986). Major *et al.* (1990) found no difference in flowering time in $ma^3R$ sorghum accessions grown under 12 and 14 h of day length.

On the other hand, Roberts *et al.* (1996) suggested that sorghum is a short-day crop where variation in the response to photoperiod and temperature determines its adaptation to the wide range of different environments in which it is grown. Characterizing the flowering responses to temperature and photoperiod of landraces from a wide range of sorghum agro-ecological environments should improve our understanding to the photo-thermal basis of natural adaptation in sorghum (Craufurd *et al.* 1999). Information on the response of progress towards flowering to the photo-thermal environment is limited even though that progress from sowing to panicle initiation in sorghum is sensitive to both photoperiod and temperature. A possible complication occurs in sorghum as a result of diurnal asynchrony between photoperiod and thermo-period (Ellis *et al.* 1997).

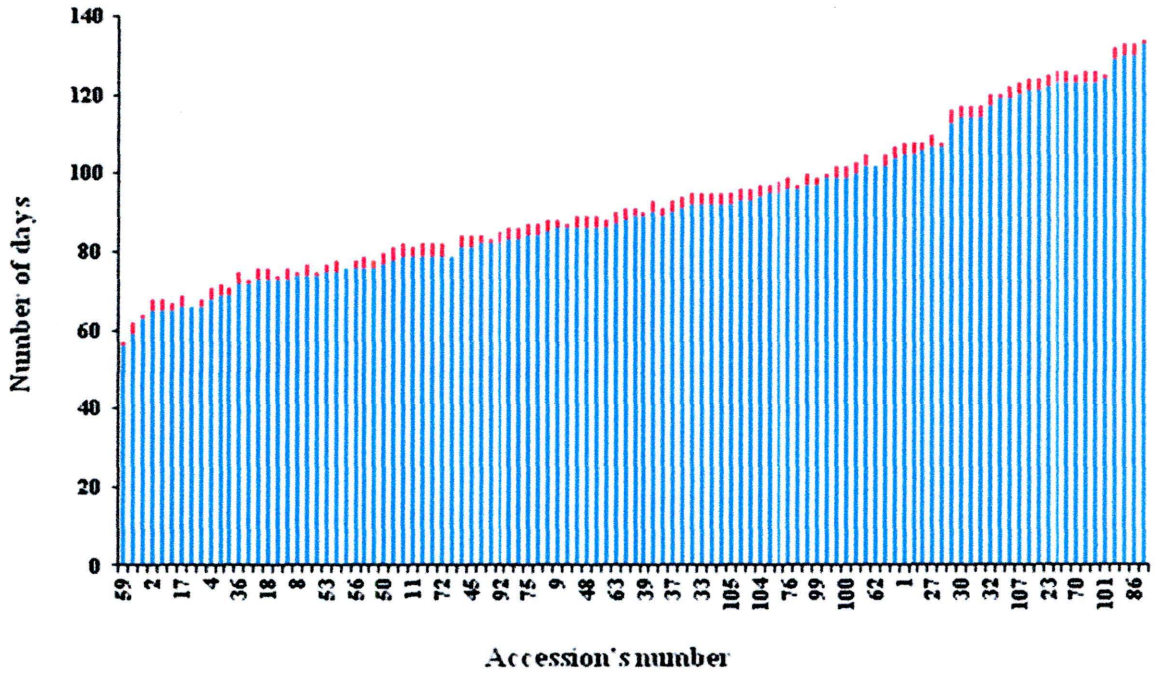


Fig 2.1: Variation in number of days to heading (blue) and number of days to flowering (red) within the core collection of sorghum grown under natural conditions of photoperiod (2008)

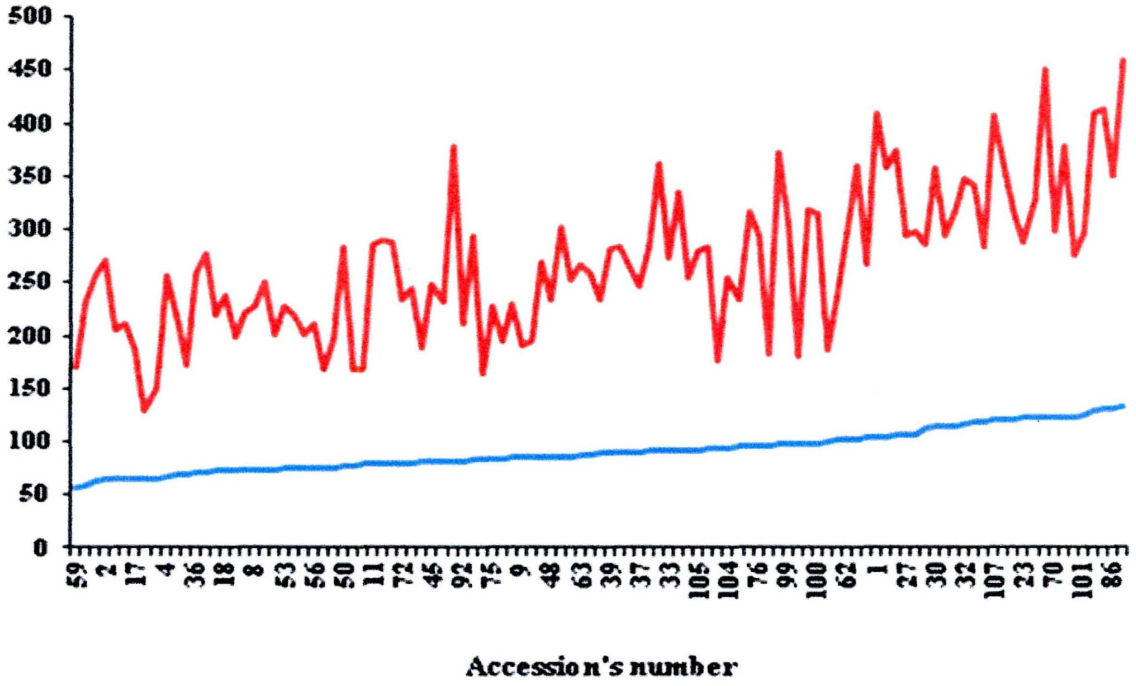


Fig 2.2: Variation in plant height (red) with number of days to heading (blue) in a core collection of sorghum

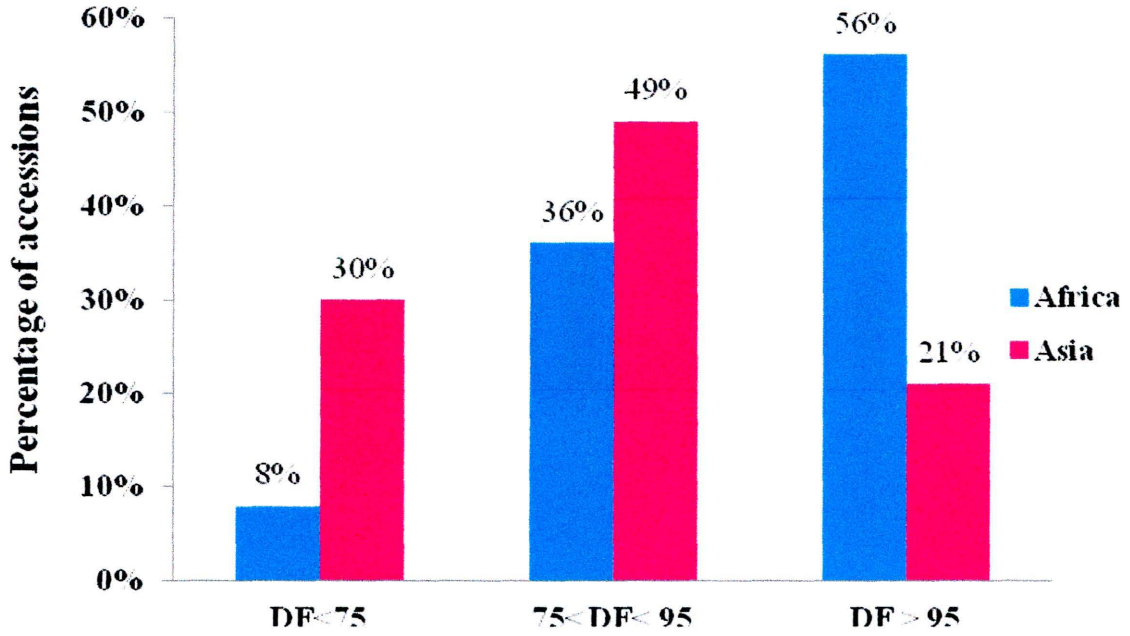


Fig 2.3: Repartition of African and Asian accessions into three groups on the basis of number of days to flowering (DF)

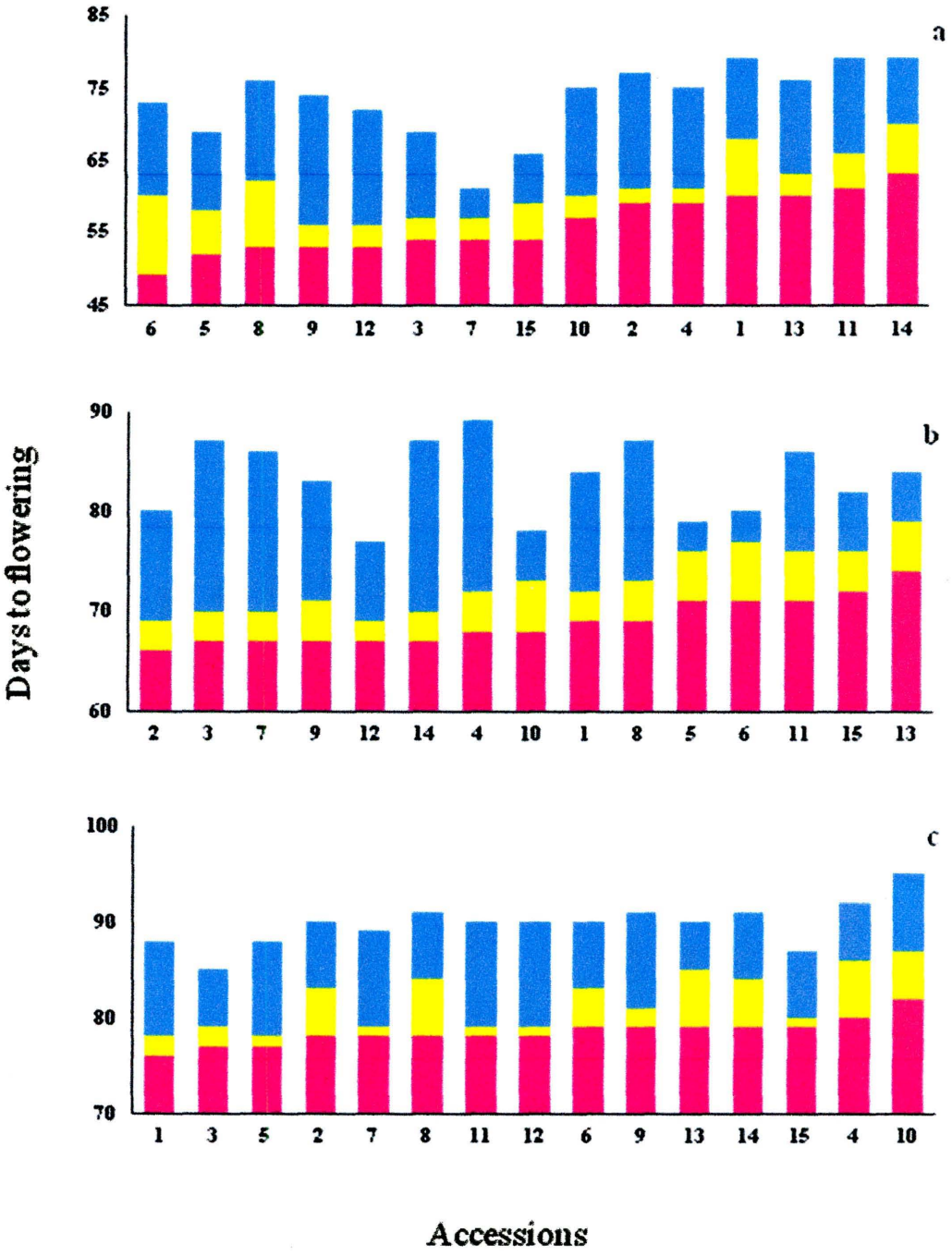


Fig 2.4: Variation in flowering time in 45 sorghum accessions grown under controlled conditions of photoperiod (a: early flowering accessions; b: Medium flowering accession; c: Late flowering accessions) **11 hs of daylength, 12 hs of daylength, 15 hs of daylength**

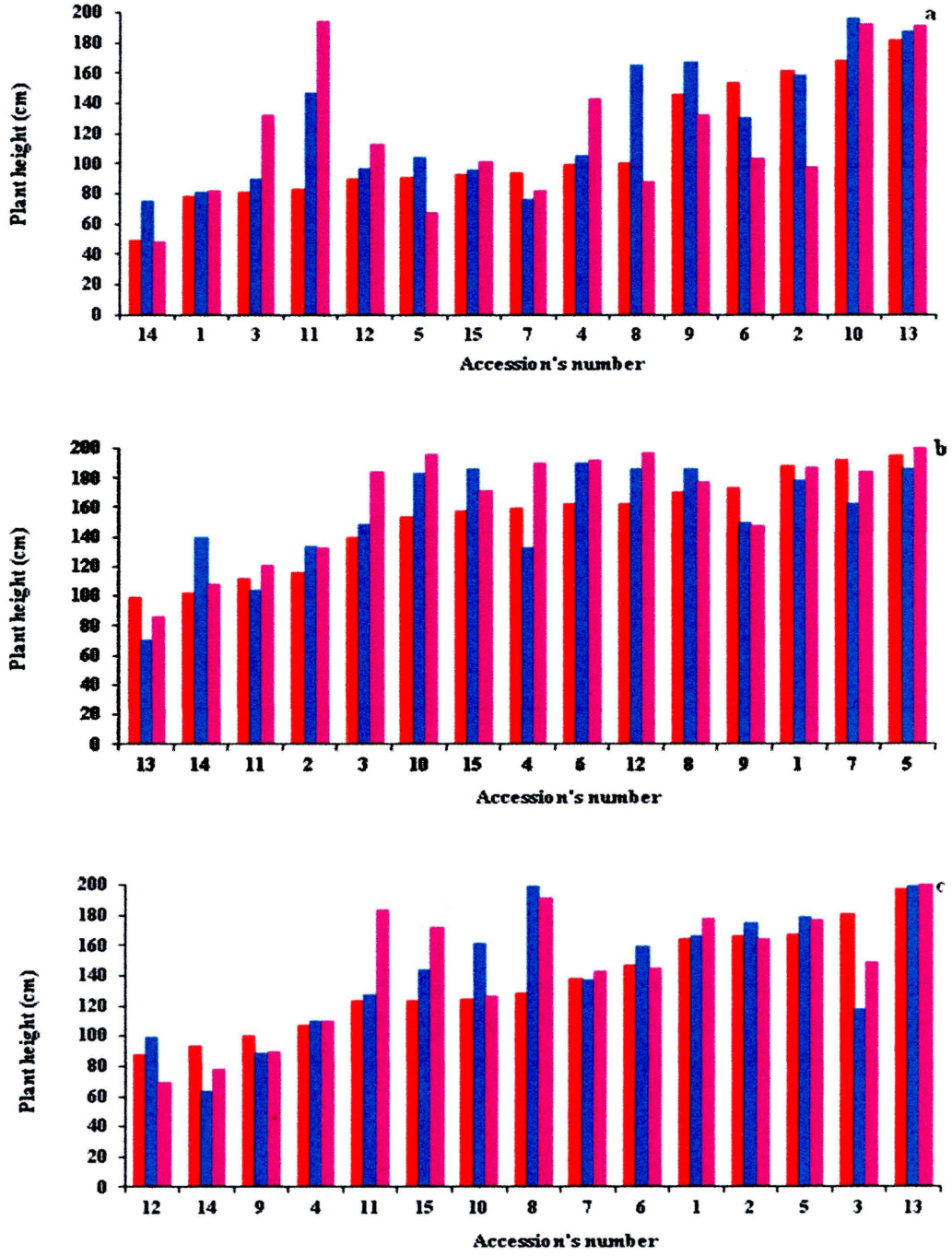


Fig 2.5: Variation in plant height in 45 sorghum accessions in response to the variation of daylength (a: early flowering accessions; b: Medium flowering accession; c: Late flowering accessions; ■ 11 hs; ■ 12 hs; ■ 15 hs)

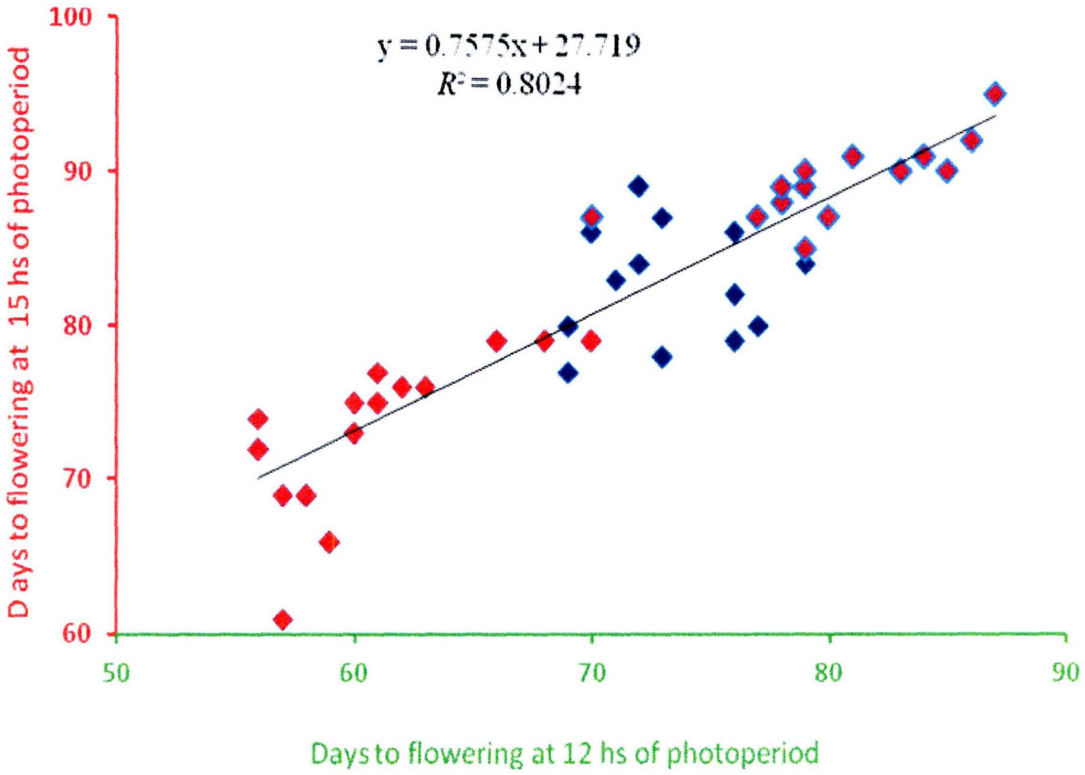


Fig 2.6:The regression of observed days to flowering (DF) values at 15 hs of PP / day, versus the observed values at 12 hs of photoperiod / day ($R^2$= 0.80); (■ 11 hs; ■ 12 hs; ■ 15 hs)

Table 2.1: List and origin of accessions in core collection of sorghum

| Accession | Cultivar Name | Origin |
|---|---|---|
| 1 | E 9 | Chad |
| 2 | OOTOYO-MURA ZAIRAI | Japan |
| 3 | HANGETSUTOSUI | Korea |
| 4 | KOUSHUU ZAIRAISHU | Korea |
| 5 | CHAL WAXY SORGHUM | Korea |
| 6 | AI HUI | China |
| 7 | Y. E. (I. P.) INT. TYPE | India |
| 8 | AIT BRAHIM | Morocco |
| 9 | CODY | Morocco |
| 10 | KOURNIANIA | Morocco |
| 11 | PHATSAI | Morocco |
| 12 | SCHOCK | Morocco |
| 13 | ESHOME | S. Africa |
| 14 | COL/PAK/1989/IBPGR/2386(2) | Pakistan |
| 15 | ZA113 DAWA PAS PARA | Nigeria |
| 16 | PI 229486 VULGARE | Iran |
| 17 | TAKAKIMI | Japan |
| 18 | COL/PAK/1991/IBPGR/2724(2) | Pakistan |
| 19 | HEGARI MALOWAR | Sudan |
| 20 | E 232 INGWARUMA PEARLY | S. Africa |
| 21 | AW 70/12 DL/59/1532 | S. Africa |
| 22 | E 233 BARNARD RED | S. Africa |
| 23 | IKEDACHO MATSUO ZAIRAI | Japan |
| 24 | KALJANPUR | India |
| 25 | EC 18868 | Nepal |
| 26 | JUNELO | Nepal |
| 27 | MN 401 | Algeria |
| 28 | 143 DINDERAWI 1 | Sudan |
| 29 | RED KAFIR | S. Africa |
| 30 | PI 282834 | Chad |
| 31 | PI 220636 Q 2/3/56 | Afghanistan |
| 32 | SC NO.0217 CI1197 | India |
| 33 | KOUCHI OUKAWA ZAIRAI | Japan |
| 34 | MAKHOTLONG I | Lesotho |
| 35 | NUO GAO LIANG | China |
| 36 | ER BAI SHE YAN | China |
| 37 | DANGOMOROKOSHI | Japan |
| 38 | TOKIBI | Japan |
| 39 | COL/PAK/1989/IBPGR/2420(1) | Pakistan |
| 40 | COL/PAK/1989/IBPGR/2427(5) | Pakistan |
| 41 | COL/PAK/1989/IBPGR/2439(1) | Pakistan |

Table 2.1 (continued)

| Accession | Cultivar Name | Origin |
|---|---|---|
| 42 | COL/PAK/1989/IBPGR/2444(1) | Pakistan |
| 43 | COL/PAK/1989/IBPGR/2550(1) | Pakistan |
| 44 | COL/PAK/1989/IBPGR/2553(4) | Pakistan |
| 45 | COL/PAK/1989/IBPGR/2411(1) | Pakistan |
| 46 | HIMEKI ZAIRAI | Japan |
| 47 | KIKUCHI ZAIRAI | Japan |
| 48 | GOOSENECK | India |
| 49 | COL/PAK/1989/IBPGR/2416(2) | Pakistan |
| 50 | COL/PAK/1989/IBPGR/2592(7) | Pakistan |
| 51 | S. VULGARE 72-726-7 | Uganda |
| 52 | S. VULGARE 72-728-1 | Uganda |
| 53 | KOUBOUSHI | Korea |
| 54 | REDBINE 655 | Sudan |
| 55 | MORABA 74 | Ethiopia |
| 56 | THIBA RED | Ethiopia |
| 57 | E 276 FRAMIDA | Uganda |
| 58 | E 1089 | Sudan |
| 59 | MARIANGARIJORA MUDDAHIHAL | India |
| 60 | AKAHO | Japan |
| 61 | BATTANBAN | Cambodia |
| 62 | AS 4547 JARDIRA | Nigeria |
| 63 | KANAGAWAZAIRAI | Japan |
| 64 | DHOOTI ANEHULA | India |
| 65 | RABI YANGAR JORA MITHUGADUR | India |
| 66 | HAZERA 6014 | Israel |
| 67 | AKLMOI WHITE | Kenya |
| 68 | LAMBAS | Sudan |
| 69 | DINDERAWI 1 | Sudan |
| 70 | 240 WAD UMM BENEIN | Sudan |
| 71 | MUGBASH WHITE | Sudan |
| 72 | S.BASUTORUM DL/60/97 | S. Africa |
| 73 | EAR FROM PIETESBURG DL/60/107 | S. Africa |
| 74 | WAD YABOO 132/53 | Zimbabwe |
| 75 | CAPE COLO 28/53 | Zimbabwe |
| 76 | MN 1277 MUHEYAR | Nigeria |
| 77 | PI 220636 Q2/3/56 | Afghanistan |
| 78 | LIAOZA 1 | China |
| 79 | MOCTAC LOCAL | Korea |
| 80 | B-112 | Sudan |
| 81 | SENKINHAKU | Korea |
| 82 | AS 5781 HUAN SA PHAUNG AHLPYSU | Myanmar |
| 83 | AS 4136 MASAKA LUWEMEA | India |

Table 2.1 (continued)

| Accession | Cultivar Name | Origin |
|---|---|---|
| 84 | SC112 | Ethiopia |
| 85 | GIZA 3/59 | Ethiopia |
| 86 | UGANDA L1 | Uganda |
| 87 | AS 4637 NHORONGO NENPI | Tanzania |
| 88 | E 37 | Tanzania |
| 89 | TSETA LOCAL NATURE TYPE 27/51 | Zimbabwe |
| 90 | E 17 | Congo |
| 91 | KA 24 | Nigeria |
| 92 | CHOONCHAN LOCAL | Korea |
| 93 | BIG WHITE HULL | China |
| 94 | XIONG YUE 334 | China |
| 95 | TENANT WHITE | Lesotho |
| 96 | NYAKASOBA BEST | Lesotho |
| 97 | 72-8-13 | Taiwan |
| 98 | 72-10-10-5 | Japan |
| 99 | 87-9-21-3-1 | Pakistan |
| 100 | 87-9-21-3-2 | Pakistan |
| 101 | E 1091 | Sudan |
| 102 | 109 TONJI | Sudan |
| 103 | PI 329762 | Ethiopia |
| 104 | E 959 | Kenya |
| 105 | PI 152748 C | Kenya |
| 106 | MILO PET. 139/51 EX TANGANYIKA | Central Africa |
| 107 | ALLAKH | Bangladesh |

Table 2.2: Variation in days to flowering (DF) in early flowering accessions under different daylength conditions

| Acc. N° | Origin | Day length (h) | | | Difference in DF | |
|---|---|---|---|---|---|---|
| | | 11 | 12 | 15 | (15-11) | (15-12) |
| 1 | Japan | 60 | 68 | 79 | 19 | 11 |
| 2 | Korea | 59 | 61 | 77 | 18 | 16 |
| 3 | Korea | 54 | 57 | 69 | 15 | 12 |
| 4 | China | 59 | 61 | 75 | 16 | 14 |
| 5 | Morocco | 52 | 58 | 69 | 17 | 11 |
| 6 | Pakistan | 49 | 60 | 73 | 24 | 13 |
| 7 | Nigeria | 54 | 57 | 61 | 7 | 4 |
| 8 | Japan | 53 | 62 | 76 | 23 | 14 |
| 9 | Pakistan | 53 | 56 | 74 | 21 | 18 |
| 10 | China | 57 | 60 | 75 | 18 | 15 |
| 11 | Ethiopia | 61 | 66 | 79 | 18 | 13 |
| 12 | India | 53 | 56 | 72 | 19 | 16 |
| 13 | Japan | 60 | 63 | 76 | 16 | 13 |
| 14 | Israel | 63 | 70 | 79 | 16 | 9 |
| 15 | Central Africa | 54 | 59 | 66 | 12 | 7 |

Table2. 3: Variation in days to flowering (DF) in medium flowering accessions under different daylength conditions.

| Acc. N° | Origin | Daylength (h) | | | Difference in DF | |
|---|---|---|---|---|---|---|
| | | 11 | 12 | 15 | (15-11) | (15-12) |
| 1 | Morocco | 69 | 72 | 84 | 15 | 12 |
| 2 | Iran | 66 | 69 | 80 | 14 | 11 |
| 3 | Japan | 67 | 70 | 87 | 20 | 17 |
| 4 | Lesotho | 68 | 72 | 89 | 21 | 17 |
| 5 | China | 71 | 76 | 79 | 8 | 3 |
| 6 | Japan | 71 | 77 | 80 | 9 | 3 |
| 7 | Japan | 67 | 70 | 86 | 19 | 16 |
| 8 | Pakistan | 69 | 73 | 87 | 18 | 14 |
| 9 | Japan | 67 | 71 | 83 | 16 | 12 |
| 10 | Korea | 68 | 73 | 78 | 10 | 5 |
| 11 | Myanmar | 71 | 76 | 86 | 15 | 10 |
| 12 | India | 67 | 69 | 77 | 10 | 8 |
| 13 | Ethiopia | 74 | 79 | 84 | 10 | 5 |
| 14 | Lesotho | 67 | 70 | 87 | 20 | 17 |
| 15 | Kenya | 72 | 76 | 82 | 10 | 6 |

Table2. 4: Variation in days to flowering (DF) in late flowering accessions under different daylength conditions

| Acc.N° | Origin | Daylength (h) | | | Difference in DF | |
|---|---|---|---|---|---|---|
| | | 11 | 12 | 15 | (15-11) | (15-12) |
| 1 | Chad | 76 | 78 | 88 | 12 | 10 |
| 2 | Japan | 78 | 83 | 90 | 12 | 7 |
| 3 | Nepal | 77 | 79 | 85 | 8 | 6 |
| 4 | Algeria | 80 | 86 | 92 | 12 | 6 |
| 5 | India | 77 | 77 | 87 | 10 | 10 |
| 6 | Japan | 79 | 83 | 90 | 11 | 7 |
| 7 | Japan | 78 | 79 | 89 | 11 | 10 |
| 8 | Uganda | 78 | 84 | 91 | 13 | 7 |
| 9 | Zimbabwe | 79 | 81 | 91 | 12 | 10 |
| 10 | Ethiopia | 82 | 87 | 95 | 13 | 8 |
| 11 | Uganda | 78 | 78 | 89 | 11 | 11 |
| 12 | Tanzania | 78 | 79 | 90 | 12 | 11 |
| 13 | Japan | 79 | 85 | 90 | 11 | 5 |
| 14 | Sudan | 79 | 84 | 91 | 12 | 7 |
| 15 | Bangladesh | 79 | 80 | 87 | 8 | 7 |

# CHAPTER 3

## Mapping of QTL controlling flowering time by linkage disequilibrium analysis

### 1. Introduction

Gene mapping using linkage disequilibrium (LD) or association mapping has become one of the most active areas of research in plant genetics. Association mapping is a powerful tool for high-resolution mapping of loci underlying quantitative traits and it is dependent on the structure of linkage disequilibrium or the non-random association of alleles or polymorphisms at different loci (Flint-Garcia *et al.* 2003). It refers to the correlation between polymorphisms in a population and relies on linkage disequilibrium to study the relationship between phenotypic variation and genetic polymorphisms (Breseghello and Sorrells 2006). Genotyped markers become proxies, or sentinels, for the functional variant because their genotypes are highly correlated with the genotypes of the functional variant. The power of an association study depends on the strength of this correlation.

The main advantage of association mapping is that it exploits all the recombination events that have occurred in the evolutionary history of a sample which is almost invariably results in a much higher mapping resolution compared with linkage mapping. Number of QTLs for a given phenotype is not limited to what segregates between parents of a cross, but rather by the number of real QTLs underlying the trait and the degree of which the mapping population captures the total genetic diversity available in nature (Zhao *et al.* 2008).

In recent years, gene mapping using association analysis has become one of the most active areas of research in plant genetics. The aim is to identify genes which contribute to phenotypes of interest using association mapping which is a pawerful strategy for identifying genes underlying quantitatif traits in plants (Casa *et al.* 2008).

Significant associations between genotypes and phenotypes can be caused (i) by marker loci harboring causal polymorphisms, (ii) by marker loci being physically linked to a polymorphism that influences a particular phenotype, and, of greater concern, (iii) from the effects of population structure or familial relationship (kinship) between individuals comprising the test population (Yu *et al.* 2006).

Individuals belonging to the same subpopulations or that are related by descent (kin), are more likely to both resemble each other phenotypically and share common alleles, independently of these alleles being linked or not to the causal polymorphism (leading to spurious associations). Knowledge of population structure and kinship in association mapping populations is critical. Yu *et al.* (2006) have shown that controlling of such demographic factors can lead to a significant reduction in the number of spurious associations in maize (*Zea Mays* L.).

Structured association using the program STUCTURE is conducted to identify populations and then estimate the proportion of each individual's variation that comes from particular population. The matrix of these estimates is called Q and the estimates are used as covariates to control for population structure in population mapping. The problem with this approach is that individuals can only vary along few axes of differentiation that may or may not be well captured by the STRUCTURE.

Random genetic markers are now most often used to generate a pairwise relatedness matrix called the kinship matrix (K). This approach of using genetic markers in estimating relatedness has been used to predict breeding values and to correct for relatedness. The application of mixed model using K matrix decreases false positive and negative over and above corrections involving only the Q matrix. While Q takes only a few axes of variation into account, the K matrix captures the relatedness between each possible pair of individuals

in a sample. The mixed model (K) is far superior to the clinal approaches (Q), but in many cases a combination (Q+K) of these approaches appears to be most powerful (Yu *et al.* 2006).

Association mapping is a way to detect causal genes by exploiting LD which is non-random association of alleles at two or more loci. It exploits both historical recombination and genetic diversity for high resolution mapping. Pattern of LD is dependent on the occurrence of new mutation that is associated with variants on the chromosome on which it arises. Since recombination breaks the association, the rate of recombination (r) is a key parameter in the process of LD decay. The pattern of LD is also affected by population size. Therefore the analysis of LD pattern is necessary to understand the feasibility and resolution of mapping based on LD (Shehzad *et al.* 2009b).

In plant breeding program, three main types of populations could be considered for implementation of association mapping: germplasm bank collections, elite breeding materials and synthetic populations. The application of association analysis differs among these populations in several aspects. In the case of germplasm banks, core collections are expected to represent most of the genetic variability with a manageable number of accessions, and thus are suitable for genetic studies (Zhang *et al.* 2000).

Sorghum is well suited to association mapping methods because of its medium-range patterns of linkage disequilibrium (Hamblin *et al.* 2005) and its self-pollinating mating system. Early characterization of complementary association genetics panels developed by a group of US scientists, and by Subprogram of the Generation Challenge Program, is in progress. More than 750 SSR alleles and 1402 SNP alleles discovered in 3.3 Mb of sequence (Casa *et al.* 2008) are freely available from the *Comparative Grass Genomics Center* relational database.

The objective of this study was to identify QTLs controlling flowering time and the sensitivity of flowering time to photoperiod in a core collection of sorghum previously described (chapter 2), using multiple association models. We also analyzed the LD pattern to understand the feasibility and resolution of the association mapping study.

## 2. Material and methods

The diversity research set of 107 sorghum accessions developed by Shehezad *et al.* (2009a) representing African and Asian countries was used to detect association between flowering time in sorghum and microsatellite primers. A total of 98 SSR markers were selected from published linkage maps of sorghum as revealed by Bhattramaki *et al.* (2000), Kong *et al.* (2000) and Taramino *et al.* (1997). The list of the 98 SSR markers with the chromosome, sequence information, size range is given in Table 3.1. Phenotypic data related to the number of days to flowering obtained in our study were used in association analysis.

### 2.1. Population structure and kinship matrix

The population structure among the 107 accessions using the genotype data of 98 SSR markers was performed using the program STRUCTURE version 2.2 (Pritchard *et al.* 2000). The analysis was conducted on 49 markers that were selected so that distances between adjacent markers were more than 10 cM in order to avoid using markers locating close to each other (Shehzad *et al.* 2009b).

The population structure was inferred with Bayesian clustering analyses with the admixture models in which the number of populations ($J$) ranged from 2 to 8. Markov chain Monte Carlo (MCMC) sampling was repeated $1 \times 10^6$ times after $1 \times 10^5$ cycles of a burwicen-in period. The analysis was repeated t for each number of $J$. A kinship matrix, $\mathbf{K}$, was calculated as allele sharing rates of 89 SSR markers as suggested by Zhao *et al.* (2008), and used in the single-QTL approach. In the calculation of the kinship matrix, 9 markers that had missing data for more than half of the accessions were eliminated.

Firstly, we used association analysis to identify QTL controlling flowering time in the world germplasm collection of sorghum grown under natural conditions. Then we have focused on the identification of QTL controlling flowering time and photoperiod sensitivity by examining the marker-trait that can be attributed to the strength of linkage disequilibrium between markers and functional polymorphisms across the 45 accessions grown under three different conditions of photoperiod or daylength.

## 2.2. Linkage Disequilibrium (LD)

LD between markers were estimated by $D'$ and $r^2$, where $D'$ is the standardized disequilibrium coefficient that is used for determining whether recombination or homoplasy has occurred between a pair of alleles; $r^2$ represents the correlation between alleles at two loci, and is informative for evaluating the resolution of association approaches. A weighted average of $D'$ or $r^2$ was calculated between the two loci (Farnir 2000) for all possible combinations of alleles, and then weighting them according to the allele's frequency. To test the significance of the LD, we also obtained $P$-values that were determined by permutation test to calculate the proportion of permuted gamete distribution that were less probable then the observed gamete distribution under the null hypothesis of independence (Weir 1996).

## 2.3. Statistical models for association analysis

Two different models were used for association analysis using TASSEL (Trait Analysis by aSSociation, Evolution and Linkage) version 2.0.1 software (Bradbury *et al.* 2007): general linear model (GLM) and mixed linear model (MLM). In general linear model two different models were used (i) naïve model where there is no control of population structure and relatedness and (ii) Q model based on population structure (Yu *et al.* 2006). In the second model we used Q matrix estimated by the structure analysis to control the effect caused by population structure. Population structure is the presence of subgroups in the sample in which individuals are more closely related to each other than the average pair of individuals taken at random in the population.

Substructure is a common cause of covariance of polygenic effects because relatives tend to share marker and gene alleles genome wide (Breseghello and Sorrells, 2006). Population structure is expected to affect the pattern of LD over the whole genome and must be controlled a priori for correct association analysis (Pritchard *et al.* 2000b).

In mixed linear model, we used two models: (i) the model which accounted for familial relatedness between accessions (K); (ii) the model that takes into account both the population structure and the familial relationship (Q+K).

# 3. Results

## 3.1. Mapping of QTL controlling flowering time in a sorghum core collection

### *Association by GLM model*

Using the model without population structure and kinship (Naïve and Q models) no QTL have been detected to be associated with flowering time in the core collection of sorghum grown under natural condition of daylength. Naïve model had no control for the heterogeneity of genetic background (population structure and familial relatedness among accessions) and thought to be largely affected by false positives (the addition of some subpopulation in the population structure).

### *Association by MLM model*

Using K model for core collection, four SSR loci were identified to be associated with flowering time in a core collection of sorghum under natural condition at a threshold of 2.5 and one locus at a threshold of 2.4. Xtxp159 on chr 7 and Xtxp51 on chr 4 showed a strong association ($p$ <0.0001) with flowering time (Table 3.2). Xtxp56, Xtxp58 and Xtxp59 were moderately associated with flowering time. However no QTL associated with flowering time was detected in the core collection of sorghum grown under natural daylenght using (Q+K) model. The $p$-value for association between SSR markers and flowering time in k model are shown in Fig 3.1.

## 3.2. Mapping of QTL controlling flowering time and sensitivity to photoperiod under controlled conditions of daylength

### *Association by GLM model*

Under controlled conditions of daylength a total of four loci were identified to be associated with flowering time by GLM model. Using the association analysis of 98 SSR markers and flowering time by the model without population structure and kinship (naïve model) three markers suggesting associations with flowering time were detected. For each condition of photoperiod one locus was identified to be moderately associated with flowering time at $p$-value $\geq 2$. Xtxp10 on chr 9 was identified to be associated with flowering time under 11 hs of photoperiod while Xtxp159 and Xtxp297 were identified under 12 hs and 15 hs of photoperiod and were identified on chr 7 and chr 2 repectively

(Table 3.4). The *p*-value for association between SSR markers and flowering time in naïve model for the 45 selected accessions under three different conditions of daylength are shown in Fig 3.2.

Using Q model for the 45 selected accessions grown under controlled conditions of daylength same locus (Xtxp13) was identified to be weakly associated with flowering time at *p*-value $\geq 2$. It was detected on chr 2 under short-day conditions only (11 hs and 12 hs). For 15 hs of photoperiod per day, no lcocus was identified to be associated with flowering time using the model based on population structure. The *p*-value for association between SSR markers and flowering time in Q model for the 45 selected accessions under three different conditions of ohotoperiod are shown in Fig 3.3.

***Association by MLM model***

Using K model under controlled conditions of dayength a total of seven loci were identified to be associated with flowering time. Four loci were detected at a threshold of 2.5. Xtxp298 on chr 2, Xtxp51 on chr 4 and Xtxp312 on chr 7 were identified under 12 hs. Xtxp100 on chr 2 was detected under 15 hs daylength. Three loci were detected at a threshold of 2. Xtxp61 and Xtxp75 on chr 1 were detected under 11 and 12 hs daylength respectively. While Xtxp27 on chr 4 was detected under 15 hs daylength (Table 3.4). The *p*-value for association between SSR markers and flowering time using K model are shown in Fig 3.4.

For (Q+K) model, the number of associated markers was the largest among all models. A total of eight markers were associated with flowering time in this model at *p*-value $\geq 2$ under short day conditions. Five loci were significantly associated with flowering time at threshold 2.5. Xtxp298 on chr 2, Xtxp61 on chr 1 and Xtxp159 on chr 7, were found to be the most strongly associated with flowering time under 12 h daylength. Only one loci was identified under 11 h of photoperiod. The *p*-value for association between SSR markers and flowering time using this model are shown in Fig 3.5.

*Linkage disequilibrium (LD) plot*

A range of LD was observed in the 45 selected 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 premutation test. Each cel represents the relationship between two markers with the color codes indicating the significance of LD. Four SSR markers with highly signifivant LD ($p$ <0.0001) were identified in this plot and are shown in Fig 3.6.

## 4. Discussion

The association analysis using GLM and MLM models was performed using firstly the total of 107 sorghum accessions representing the core collection and grown under natural daylength condition and secondly the 45 selected accessions grown under three ranges of daylength. The objective of this study was to identify QTLs controlling flowering time by the mean of total of 98 SSR markers involved in previous studies (Shehzad *et al.* 2009b). In this study, we used different models for association analysis to control both false positives (spurious association) and false negatives (increase statistical power of the model). The (*p*-values) were variable between models and treatments. Most of markers identified to be associated with flowering time showed different levels of significance by different models. Few markers only were strongly associated with flowering time under natural and controlled conditions of photoperiod.

For core collection markers associated with flowering time were identified only by K model which did not control the effects caused by population structure. A total of five loci have been identified among them two loci were strongly associated with flowering time (Xtxp159 on chr 7 and Xtxp51 on chr 4). K model was probably affected by a large number of spurious association in comparison with the other model used for identifiying the QTL(s) associated with flowering time in sorghum core collection.

On the other hand, using 45 selected accessions grown under three different conditions of photoperiod K and (Q+K) models detected a larger number of association

between markers and flowering time under different photoperiod conditions. K model detected a total of seven markers associated with flowering time, (Q+K) model detected eight markers for the same trait, wheras only three markers were detected using naïve model and only two markers were identified using Q model (Table 3.3)

The naïve model has no control for the heterogeneity of genetic background (i.e. population structure and familial relatedness among accessions) and thought to be affected largely by false positives. To control the false positives in association mapping, Q (population) and K (kinship) matrices were constructed. The results obtained using K model and (Q+K) model may indicate that both population structure and familial relatedness (i.e. kinship) shoud be taken into account in the model for association mapping. Same results were proved by Casa *et al.* (2008) and by Shehzad *et al.* (2009b) in sorghum. Zhao *et al.* (2008) used the MLM models in 95 highly structred Arabidopsis population and found better performance of (Q+K) model than any of the other tests using K or Q matrix alone.

In K model four loci were identified to be associated with flowering time at 0.1% level under 12 h of photoperiod. The branded loci were located on chr 1, chr 2, chr 4 and chr 5 with *p-value* ranging from 2.044 to 2.783. The kinship matrix K, explained more variation than with Q alone. Most of QTL detected in this study were identified under 12 hs of photoperiod (Table 3.4). In fact 13 loci associated with flowering time were identified using data related to the 45 accessions of sorghum grown under 12 hs of photoperiod, among them only Xtxp13 (chr 2) and Xtxp61 (chr 1) were identified under the condition of 11 hs of photoperiod. However only Xtxp279 (chr 1) was identified under both 12 hs and 15 hs of photoperiod as presented in Table 3.3. No common locus controlling flowering time in the selected population of sorghum was identified under 11 hs and 15 hs of photoperiod. These results suggested that the sensitivity to daylength changes was highly expressed under 12 h of photoperiod.

Furthermore Xtxp51 and Xtxp159 were found to be significantly associated with flowering time under natural daylength condition and under 12 hs daylength. These loci were detectable under varying photoperiod indicating that their expression is photoperiod insensitive. Two loci controlling flowering time were located-Xtxp61 on chr 1 and Xtxp13

on chr 2, and were expressed exclusively in short-day conditions suggesting that their expression was relatively sensitive to photoperod. These two loci accelerated flowering under short photoperiod. We also detected two photoperiod sensitive QTLs on chr 2 and chr 6 since they were only detectable under 11 hs of photoperiod suggesting that there is a minimum photoperiod necessary for their expression. These loci are sensitive to photoperiod of some degree. Three other loci were detectable exclusively under long-day condition, suggesting that there is a maximum photoperiod necessary for their expression (Table 3.4).

In addition, we analyzed also the LD plot pattern in the 45 selected accessions grown under controlled conditions of daylength. This analysis aimed to understand the feasibility and resolution of the association mapping study. There was no a close degree of LD between markers (Fig 3.6). Only four markers mentioned a significant association with the flowering time ($p$ <0.0001).

The success of association mapping deponds 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 (Shehezad *et al.* 2009b) and also due to the small range of accessions used for association analysis. In our study the range of LD was very limited compared with the LD described by Shehzad *et al.* (2009 b) using the total of the core collection of sorghum with the same 98 SSR markers. However in this study Shehzad *et al.* (2009b) used a wide range of morphological traits relted to yield. They found a wide range of LD ranging over chromosomes.

We compared the results of this analysis using the 45 selected accessions with the results obtained by Shehzad *et al.* (2009b) where the total of core collection was used for association analysis for 26 morphological traits. Shehzad *et al.* (2009b) reported the presence of a wide-range of LD over choosomes. In this study, a short-range of LD between markers closely locating on the same chromosome was not obvious.

Shehzad *et al.* (2009b) explained that a wide range of LD might be caused by population structure, and might be responsible for a large number of folse positives when the association mapping models did not take into account the population stucture (i.e. naïve and K models). On the other hand, a short range of LD is mainly caused by the physical linkage on the chromosome. Low LD in a short range may indicate that marker density in this study is not enough for detecting all QTLs associated with flowering time and sensitivity to photoperiod.

The difference in result found in the present study and the study described by Shehzad *et al.* (2009b) can be explained by the difference in the population structure. In fact using the 45 accessions, the genetic structure changed compared with the total of 107 accessions of sorghum used in the previous study where 53% of the accessions were originated from Asia and 47% from Africa. While in the present study 66.6% of the accessions were originated from Asia and only 33.3% from Africa. The change on genetic structure can be explained by the difference in genotype in African acessions: population structure was largely affected by African accessions.

Association analysis is a method potentially useful for detection of marker-trait associations based on linkage disequilibrium, but little information is available on the application of this technique to plant breeding populations. With appropriate statistical methods, valid association analysis can be done in plant breeding populations; however, the most significant marker may not be closed to the functional gene. Bias can arise from (i) covariance among markers and QTL, frequently related to population structure or intense selection and (ii) differences in initial frequencies of marker alleles in the population, such that exclusive alleles tend to be in higher association (Flavio and Mark 2006).

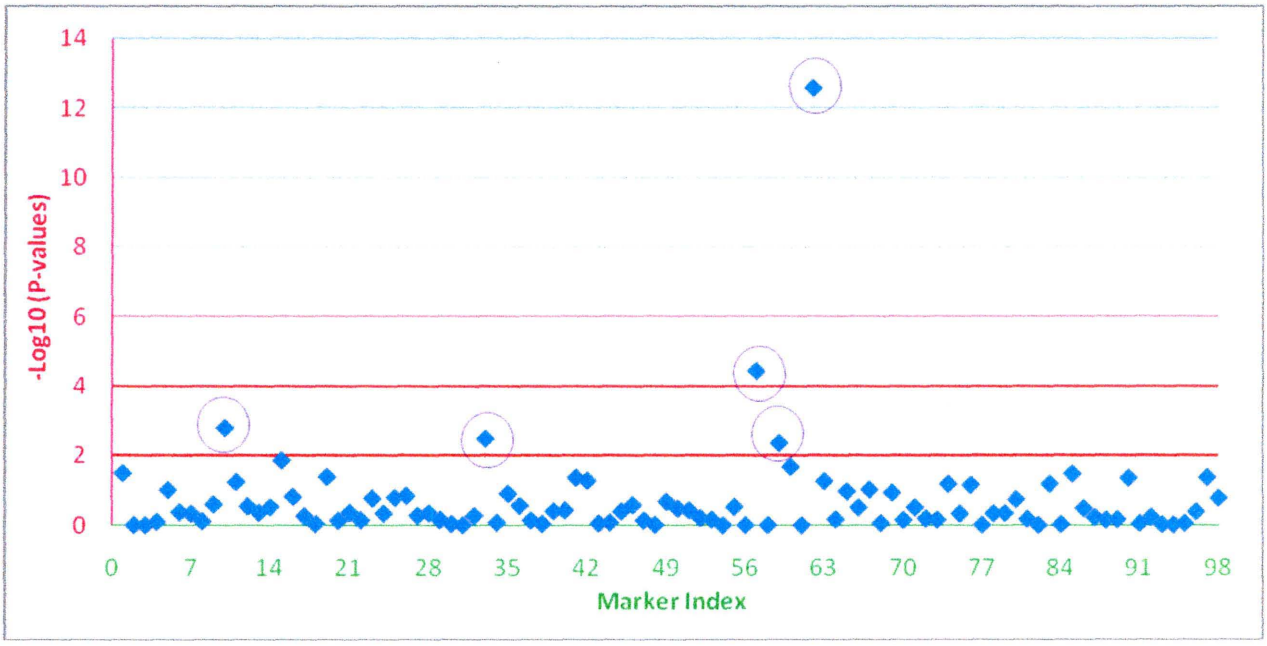


Fig 3.1: Association analysis of 98 SSR markers and flowering time using K model for 107 sorghum accessions under natural condition of daylength

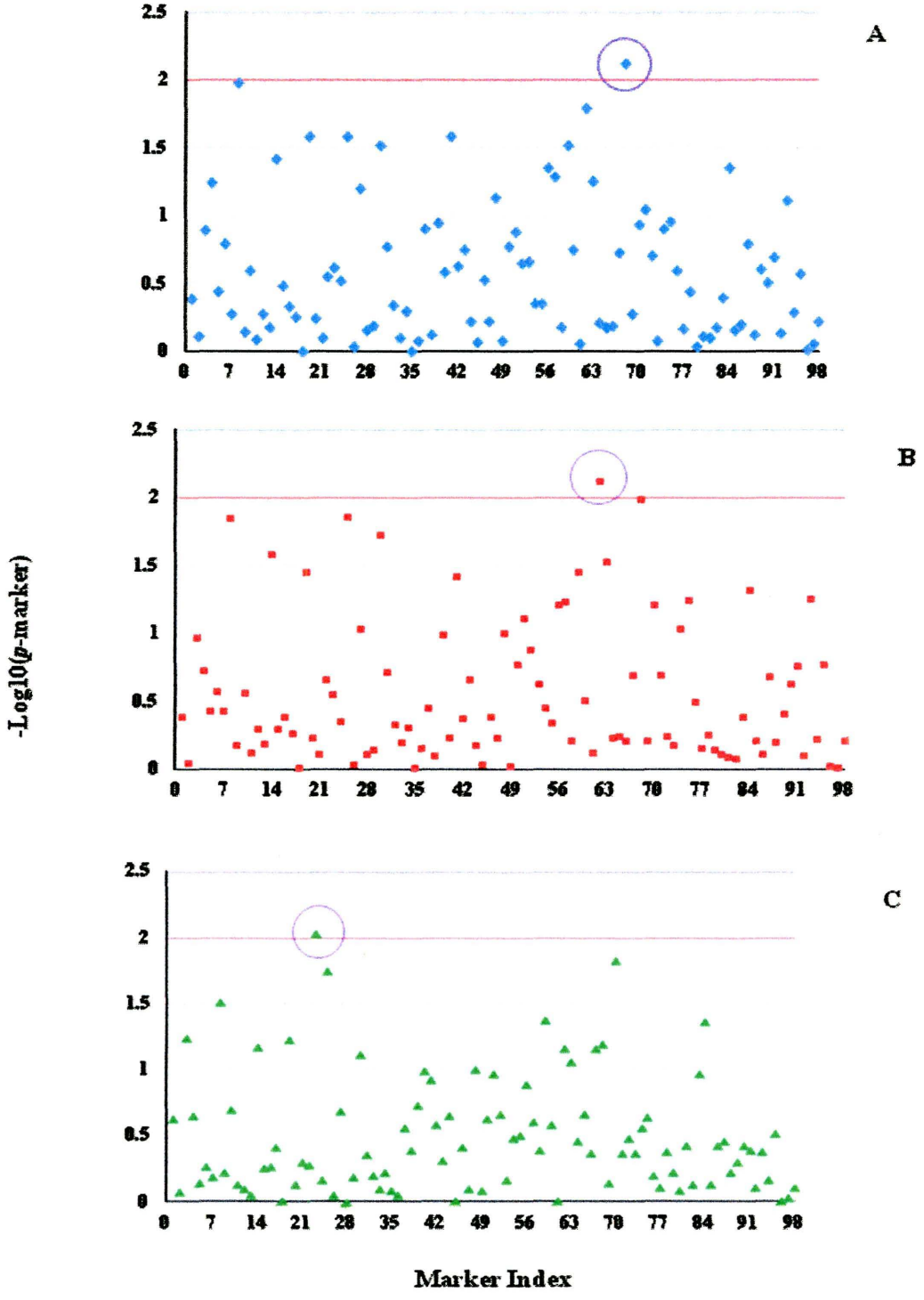


Fig 3.2: Association analysis of 98 SSR markers and flowering time using Naïve model for 45 sorghum accessions under controlled conditions of daylength (A:11hs, B:12hs and C:15hs)

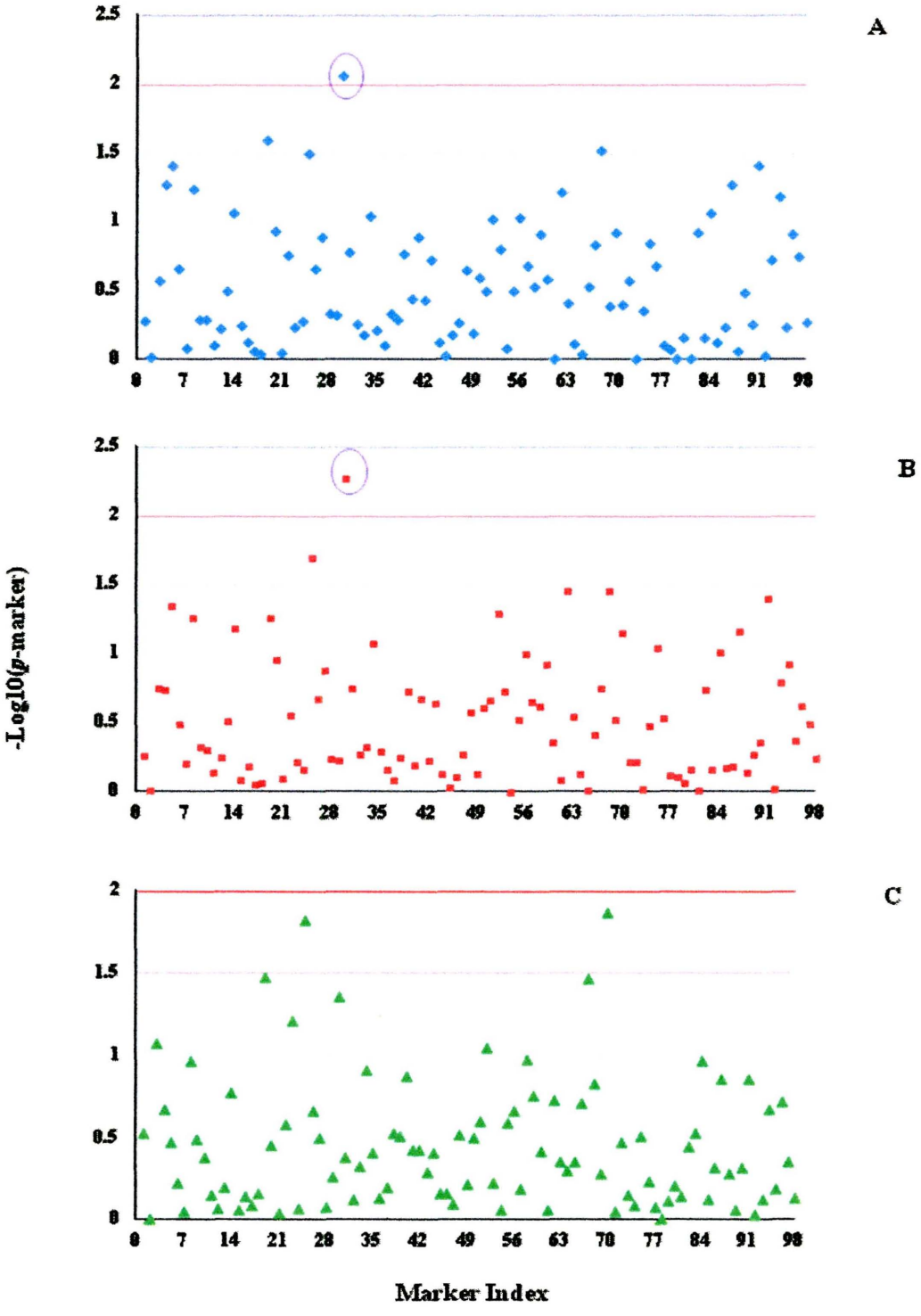


Fig 3.3: Association analysis of 98 SSR markers and flowering time using Q model for 45 sorghum accessions under controlled conditions of daylength (A:11hs, B:12hs and C:15hs)

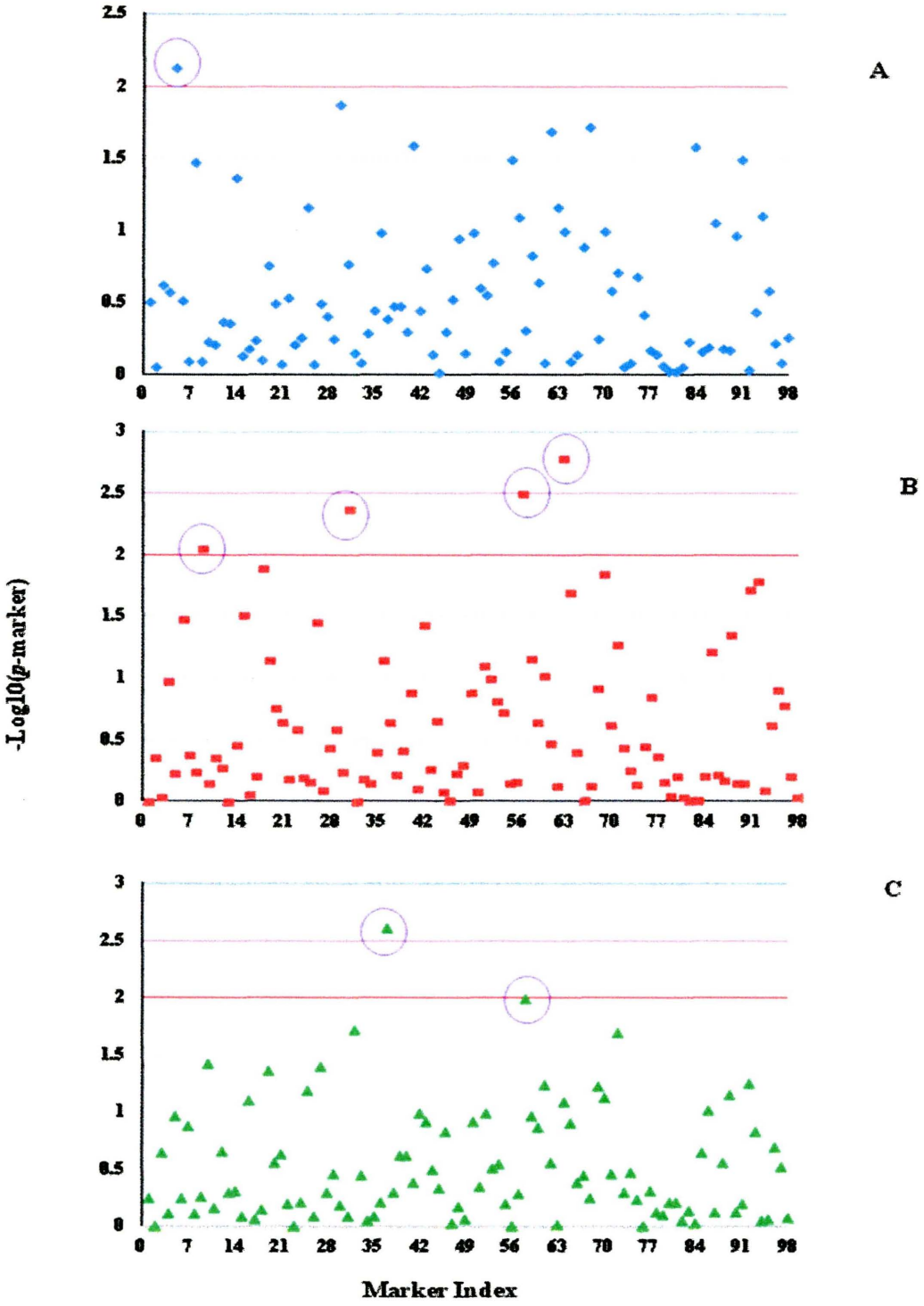


Fig 3.4: Association analysis of 98 SSR markers and flowering time using K model for 45 sorghum accessions under controlled conditions of daylength (A:11 hs, B:12 hs and C:15hs)

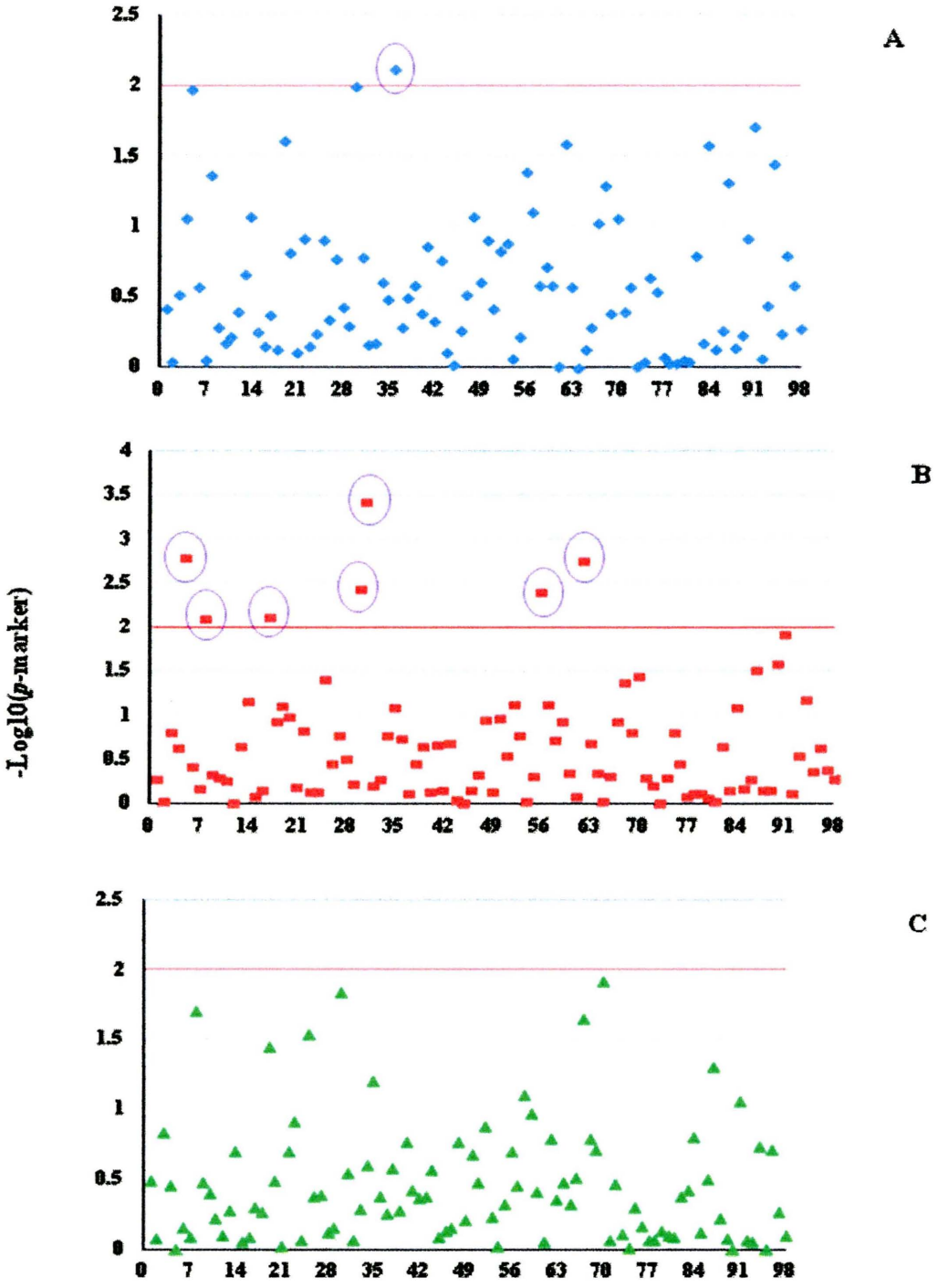


Fig 3.5: Association analysis of 98 SSR markers and flowering time using (Q+K) model for 45 sorghum accessions under controlled conditions of daylength (A:11 hs, B:12 hs and C:15hs)

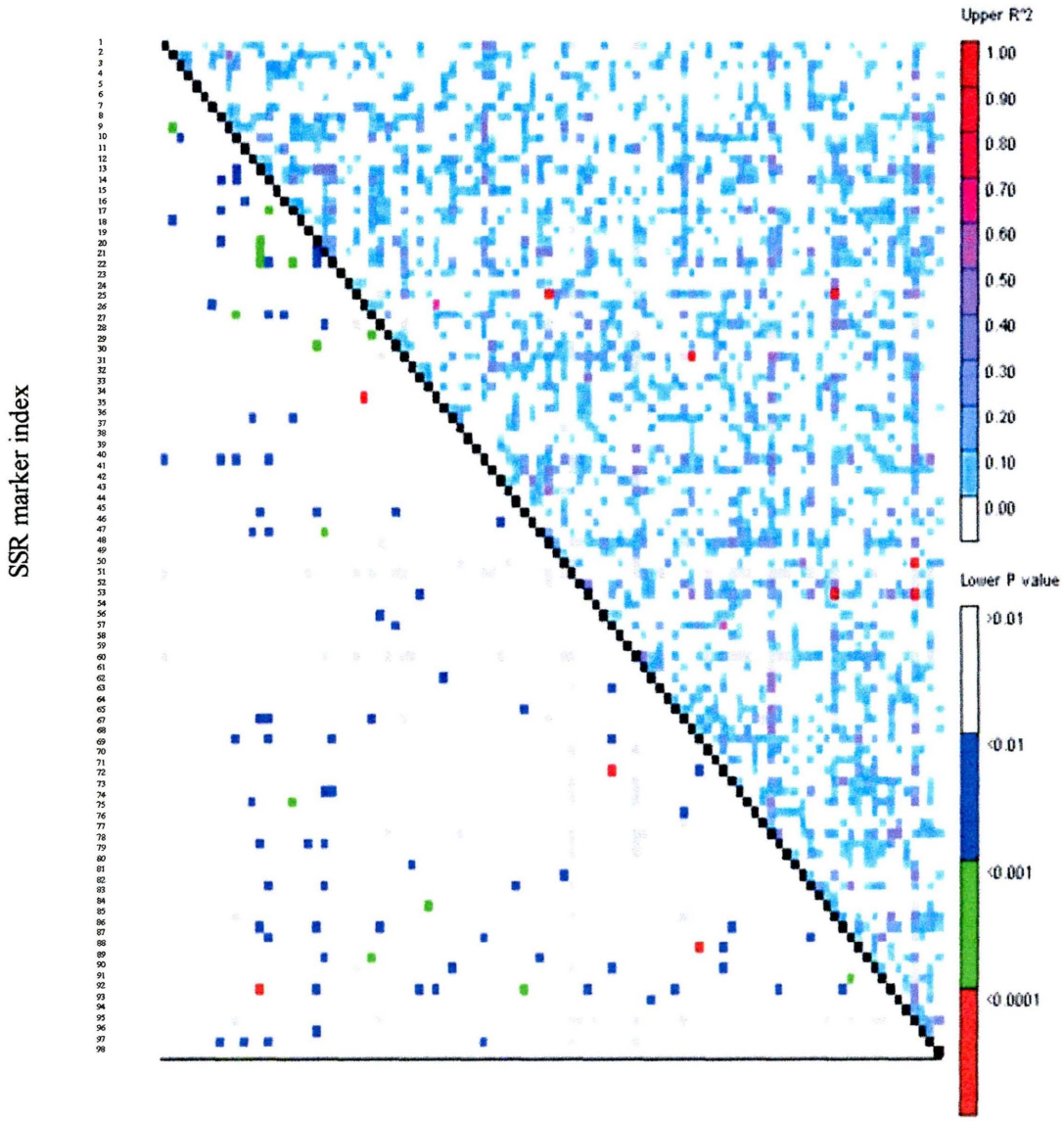


Fig 3.6: LD plot generated by 98 SSR markers. Each cell represents the relationship between two markers with the color codes for the presence of significant LD. Colored bar codes for the significant threshold levels.

Table 3.1: List of 98 sorghum SSR primers ( Bhattramakki et al. 2000, Kong et al. 2000 and Taramino et al. 1997)

| Index | Locus | Chromosome | Number of alleles | Type of SSR and number of repeats |
|---|---|---|---|---|
| 1 | Xtxp316 | 1 | 7 | $(AGA)_{12}$ |
| 2 | Xtxp248 | 1 | 8 | $(AG)_5(GA)_{28}$ |
| 3 | Xtxp340 | 1 | 5 | $(TAC)_{15}$ |
| 4 | Xtxp319 | 1 | 5 | $(TC)_{17}$ |
| 5 | Xtxp61 | 1 | 6 | $(GA)_{13}$ |
| 6 | Xtxp284 | 1 | 4 | $(AAG)_{19}$ |
| 7 | Xtxp229 | 1 | 3 | $(GT)_8$ |
| 8 | Xtxp279 | 1 | 5 | $(CTT)_{10} + (CTT)_3 + (CTT)_6$ |
| 9 | Xtxp75 | 1 | 6 | $(TG)_{10}$ |
| 10 | Xtxp58 | 1 | 5 | $(AG)_{13}(GA)_{16}$ |
| 11 | Xtxp335 | 1 | 5 | $(GT)_{12}$ |
| 12 | Xtxp37 | 1 | 5 | $(TC)_{23}$ |
| 13 | Xtxp32 | 1 | 7 | $(AG)_{16}$ |
| 14 | Xtxp88 | 1 | 7 | $(AG)_{31}$ |
| 15 | Xtxp149 | 1 | 3 | $(CT)_{10}$ |
| 16 | Xtxp43 | 1 | 7 | $(CT)_{28}$ |
| 17 | Xtxp302 | 1 | 6 | $(TGT)_8$ |
| 18 | SbAGF06 | 1 | 10 | $(AG)_{35}$ |
| 19 | SbAGF02 | 1 | 4 | $(AG)_{35}$ |
| 20 | Xtxp197 | 2 | 3 | $(AC)_{10}$ |
| 21 | Xtxp96 | 2 | 5 | $(GA)_{24}$ |
| 22 | Xtxp25 | 2 | 9 | $(GT)_{12}$ |
| 23 | Xtxp297 | 2 | 8 | $(AAG)_{24}$ |
| 24 | Xtxp50 | 2 | 3 | $(CT)_{13}(CA)_9$ |
| 25 | Xtxp211 | 2 | 6 | $(CT)_{23}$ |
| 26 | Xtxp84 | 2 | 3 | $(AG)_9$ |
| 27 | Xtxp4 | 2 | 6 | $(GA)_{23}$ |
| 28 | Xtxp201 | 2 | 5 | $(GA)_{36}$ |
| 29 | Xtxp19 | 2 | 3 | $(AG)_5 + (AG)_{10}$ |
| 30 | Xtxp13 | 2 | 4 | $(TG)_{13}$ |
| 31 | Xtxp298 | 2 | 6 | $(AGA)_{23}$ |
| 32 | Xtxp1 | 2 | 8 | $(AG)_{34}$ |
| 33 | Xtxp56 | 2 | 4 | $(GA)_{39}$ |
| 34 | Xtxp286 | 2 | 5 | $(GCA)_4ACA(GCA)_5A(CAA)_5+(AAC)_9$ |
| 35 | Xtxp348 | 2 | 4 | $(TAA)_{37}$ |

Table 3.1 (continued)

| Index | Locus | Chromosome | Number of alleles | Type of SSR and number of repeats |
|---|---|---|---|---|
| 36 | Xtxp315 | 2 | 5 | (TAT)22(CAT)18CGT(CAT)4 |
| 37 | Xtxp100 | 2 | 4 | (CT)19 |
| 38 | Xtxp7 | 2 | 5 | (CT)14 |
| 39 | Xtxp207 | 2 | 4 | (CT)14 |
| 40 | Xtxp296 | 2 | 3 | (CA)18 |
| 41 | Xtxp8 | 2 | 9 | (TG)31 |
| 42 | Xtxp69 | 3 | 6 | $(TC)_{12}$ |
| 43 | Xtxp285 | 3 | 5 | $(CTT)_{11}CTC(CTT)_{16}$ |
| 44 | Xtxp38 | 3 | 4 | $(AG)_{17}$ |
| 45 | Xtxp59 | 3 | 2 | $(GGA)_5$ |
| 46 | Cba | 3 | 3 | $(TA)_{18}$ |
| 47 | Xtxp336 | 3 | 3 | $(CGG)_4 + (GAG)_6$ |
| 48 | Xtxp31 | 3 | 7 | $(CT)_{25}$ |
| 49 | Xtxp205 | 3 | 3 | $(AG)_{12}$ |
| 50 | Xtxp33 | 3 | 8 | $(TC)_{20}$ C$(TG)_5 + (CT)_9CC(TG)_7$ |
| 51 | Xtxp228 | 3 | 3 | $(TC)_{12}$ |
| 52 | Xtxp266 | 3 | 2 | $(GT)_8$ |
| 53 | Xtxp12 | 4 | 7 | $(CT)_{22}$ |
| 54 | Xtxp24 | 4 | 7 | $(TC)_{21}$ |
| 55 | Xtxp60 | 4 | 2 | $(GT)_4GC(GT)_5$ |
| 56 | Xtxp212 | 4 | 3 | $(GT)_{10}$ |
| 57 | Xtxp51 | 4 | 2 | $(TG)_{11}$ |
| 58 | Xtxp27 | 4 | 5 | $(AG)_{37}$ |
| 59 | Xtxp21 | 4 | 5 | $(AG)_{18}$ |
| 60 | Xtxp40 | 5 | 3 | $(GGA)_7$ |
| 61 | Xtxp36 | 5 | 2 | $(GGA)_7GTA(T)_7 + (A)_7$ |
| 62 | Xtxp159 | 7 | 5 | $(CT)_{21}$ |
| 63 | Xtxp312 | 5 | 6 | $(CAA)_{26}$ |
| 64 | Xtxp278 | 5 | 2 | $(TTG)_{12}$ |
| 65 | Xtxp92 | 5 | 2 | $(GAA)_5$ |
| 66 | Xtxp295 | 5 | 4 | $(TC)_{19}$ |
| 67 | SbAGE03 | 5 | 6 | $(AG)_{34}GA(CA)_4$ |
| 68 | Xtxp10 | 6 | 3 | $(CT)_{14}$ |
| 69 | Xtxp67 | 6 | 4 | $(GA)_{28}$ |
| 70 | Xtxp287 | 6 | 4 | $(AAC)_{21}$ |

Table 3.1 (continued)

| Index | Locus | Chromosome | Number of alleles | Type of SSR and number of repeats |
|---|---|---|---|---|
| 71 | Xtxp258 | 6 | 5 | $(AAC)_{19}$ |
| 72 | SbAGB03 | 6 | 9 | $(AG)_{41}$ |
| 73 | Xtxp217 | 7 | 4 | $(GA)_{23}$ |
| 74 | Xtxp20 | 7 | 5 | $(AG)_{21}$ |
| 75 | Xtxp270 | 7 | 6 | $(GAA)_{12}(GAAA)_{6}+(GAA)_{21}+(GTA)_{5}+(GTA)_{3}+(GTA)_{3}$ |
| 76 | Xtxp331 | 7 | 9 | $(GAT)_{32}$ |
| 77 | PepC | 7 | 3 | $(AT)_{10}$ |
| 78 | Xtxp273 | 8 | 4 | $(TTG)_{20}$ |
| 79 | Xtxp47 | 8 | 2 | $(GT)_{8}(GC)_{5}+(GT)_{6}$ |
| 80 | Xtxp294 | 8 | 3 | $(TG)_{10}(GT)_{4}$ |
| 81 | Xtxp354 | 8 | 5 | $(GA)_{21}$ + $(AAG)_{3}$ |
| 82 | Xtxp18 | 8 | 7 | $(AG)_{21}$ |
| 83 | Xtxp250 | 8 | 5 | $(AAG)_{17}$ AAT $(AAG)_{4}$AAA$(ACA)_{9}$ |
| 84 | Xtxp321 | 8 | 7 | $(GT)_{4}$ + $(AT)_{6}$ + $(CT)_{21}$ |
| 85 | Xtxp105 | 8 | 4 | $(TG)_{5}$ + $(CT)_{6}$ GTCT$(GT)_{7}$ |
| 86 | SbAGA01 | 8 | 6 | $(AG)_{33}$ |
| 87 | Xtxp145 | 9 | 5 | $(AG)_{22}$ |
| 88 | Xtxp274 | 9 | 6 | $(TTC)_{19}$ |
| 89 | Xtxp104 | 9 | 3 | $(GGC)_{6}$ + $(GT)_{7}$ |
| 90 | Xtxp97 | 9 | 3 | $(CA)_{8}$ + $(GCC)_{6}$ |
| 91 | Xtxp95 | 9 | 5 | $(GA)_{18}$ $(GC)_{4}$ |
| 92 | Xtxp65 | 10 | 3 | $(ACC)_{4}$ + $(CCA)_{3}$ CG$(CT)_{8}$ |
| 93 | Xtxp303 | 10 | 4 | $(GT)_{13}$ |
| 94 | Xtxp15 | 10 | 4 | $(TC)_{16}$ |
| 95 | Xtxp145 | 10 | 6 | $(GA)_{15}$ |
| 96 | Xtxp23 | 10 | 4 | $(CT)_{19}$ |
| 97 | Kaf2 | 10 | 3 | $(CAA)_{9}$ |
| 98 | SbKAFGK1 | 10 | 4 | $(ACA)_{9}$ |

Table 3.2: List of QTLs controlling flowering time identified under natural condition of day length by association analysis based on K model using genotypes at 98 SSR marker loci for a core collection of sorghum

| Marker | Chromosome | -Log10(*p*-values) |
|---|---|---|
| Xtxp58 | 1 | 2.8 |
| Xtxp56 | 2 | 2.5 |
| Xtxp51 | 4 | 4.4 |
| Xtxp59 | 4 | 2.4 |
| Xtxp159 | 5 | 12.6 |

Table 3.3: Loci associated with flowering time using GLM and MLM models for accessions under controlled conditions of daylength

| Markers | Naïve | Q | K | (Q+K) |
|---|---|---|---|---|
| Xtxp10 | + | - | - | - |
| Xtxp13 | - | + | - | + |
| Xtxp61 | - | - | + | + |
| Xtxp315 | - | - | - | + |
| Xtxp159 | + | - | - | + |
| Xtxp75 | - | - | + | - |
| Xtxp298 | - | - | + | + |
| Xtxp51 | - | - | + | - |
| Xtxp312 | - | - | + | - |
| Xtxp279 | - | - | - | + |
| Xtxp302 | - | - | - | + |
| Xtxp212 | - | - | - | + |
| Xtxp297 | + | - | - | - |
| Xtxp100 | - | - | + | - |
| Xtxp27 | - | - | + | - |

+ Identified

- Not identified

Table 3.4: The total of loci associated with flowering time identified using 98 SSR markers for 45 selected accessions grown under controlled conditions of daylength

| **Photoperiod** | **Marker** | **Chromosome** | ***p*-values** |
|---|---|---|---|
| 11(h/day) | Xtxp61 | 1 | 2.1 |
| | Xtxp13 | 2 | 2.1 |
| | Xtxp315 | 2 | 2.1 |
| | Xtxp10 | 6 | 2.1 |
| 12(h/day) | Xtxp61 | 1 | 2.8 |
| | Xtxp75 | 1 | 2.0 |
| | Xtxp279 | 1 | 2.1 |
| | Xtxp302 | 1 | 2.1 |
| | Xtxp13 | 2 | 2.4 |
| | Xtxp13 | 2 | 2.3 |
| | Xtxp298 | 2 | 3.4 |
| | Xtxp298 | 2 | 2.4 |
| | Xtxp51 | 4 | 2.5 |
| | Xtxp212 | 4 | 2.4 |
| | Xtxp159 | 5 | 2.1 |
| | Xtxp159 | 5 | 2.8 |
| | Xtxp312 | 5 | 2.8 |
| 15(h/day) | Xtxp297 | 1 | 2.0 |
| | Xtxp100 | 2 | 2.6 |
| | Xtxp27 | 4 | 2.0 |

# CHAPTER 4

## Construction of linkage map and mapping of QTL controlling flowering time in $F_2$ population

### 1. Introduction

Molecular linkage map and quantitative trait loci mapping technology represent tools used to estimate the number of loci governing a particular trait of agronomic importance and to determine their map positions in the genome. The identification of QTLs can create a base for rapid, detailed and direct genetic manipulation of them though marker assisted selection. Construction of genetic maps has provided a device for identification of the number, significance and location of QTLs associated with a variety of phenotypic characteristics (Tanksley 1993). Construction of linkage map is the most fundamental step required for a detailed genetic study and marker-assisted breeding approach in any crop (Tanksley, 1993).

Sorghum genome mapping based on DNA markers began in early 1990s, and since then several genetic maps of sorghum have been constructed. Initially, the genetic maps of sorghum were based largely on DNA probes previously mapped in maize genome (Pereira *et al.* 1994). Later more maps were constructed using mainly sorghum genomic DNA probes (Xu *et al.* 1994). In sorghum, maps have been developed by both processes: Association mapping and linkage mapping (Pereira and Lee 1995; Tuinstra *et al.* 1998; Rami *et al.* 1998; Hart *et al.* 2001 and Feltus *et al.* 2006 cited by Srinivas *et al.* 2009b).

However genetic linkage maps are prerequisite for studiying the inheritence of both qualitative and quantitative traits (Mace *et al.* 2009).

QTLs have been identified using these genetic linkage maps predominantly containing anonymous molecular markers for many agronomical important traits including plant early development (anthesis and maturity), yield and its component traits, plant height and other growth characters (Pereira and Lee 1995; Tuinstra *et al.* 1998; Rami *et al.* 1998; Hart *et al.* 2001; Brown *et al.* 2006; Feltus *et al.* 2006), pre- and post-flowering drought stress (stay green) tolerance (Tuinstra *et al.* 1996, 1997; Crasta *et al.* 1999; Subudhi *et al.* 2000; Xu *et al.* 2000; Tao *et al.* 2000; Kebede *et al.* 2001; Haussmann *et al.* 2002) and for important biotic stresses as explained by Srinivas *et al.* (2009b).

During the last few years, emphasis has shifted towards the development of molecular markers from the transcribed region of the genome in order to associate the molecular polymorphisms of genes with phenotypic variability of the traits. Construction of genetic map by mapping functionally needed genes permits evaluation of co-location between genic-markers and QTLs of any trait (Aubert *et al.* 2006). It may also increase our understanding of the biochemical pathways and mechanisms affecting agronomically important traits (Matthews *et al.* 2001; Zhang *et al.* 2004).

To dissect the morphological and physiological trait of sorghum at a genetic level, different type of molecular markers have been developed including restriction-fragment-length polymorphisms (RFLP), amplified-fragment-length polymorphisms (AFLP) and simple sequence repeats (SSR). SSR markers are mostly codominant, are readily amplified by polymerase chain reaction (PCR) and are effective at detecting genotype variation caused by a high degree of polymorphism (Yonemaru *et al.* 2009).

SSR markers with a high degree of polymorphism contribute to the molecular dissection of agriculturally important traits in sorghum (*Sorghum bicolor* (L.) Moench). Yonemaru *et al.* (2009) have developed a new set of SSR markers to facilitate the genetic and molecular dissection of sorghum genes that encode trait with economic value, including quantitative traits. They designed 5599 non-redundant SSR markers, including regions flanking the SSRs, in whole-genome shotgun sequences of sorghum line AT x 623.

$(AT/TA)_n$ repeats constituted 26.1% of all SSRs, followed by $(AG/TC)_n$ at 20.5%, $(AC/TG)_n$ at 13.7% and $(CG/GC)_n$ at 11.8%. The chromosomal locations of 5012 SSR markers were determined by comparing the locations identified by means of electronic PCR.

Construction of linkage maps are fundamental for the localization of genes related to the control of flowering time in sorghum, *Sorghum bicolor* (L.) Moench. Genetics studies of flowering time in sorghum culminated in the identification of genes which influence the flowering time in sorghum. The mapping of sorghum flowering time genes may be useful in producing photoperiod–sensitive hybrids for regions of the world where photoperiod-sensitive landraces grow. The objectives of our study were to construct linkage map of sorghum population and to carry out a QTL mapping analysis to identify genomic region involved in sorghum flowering time.

## 2. Material and methods

### 2.1. 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 the diversity research set of sorghum germplasm used for analyzing the variation in flowering time in chapter 2 and for mapping of QTL controlling flowering time by linkage disequilibrium analysis in chapter 3. The parental cultivars were selected on the basis of their morphological variation regarding flowering time and also regarding the difference and importance of origin of these two cultivars. The $F_2$ plants and their parental cultivars were sown in early May 2008 at the experimental field of Tsukuba University under natural daylength with a planting density of 1.5 m x 20 cm. During the growing season daylength ranged from 14.25 h in May, 14.40 h in June, July and August, to 13 h in September. And from September it has decreased further to 12.5 h. The total $F_2$ plants and their parents were also grown during the growing season (May-Nov) in a controlled 12 h daylength facility in 2008. Standard agronomic practices were applied from sowing to

harvest. The days to flowering was scored as the number of days from sowing to the time when 50% of the panicle flowered.

### 2.2. Genomic DNA isolation

The leaves of 40-day-old plants were sampled and used for genomic DNA isolation. Extraction of the DNA from the 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.

### 2.3. Screening of SSR markers

Microsatellite primers were selected from the genome-wide simple sequence repeat markers developed by Yonemaru *et al.* (2009) using whole-genome shotgun sequences of sorghum. A total of 580 genome-wide SSR markers randomly selected from all ten of the sorghum chromosomes and were screened for the detection of polymorphisms between the parental cultivars of the $F_2$ mapping population. The polymorphic markers were employed in genotyping of entire mapping population. The other primer sets were discarded because no band, complex banding pattern or no polymorphic nature. The whole informations related to the SSR markeres used in our study are published online by Yonemaru *et al.* (2009). A total of 213 markers were polymorphic and were used for constructing the linkage maps.

### 2.4. PCR conditions and electrophoresis

PCR amplifications of the sorghum SSRs were performed in a 10 µl reaction mixture containing 10 ng DNA template, 10 x 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 (10x) was used in casting the gel, and 1x TBE buffer was used during the electrophoresis; the gel was stained in 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.

## 2.5. Construction of genetic Linkage maps and mapping of QTLs controlling flowering time

Two linkage maps were constructed for the $F_2$ plants grown under natural daylength and under controlled daylength 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 to not 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. The 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 $\geq 2.5$.

The position at which the logarithm of 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 daylength environments were considered to be the same if the estimated map position of their peaks fell within 20 cM of each other.

# 3. Results

## 3.1. Phenotypic data analysis

The days to flowering varied widely among the parental accessions and $F_2$ plants grown under a natural daylength, 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 the $F_2$ plants ranged from 68 to 135 days and was almost within the variation of their parents (Fig 4.1). The analysis indicated considerable differences between the parental cultivars and their $F_2$ plants with regard to the variation in flowering time. Under a 12 h daylength, 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 the $F_2$ plants ranged from 56 to 71 days (Fig 4.2).

## 3.2. Linkage mapping and identification of QTLs controlling flowering time

Of the 580 markers screened using the parental cultivars Kikuchi Zairai and SC112, a total of 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 a natural daylength and under a 12 h daylength.

The final map constructed using the $F_2$ plants grown under the natural daylength contained 178 SSR markers that were distributed throughout 17 linkage groups, spanning a length of 2468 cM (Fig 4.3). The linkage groups were assigned to the 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 $\geq$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 the $F_2$ plants grown under the 12 h daylength and included 175 SSR markers, covering a total genetic distance estimated at 2340 cM (Fig 4.4). 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 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 the $F_2$ plants grown under the natural daylength (Table 4.1): *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 4.1. The 9 QTLs identified under the natural daylength 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 the 12 hs daylength, 7 QTLs controlling flowering time were identified (Table 4.2). 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 the natural daylength. Nevertheless, *qFT5* on chr 5 and *qFT6b* on chr 6b were mapped only under the 12 hs daylength. The 7 QTLs determined under the 12 hs daylength explained an additive effect that ranged from (1.2) for *qFT6b* on chr 6b to (4.43) for *qFT5b* on chr 5b and a 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 4.2). The 7 QTLs identified under the 12 hs daylength explained 46.6% of the total phenotypic variation.

Accordingly, a total of 5 QTLs were mapped under the natural and 12 hs daylengths. However, *qFT5* on chr 5 and *qFT6b* on chr 6b were identified only under the 12 hs daylength 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 the natural daylength and explained 27.1% of the phenotypic variation.

# 4. Discussion

## 4.1. Variation in flowering time in $F_2$ population

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 daylengths. A wide variation in flowering time was noted among the parental cultivars and their $F_2$ plants under the natural daylength. The $F_2$ population demonstrated a transgressive segregation for flowering time. Transgressive segregation 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 the $F_2$ plants.

Although a smaller range of variation in flowering time under the 12 hs daylength was found for the $F_2$ plants, all of the plants flowered earlier under the 12 hs daylength than when grown under the natural daylength. The decrease in days to flowering under the 12 hs daylength 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 daylength. In the present study, flowering in a larger number of the $F_2$ plants was accelerated under the 12 hs daylength when compared to the flowering time of the early-flowering Ethiopian cultivar. Accordingly, the Japanese

cultivar allele appeared to delay the flowering time under the natural daylength, whereas the Ethiopian cultivar allele suppresses the delayed effect on the flowering by the Japanese cultivar allele and accelerates flowering under the 12 hs daylength. Under the 12 hs daylength, the Ethiopian cultivar flowered nine days earlier than under the natural daylength, and the Japanese cultivar flowered 30 days earlier.

### 4.2. Identification of QTLs controlling flowering time

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 daylength conditions, the total map length was larger than the range previously reported: the distances between the adjacent markers are larger in our map compared to the 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 markers were highly distorted and skewed. The SSR markers used were most affected by the distortion compared with the other markers used in previous studies. Most of markers showed 3:1 segregation ratios, 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.

A total of five QTLs controlling flowering time were detected under both the natural and 12 hs daylengths, 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 daylength. These four QTLs were considered to be sensitive to the photoperiod due to the response to the change in the daylength. These QTLs explained 27.1% of the total phenotypic variation and controlled the photoperiodic sensitivity, as the discrepancy in the daylength or photoperiod was required for their expression. Conversely, *qFT5* on chr 5 and *qFT6b* on chr 6b were identified only under the 12 hs daylength and were expressed under a fixed daylength,

suggesting that their expression was not affected by the change in daylength and that they were insensitive to the photoperiod.

The 9 QTLs identified under the natural daylength explained 60% of the variation for the flowering time. The 7 QTLs identified under the 12 h daylength explained 46.6% of the variation for the flowering time, which explains the complex genetic nature of 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 in flowering time in the $F_2$ plants. Furthermore, the small additive effects of individual QTLs indicated the complexity in 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 a total of nine QTLs controlling flowering time were identified in sorghum, with very small additive effects ranging from 1.2 4 to 1.96. These results are also similar to the finding of Mace *et al.* (2011) who described that small additive effect of QTLs controlling morphological traits can be explained by a 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 for any single large-effect QTLs. Indeed, the authors identified 36 QTLs that explained 89% of the total variance for the flowering time in maize. Buckler *et al.* (2009) identified evidence for 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 are determined by the geographic origin or large effects for 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 single large-effect QTL in rice and *Arabidopsis*.

Numerous QTLs controlling flowering time in sorghum have been identified in previous studies (Lin *et al.* 1995; Paterson *et al.* 1995; Dufour 1996; Crasta *et al.* 1999; Hart *et al.* 2001; Feltus *et al.* 2006 and Srinivas *et al.* 2009). However, no QTL controlling flowering time or sensitivity to photoperiodic changes with a major effect was identified in previous studies in sorghum. Moreover, it is expected that new recombination will help in identifying new QTLs. Therefore, we have 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.

*qFT2* on chr 2 was mapped to a position adjacent to the one 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 to chr 7, chr 8 and chr 10, respectively, are considered newly mapped, as they were not reported in previous studies. 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 the 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 the 12 h daylength and could correspond to the $Ma_1$ allele because it was mapped to a region adjacent to SBI-06 (Lin *et al.* 1995 and Klein *et al.* 2008).

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 preset study, *qFT1-1* was mapped to the region delimited by SB105 and SB258 (112.0-120.3 cM) on chr 1 under natural conditions, 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 flowering time in sorghum was similar to maize. This study represents a preliminary and basic study for the QTLs controlling flowering time in sorghum, and the results of this study give emphasis to 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.

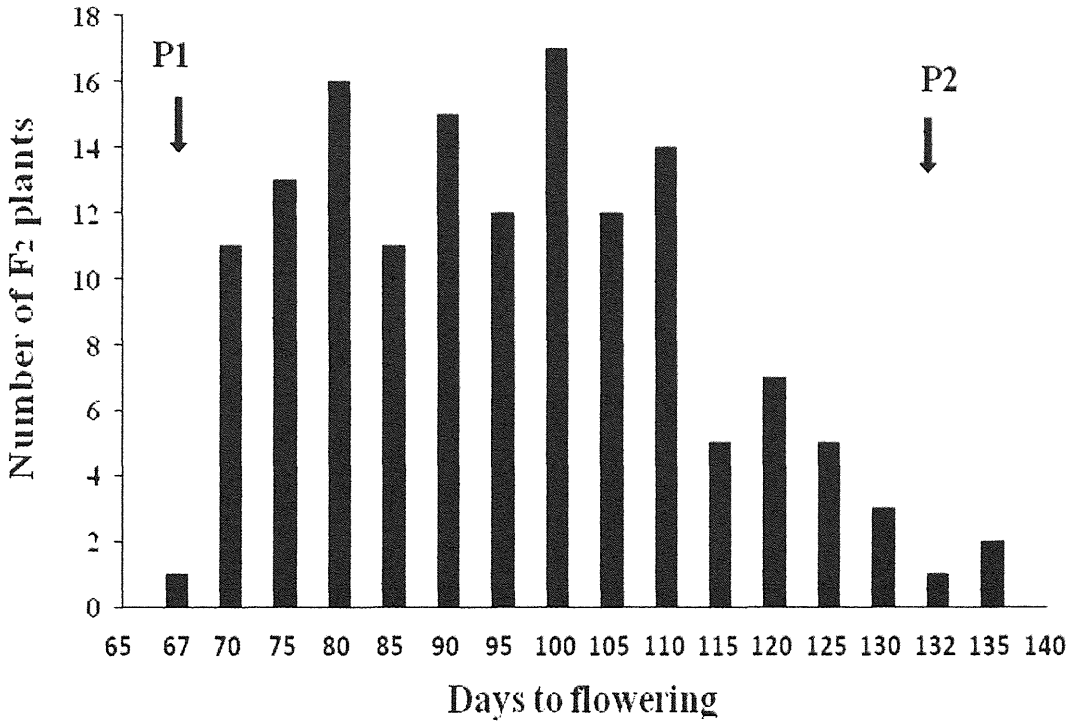


Fig 4.1: Variation in flowering time in $F_2$ plants and their parents grown under natural daylength (P1: SC 112, P2: Kikuchi Zairai)

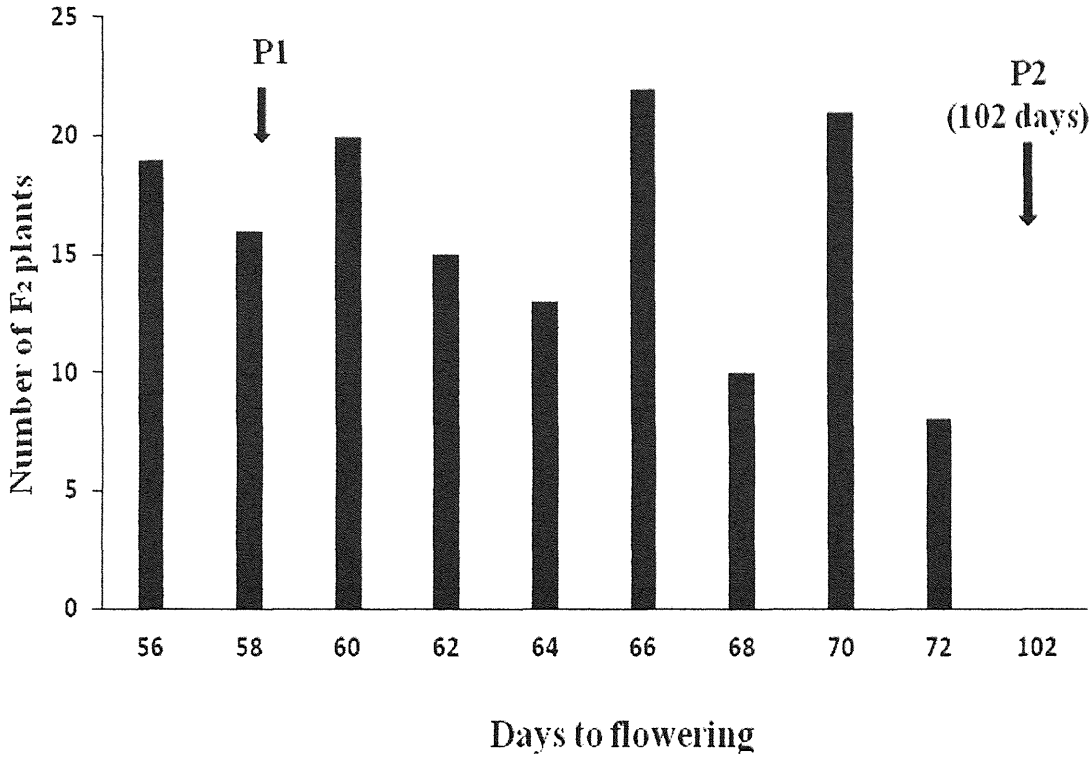


Fig 4.2: Variation in flowering time in $F_2$ plants and their parents grown under 12 hs daylength (P1: SC 112, P2: Kikuchi Zairai)

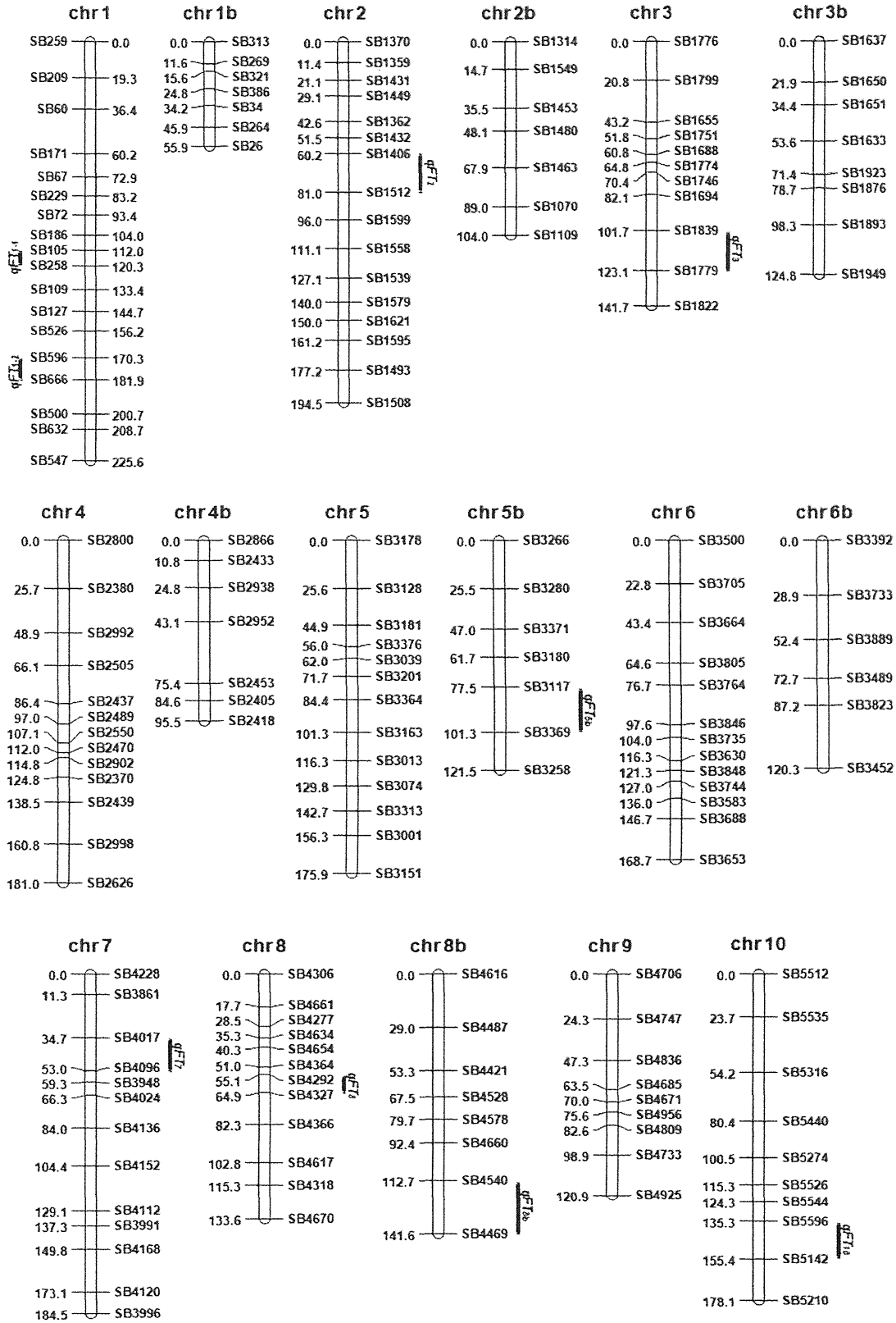


Fig 4.3: Location of QTLs for flowering time measured in this study on a genetic linkage map based on $F_2$ mappinjg population grown under natural daylength. QTLs are represented by bars (1-Lod interval) and extended lines (2-LOD interval).

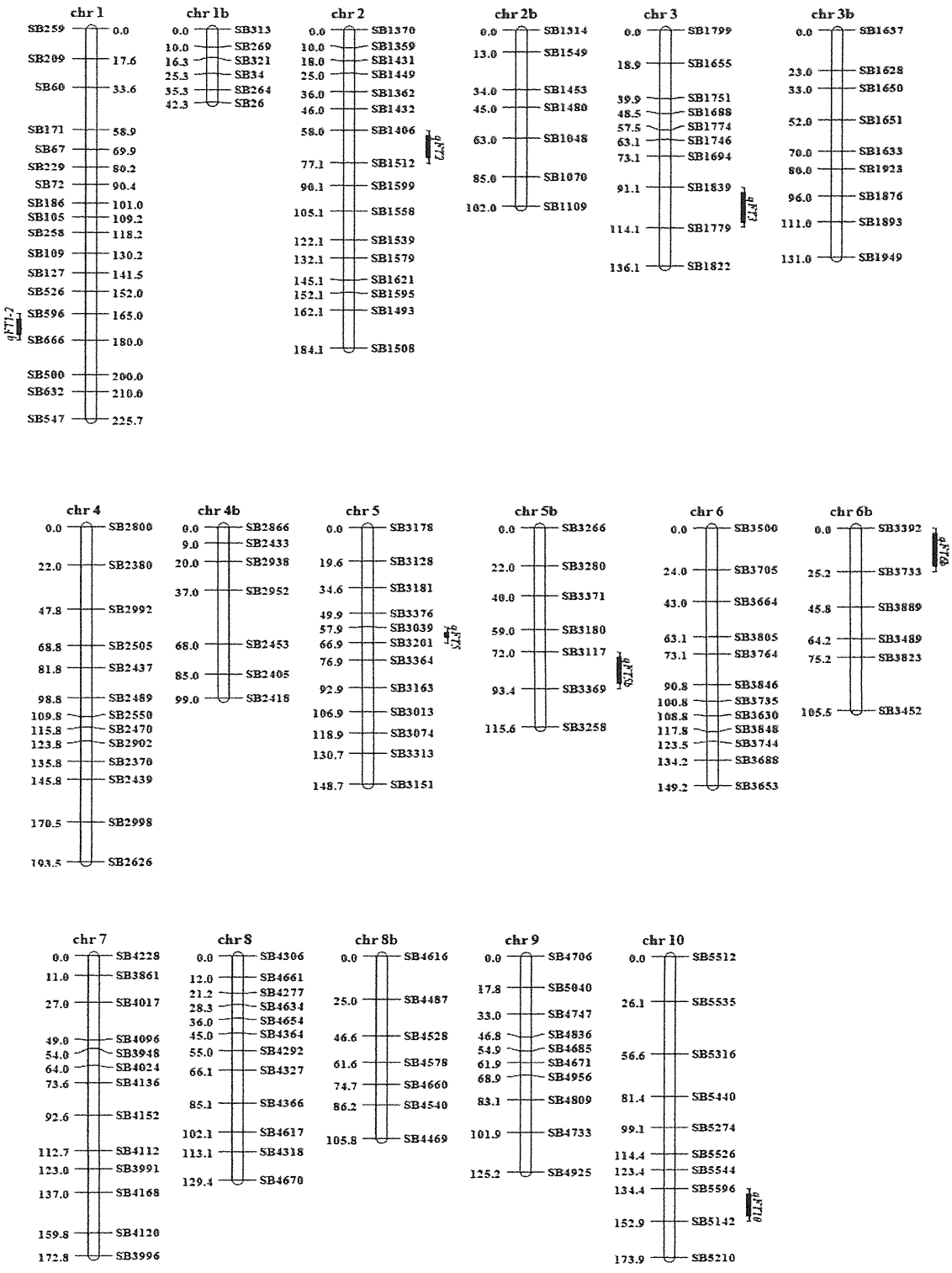


Fig 4.4: Location of QTLs for flowering time measured in this study on a genetic linkage map based on $F_2$ mappinjg population grown under 12 hs daylength. QTLs are represented by bars (1-Lod interval) and extended lines (2-LOD interval).

Table 4.1: QTLs identified under natural daylength

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

*CS112 alle decreased the number of days to flowering

**Phenotypic variation explained by each QTL

Table 4.2: QTLs identified under 12 hs daylength

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

*CS112 alle decreased the number of days to flowering

**Phenotypic variation explained by each QTL

# CHAPTER 5

## General discussion

Sorghum [*Sorghum bicolor* (L.) Moench] is a C4 grass native to Africa that provides an indispensable food source for over 300 million people inhabiting food-insecure regions worldwide (Smith and Frederiksen 2000). Although primarily grown for its grain and forage, high biomass sorghum is also an excellent drought-tolerant energy crop for sustainable production of lingo-cellulosic-based biofuels (Rooney *et al.* 2007). Forage and energy sorghums are selected for delayed flowering to increase biomass yield through longer duration of vegetative growth, whereas grain sorghums are selected for early flowering to ensure sufficient time for grain maturation and to avoid drought and frost. Optimal production of each of these sorghum crops requires the precise regulation of flowering time, which varies depending on planting location and climate. Differences in photoperiod sensitivity confer a wide range of flowering times on diverse accessions of the sorghum germplasm collection (Garner and Allard 1920). Due to its critical importance to crop yield and hybrid seed production, photoperiodic regulation of flowering has been an important trait characterized by sorghum improvement programs dating back to the early 1900s (Quinby 1974). Sorghum genotypes show a wide range of photoperiod sensitivity and critical floral-inductive day lengths (Craufurd *et al.* 1999).

Historic genetic studies uncovered four flowering time (maturity) loci, which were designated *Ma*1, *Ma*2, *Ma*3, and *Ma*4 (Quinby 1967). After several years, two additional maturity genes, *Ma*5 and *Ma*6, which increase photoperiod sensitivity and extended the duration of vegetative growth in forage and high-biomass sorghum hybrids, were described

(Rooney and Aydin 1999). Dominant alleles at each maturity locus contribute to late flowering in long-day. Of the four original maturity loci, Quinby 1974 reported that *Ma*1 has the largest impact on flowering time in sorghum. Mutations in *Ma*1 were critical for the early domestication and dispersal of sorghum from its center of origin during the migration of people across Africa and Asia (Quiby 1967).

During the first 40 years of the 20$^{th}$ century, growers and plant breeders in US and in different places selected recessive alleles of *Ma*1 that resulted in the development of early flowering sorghum cultivars suitable for grain production in temperate regions worldwide (Smith and Frederiksen 2000). More recently, the manipulation of flowering time loci has been of fundamental importance to the production of high-biomass sorghum for bio-power and lingo-cellulosic biofuels (Rooney *et al.* 2007).

However the variation in flowering that cannot be explained by the maturity loci was observed in many sorghum cultivars. Therefore we studied the flowering response and showed the effects of daylength or photoperiod on flowering time in sorghum. Accordingly the present study was conducted to (1) to analyze the variation in flowering time and the sensitivity to photoperiod changes in a core collection of sorghum; (2) to identify quantitative trait loci (QTLs) associated with flowering time and photoperiod sensitivity by linkage disequilibrium analysis (LD); and (3) to construct a sorghum linkage map using SSR markers and to map QTL controlling flowering time in $F_2$ population deriving from a cross between Kikuchi Zairai (late flowering accession originated from Japan) and SC112 (early flowering accession originated from Ethiopia). The parental accessions were selected from the core collection of sorghum.

**1. Analysis of the variation in flowering time in sorghum**

To examine the variation in flowering time a diversity research set of 107 sorghum accessions representing African and Asian countries was grown under natural daylength condition. According to their flowering time, accessions were divided into early, medium and late flowering groups. Fifteen accessions were randomly selected from each group. The 45 selected accessions were grown as replicated sets under 11 hs, 12 hs and 15 hs of daylength respectively. A wide range of variation in number of days to flowering ranging

from 56 to 133 was detected within the core collection of sorghum (Fig 1). Under controlled conditions of photoperiod, sorghum accessions gradually responded to the short daylength. In general a photoperiod of 11 hs and 12 hs accelerated the flowering time for most of the sorghum accessions from different geographical origins. Whereas a photoperiod of 15 hs delays flowering time by increasing the number of days to flowering. In particular we identifyed that variation in response to photoperiod and sensitivity to it fluctuated within accessions. Some accessions seemed to be weakly affected by change in photoperiod, nevertheless other accessions are stongly affected by the changes in daylength.

Furthermore a set of 144 $F_2$ plants was developed from the cross between Kikuchi Zairai (Japan, late flowering cultivar) and SC112 (Ethiopia, early flowering cultivar). The $F_2$ plants and their parental cultivars were grown under natural daylength condition and also under 12 hs daylength. The results of this experiment confirmed that flowering in sorghum is accelerated when daylength decreases since the parental cultivars and their $F_2$ plants flowered earlier under 12 hs daylength than under natural daylength condition. These results were described previously by Garner and Allard (1923). They were also validated by Folliard *et al.* (2004).

On the basis of the results obtained in this study we concluded that the increased photoperiod significantly increased the time requested by sorghum to flower revealing that sorghum is a short-day plant. We suggested that the exacted photoperiod compulsory for flowering of sorghum belongs to the interval of 11 to 12 hs. Moreover sorghum genotypes varied in their degree of photoperiod sensitivity which seemed to be affected principally by the geographical origins of accessions. The degree of sensitivity to photoperiod in sorghum refers to the length of the short days that are required to induce flowering. A highly photoperiod sensitive sorghum required photoperiod less than 12 hs to flower whereas plants with low photoperiod sensitivity were able to flower indifferently under different photoperiod (Teshome *et al.* 2007).

In conclusion, most crop assume that photoperiod effects are additive to those of temperature because of the photoperiod and temperature interaction. This interaction manifests itself as a hyperbolic response to photoperiod, variation in the critical

photoperiod with temperature or variation in the optimum temperature with photoperiod (Craufurd and Wheeler 2009). Most of the models for crops assume that photoperiod only affects rate of development at/and below a specific temperature above which only temperature affects the rate. Crop species that originated in the tropics (e.g. sorghum, millet) have higher values for this temperature. Temperature has been suggested to be the main factor influencing flowering time in maize as reported by Birch *et al.* (1998).

For instance study related to the response of flowering time in 47 varieties of rice to different photoperiod (9, 10, 11, 12, 13 and 15 hs) conducted by Maheswaran *et al.* (2000), revealed that under a specific temperature, each variety has its own optimum daylength under which it flowers the earliest, and as the daylength deviates from the optimum to either the longer or shorter side, the time to flowering is retarded according to the sensitivity of the variety. The number of days to flower in rice plants does not show a simple linear correlation with daylength (Suge 1976). In same way, numerous studies proved that the critical daylength varied with the cultivar in buckwheat (Michiyama *et al.* 2003) and maize (Birch *et al.* 1998).

Therefore Murfet (1977) explained that flowering is a complex phenotype which is the end result of numerous physiological and biochemical processes within a plant. These processes are regulated by the interaction of many genes within an organism, and are also influenced by environmental stimuli. Temperature and photoperiod are the most important environmental variables that determine flowering time. Sensitivity to photoperiod is under genetic control and interacts with other temperature and flowering genes to accelerate or delay the flowering response (Chang *et al.* 1969).

## 2. Identification of QTLs controlling flowering time in sorghum

Initially association analysis was preformed to identify QTLs controlling flowering and photoperiod sensitivity using (i) 107 accessions of sorghum grown under natural condition of daylength and (ii) 45 accessions grown under controlled conditions of daylength. Four QTLs controlling flowering time were detected under natural condition of daylength at threshold 2.5 using K model. A total of seven flowering time loci were

detected under controlled condition of daylength. One QTL controlling sensitivity was detected on chr 1 and one QTL controlling photoperiod insensitivity was detected on chr 4.

Afterward construction of linkage maps and mapping of QTLs controlling flowering time was performed using $F_2$ population derived from a cross between Kikuchi Zairai (late flowering cultivar originated from Japan) and SC112 (early flowering cultivar originated from Ethiopia). A total of 144 $F_2$ plants and their parental cultivars were grown under natural daylength and also under daylength of 12 hs. Two linkage maps were constructed by using 213 simple sequence polymorphism markers. Using linkage mapping a total of five QTLs controlling flowering time were detected under both natural and 12 hs daylength. $qFT_{1\text{-}1}$ on chr 1, $qFT_7$ on chr 7, $qFT_8$ on chr 8 and $qFT_{8b}$ on chr 8b were considered to be sensitive to photoperiod. On the other hand $qFT_5$ on chr 5 and $qFT_{6b}$ on chr 6b were identified only under 12 h were identified to be insensitive to photoperiod.

We have compared the results of QTLs controlling flowering time identified using association analysis and linkage mapping. The physical positions of the markers used for association analysis are shown in the linkage map of the sorghum (BTx623) x (IS3620C) recombinant inbred population established by Bhattramakki *et al.* (2000). In this map linkage group designations are identical to those described in Menz et al. (2002) and ordered on chromosome in Kim et al. (2005).

The result of this assessment show that Xtxp302 identified on chr 1 using association analysis was mapped on an adjacent physical position as $qFT_{1\text{-}2}$ identified by linkage mapping. While $qFT_{1\text{-}1}$ was identified only using linkage mapping. The $qFT_{1\text{-}1}$ seems to be newly mapped in this study since no QTL controlling flowering time in sorghum was mapped in the same position in previous studies. On the other hand, Xtxp13 associated with flowering time using LD was located at an adjacent physical distance to $qFT_2$ identified on chr 2 using linkage mapping and previously mapped by Srinivas *et al.* (2009). While q$FT_3$ on chr 3 and q$FT_5$ and q$FT_{5b}$ on chr 5 were mapped only using linkage mapping. Similarly q$FT_{6b}$ on chr6 was identified to be associated to flowering time only by linkage mapping. Three more QTLs were considered newly mapped in the current study since they were not reported in previous studies on flowering time in sorghum. These three

QTLs controlling flowering time ($qFT_7$ on chr 7, $qFT_8$ on chr 8 and $qFT_{10}$ on chr 10) were identified using linkage mapping however they were not mapped using association analysis.

The difference in the results of association analysis and linkage mapping can be explained by different raisons. Indeed Subudhi *et al.* (2000) explained that the objective of many genetic mapping studies is to identify quantitative trait loci that are responsible for phenotypic variations. Although often viewed as fundamentally different, linkage and association mapping share a common strategy that exploits recombination's ability to break up the genome into fragments that can be correlated with phenotypic variation. However Casa *et al* (2008) expounded that the key difference between the two methods is the control applied by the researcher over the recombination. On one hand, linkage mapping is a highly controlled experiment where individuals are crossed to generate a mapping population in which relatedness is known. In plants, these are generally bi-parental crosses. By this means, the experimenter creates a closed system and uses a small number of genetic markers to identify the locations of the relatively few recombination breakpoints. With genotype data from across the genome, the experimenter can then determine if a chromosomal fragment between two specific breakpoints is associated with a phenotype.

On the other hand, association analysis, also known as association mapping or linkage disequilibrium mapping, is a method that relies on linkage disequilibrium to study the relationship between phenotypic variation and genetic polymorphism. Linkage disequilibrium is the nonrandom combination of alleles at two genetic loci (Flavio and Mark 2006). Therefore, association mapping is not a controlled experiment but rather a natural experiment. Genotype and phenotype data are collected from a population in which relatedness is not controlled by the experimenter, and correlations between genetic markers and phenotypes are required within this population. This open system design provides higher mapping resolution compared with the closed system of controlled crosses, but it is difficult to deduce where and when recombination has occurred (Myles *et al.* 2009).

While using linkage mapping it is only possible to exploit the recombination events that have occurred during the establishment of the mapping population. In this case,

recombination has not had enough time to shuffle the genome into small fragments, and QTLs are generally localized to large chromosomal regions (10 to 20 centimorgans). In addition linkage mapping can only identify QTL from the phenotypic diversity generated from the controlled crosses, which may often represent only a small fraction of the phenotypically relevant variation in species. Indeed, because different QTLs segregate in different linkage mapping populations, QTLs often are not consistent across mapping populations (Holland 2007). It has long been recognized that association mapping offers advantages over linkage mapping for the identification of QTLs.

Furthermore the difference in results of association analysis and linkage analysis can be explained by the difference in markers and also by the difference in mapping populations used for each type of analysis since the number of QTL controlling a specific trait is largely affected by the number and type of markers used and also by the population type and size as reported by Shehzad *et al.* (2009b). Additionally the experiments used for association analysis and linkage analysis were conducted under different conditions among which only photoperiod was controlled. Other climatic conditions than photoperiod could affect the number of QTL identified in each type of analysis. In fact temperature is considered as a major determinant of flowering time.

In addition flowering in the japanese parent with strong photoperiod sensitivity and very late flowering was affected not only by photoperiod sensitivity but also thermo-sensitivity like other Japanese improved cultivars as Tentaka and Kazetachi (Tarumoto 2011). Many breeders explained that the interaction involving daylength and temperature strongly affect flowering time and therefore crop adaptation. In previous study Nakano *et al.* (1997) cited by Yanase *et al.* (2008) classified 70 Japanese commercial sorghum varieties into three groups: a variety group insensitive to photoperiod, a variety group sensitive to photoperiod, and a variety group sensitive to both photoperiod and temperature. In a next study Nakano *et al.* (1997) classified 104 Japanese commercial sorghum varieties by using three different traits: (i) earliness in heading; (ii) photosensitivity; and (iii) temperature dependency on photoperiodic reaction. They found that more than 50% of the varieties had the temperature dependency. These results suggested that the heading time

and therefore the flowering time temperature are affected by both photoperiod and temperature. Consequently the effect of temperature should be taken into account for further studies (Yanase *et al.* 2008).

In this study the effect of thermo-sensitivity on flowering time in sorghum plants were not analyzed. These results suggest that the QTLs controlling flowering time in sorghum are affected by changing responses to other environment signals. We considered that flowering time in sorghum is a complexe trait controlled by multiple genes and was expressed by the interactive regulation of different genes such as thermo-sensitivity genes, photoperiod sensitivity genes and maturity genes. Tarumoto (2011) proposed that the genes for thermo-sensitivity and photoperiod sensitivity accounted for the relationship between flowering time and the environment.

Based on the results obtained in this study and in previous studies we concluded that flowering time is a complex trait that controls adaptation of crops to local environment. Buckler *et al.* (2009) discussed that there were two different types of genetic architecture of flowering time in plants. Among these two types one was based on numerous small-effect QTLs controlling flowering time in outcrossing species, maize, in contrast to any single large-effect QTL in the selfing plants, rice (Ebane *et al.* 2011) and Arabidopsis (Salome' *et al.* 2011). The present study indicated that flowering time in sorghum was controlled by a large number of QTLs with small effects, suggesting that the genetic architecture of flowering time in sorghum was similar to maize.

Finally, the interaction of the QTLs controlling flowering time in sorghum with photoperiod appears to be fundamental study to improve this crop and to feed the world's expanding populations especially that sorghum is particularly adapted at low input level and suited to hot and dry agro-ecologies where it is difficult to grow other food crops.

## 3. Conclusion and perspectives

The objective of many genetic mapping studies is to identify quantitative trait loci that are responsible for phenotypic variations. The obvious targets of genetic crop improvement have been increased resistance to insects and diseases. Stress resistance is also an important target because of the large impact that drought stress has on crop yields (Boyer 1982). However, in order to increase basic productivity, changes will have to be made in development and metabolism. Several aspects of the development of grain crop species have major impacts on adaptability, stress tolerance and yield (Morgan and Finlayson 2000). The processes include photoperiodism, flowering time, shoot elongation and root development.

Many QTLs controlling flowering time were suggested by previous studies, however the effect of photoperiod change on flowering time and the sensitivity of sorghum to the variation in daylength were not intensely examined. Moreover the range of the variation of photoperiod above which variation in daylength tremendously affects the flowering time and consequently the crop yield is not entirely investigated in sorghum.

The objectives of the present research were to study the variation in flowering time in core collection of sorghum and to analyze the variation in the response to daylength. In the second part of this research we focused on the identification of QTL controlling flowering time using linkage disequilibrium (under natural and controlled conditions of daylength) and linkage mapping (under natural condition and 12 h daylength). Several QTLs were identified to be associated with flowering time in sorghum under natural and controlled conditions of photoperiod. For this purpose we analyzed the variation in flowering time and response to photoperiod using a core collection of sorghum germplasm.

From the results of this experiment we concluded that sorghum is a short day plant gradually responding to the short daylength. In addition sensitivity to photoperiod appeared to be affected by multiple factor such as the geographical origin of accessions. The degree sensitivity to photoperiod in sorghum refers to the length of the short days that are required to induce flowering. We also concluded 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. Given that photoperiod sensitivity is the most important mechanism governing adaptation.

We performed association analysis to identify QTLs controlling flowering time and photoperiod sensitivity using a core collection of sorghum under natural and controlled conditions of daylength. The success of association mapping efforts depends on the possibilities of separating LD due to linkage from LD due to other causes (population structure, size and number of marker used for association analysis..). We also performed the linkage analysis and we could identify new candidate QTLs controlling flowering time in sorghum. We also identified QTLs controlling photoperiod sensitivity and photoperiod insensitivity in sorghum. Consequently we have achieved the aims of this research by studying and explaining the variation in response of flowering time in sorghum to different range of photoperiod in relation with the geographical origin of accessions representing the sorghum core collection and by ascertaining the threshold of photoperiod above which flowering time is strongly affected by change in day length in sorghum.

Nevertheless, the identification of genes controlling sensitivity to both photoperiod and temperature is getting more expanding importance in breeding programs because of the effects of global warming and its consequences. Hence the outlooks of the present study are (i) to better understand the effect of photoperiod and temperature on heading and flowering times in sorghum, (ii) to construct a high density linkage map of sorghum using recombinant inbreed lines (RIL), developed by sorghum group in the laboratory of Plant Genetics and Breeding Science at the University of Tsukuba, and deriving from different crosses between parents of diverse origin and genetic background (iii) to identify thermo and photosensitivity genes controlling flowering time in sorghum which represent a crop of a growing importance for the world's expanding populations.

## Abstract

The overall importance of flowering time and the critical role that genes controlling flowering time play led to this large scale effort to understand the molecular basis of the QTLs controlling flowering time in sorghum. We report here the QTLs controlling flowering time and sensitivity to photoperiod in sorghum. This study provides insight into the genetic architecture of lowering time in sorghum.

We mapped QTLs controlling flowering time both within a core collection of sorghum (using association analyses) and an $F_2$ population derived from a cross between Kikuchi Zairai (a late flowering cultivar originated from Japan) and SC112 (an early flowering cultivar originated from Ethiopia) using linkage mapping. The parental cultivars were selected from the core collection of sorghum. The two methods used for identifying QTLs controlling flowering time, produced concordant results in terms of the magnitude of effects; however they have different power and resolution capabilities.

Association analysis identified four QTLs under natural condition of daylength, while seven QTLs were detected under controlled conditions of daylength (at *p-value* $\geq$ 2.5). One QTL controlling photoperiod sensitivity was newly identified on chr 1 and one QTL controlling photoperiod insensitivity was detected on chr 4. Whereas linkage mapping identified 9 QTLs controlling flowering time under natural daylength explaining 60% of the total variance for flowering time; and 7 QTLs under 12 hs daylength explaining 46.6% of the phenotypic variation. A total of five QTLs controlling flowering time were detected under both the natural and 12 hs daylengths and the *qFT7*, q*FT*8 and *qFT10* mapped in the present study to chr 7, chr 8 and chr 10, respectively, are considered newly mapped, as they were not reported in previous studies.

The identification of QTLs controlling flowering time in this study permitted unprecedented estimation of the genetic architecture in sorghum in term of the magnitude of QTLs effects and QTL-environment interactions. Our results demonstrate that large

difference in flowering time in sorghum core collection and $F_2$ population grown under different daylength conditions are not caused by a single gene of a large effect, but by the cumulative effects of numerous QTLs each with only a small impact on the trait.

In the same way Buckler *et al.* (2009) studied the variation in flowering time with a set of 5000 RIL and identified 36 QTLs that explained 89% of the total variance for the flowering time in maize. Buckler *et al.* (2009) suggested that in outcrossing species (maize), the genetic architecture of flowering time is dominated by small, additive QTLs, 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 as 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 single large-effect QTL in rice and *Arabidopsis*.

We have achieved the aims of this research by studying and explaining the variation in response of flowering time in sorghum to different range of photoperiod by ascertaining the threshold above which flowering time is strongly affected by changes in daylength in sorghum. We also performed the association and linkage analyses and we could identify new candidate QTLs controlling flowering time in sorghum. In addition we identified candidate QTLs controlling photoperiod sensitivity and photoperiod insensitivity in sorghum. Therefore this study represents a preliminary and basic study for the QTLs controlling flowering time in sorghum, and the results give emphasis to 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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