# GENETIC ANALYSIS FOR PANICLE ARCHITECTURE AND GRAIN YIELD IN SORGHUM [*Sorghum bicolor* (L.) Moench] IN MALI

**By**

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## DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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# GENERAL ABSTRACT

Sorghum panicle architecture is a complex trait involving growth, elongation and branching pattern. The main objective of this research was to determine the genetic control of sorghum panicle architecture and its involvement in grain yield. Molecular markers associated with statistical analyses were used to identify the genomic regions or QTLs controlling quantitative traits. A population composed of 401 $F_4$ families was derived from a cross between two contrasted parents for panicle traits (Tiandougou and Lata-3). Phenotyping of sorghum panicle architecture was based on two groups of variables: architectural geometry traits related to the length and the diameter and architectural topology traits that relate to the number, density and position. Forty eight sorghum panicle architecture traits and grain yield components were studied in three sowing dates in two years at the research station of Sotuba, Bamako, Mali. A linkage map was constructed using 228 SNP. A total length of the genetic map was 1362.3 cM with average distance between markers of 6.01 cM. In this study, 164 QTLs including 53 major QTLs were detected for the different traits. These putative QTL explained 1.91 to 45.64% of the phenotypic variation observed for each trait. Many of the major QTLs were consistent across the sowing dates. Key regions of the genome in the population Tiandougou/Lata-3 affecting panicle growth, elongation and branching may be utilized to improve agronomic performance through MARS approach. Sorghum grain yield was positively influenced by the following panicle architecture traits: primary branches number and length, rachis basal diameter and the number of nodes per panicle. These traits can be used for indirect selection for yield potential improvement in conventional or molecular breeding methods. QTLs and candidate genes involved in inflorescence architecture in related grasses were co-localized. These associations may be investigated in sorghum to decipher the function and pattern of these candidate genes.

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## DEDICATION

This thesis is dedicated to the families of SAKO at Moribabougou, Bougouni and Bamako Hamdallaye. My Mothers Coumba DIALLO, Haby COULIBALY and Fanta MARIKO, my wife, Diarah GOITA and children, Fanta, Mamadou, Minata, Coumba and Fotigui.

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## LIST OF ABBREVIATIONS

AFLPs: Amplified fragment length polymorphism

ANOVA: Analysis of Variance

BIP: bi-parental populations

BLAST: Basic Local Alignment Search Tool

BLUP: Best Linear Unbiased Predictor

BM: Branch Meristem

CIRAD: Agricultural Research for Development

cM: Centimorgan

DArTs: Diversity Array Technology

DAP: Di-Ammonium Phosphate

DNA: Deoxyribo Nucleic Acid

FM: Floral Meristem

GBS: Genotyping by Sequencing

IBPGR: International Board for Plant Genetic Resources

ICRISAT: International Crops Research Institute for the Semi-Arid-Tropics

IPGRI: International Plant Genetic Resources Institute

LOD: Logarithm of Odds

MARS: Marker Assisted Recurrent Selection

NCBI: National Center for Biotechnology Information

PCA: Principal component analysis

PCR: Polymerase Chain Reaction

QTL: Quantitative Trait Locus

REML: Restricted Maximum Likelihood

RFLPs: Restriction Fragment Length Polymorphisms

RIL: Recombinant inbred line

SAM: Shoot Apical Meristem

SIM: Simple interval mapping

SM: Spikelet Meristem

SNPs: Single Nucleotide Polymorphisms

SPM: Spikelet Pair Meristem

SSRs: Simple Sequence Repeats

# CHAPTER ONE

## GENERAL INTRODUCTION

Cultivated sorghum [*Sorghum bicolor* (L.) Moench] is an annual $C_4$ photosynthetic monocot, diploid with haploid chromosome number of 10 (2n =2x =20). The physical size of its genome is 730 Mbp (Paterson *et al.*, 2009). Sorghum crop ranks fifth in production after wheat, maize, rice, and barley and has a predominant contribution towards food and fodder security in the arid and semi-arid regions of the world (Srinivas *et al.*, 2009). Food security for an increasing world population depends largely on the ability of plant breeders to increase sorghum grain yield, which depends on several yield components (Pushpendra *et al.*, 2006). Sorghum grain yield increase is of primary interest in food security for millions of rural families in the arid and semi-arid areas of the world. Grain yield is determined by the total number of grains produced and their average weight (Peltonen-Sainio *et al.*, 2007). Final grain size and number are interrelated since genetic variation in seed size is often compensated by an adjustment in seed number (Egli, 2006; Sadras, 2007).

Grain yield improvement remains one of the major breeding objectives of many cereal improvement programs. Thus the study of association among traits becomes a prerequisite to develop comprehensive selection criteria to achieve this objective (Ezeaku and Mohammed, 2006). Sorghum grain yield has been reported to have low heritability (Bello *et al.*, 2007). The indirect selection for yield related characters with high heritability might be more effective than direct selection for yield. Grain size and number have been targeted for breeding to improve sorghum grain yield (Pushpendra *et al.*, 2006). The genetic control of the number and growth of branches is very important owing to its effect on grain yield (Kellogg, 2007). Sorghum grain yield improvement involves the dissection of yield components such as the number of branches per panicle, the length of the panicle, number and weight of the grain. All of these yield components are under genetic control and are affected either positively or negatively by panicle architecture. Sorghum panicle architecture which includes panicle shape, compactness, branching pattern and seed density is a backbone for targeting grain yield improvements in sorghum.

Primarily determined by branching patterns, panicle architecture affect the number of seeds (Zhao *et al*., 2006), by the developmental fate of shoot apical meristems and variation in branching patterns leading to architectural diversity (Prusinkiewicz *et al*., 2007). The regulatory mechanism affecting panicle branching may also influence the number of spikelets and consequently has a direct effect on grain production. Therefore sorghum panicle architecture should be of primary interest to breeders for enhancing yield potential.

Rice and maize are the leading cereals studied most often to characterize the number of genes involved in the control of inflorescence architecture (Bommert *et al*., 2005) and inflorescence morphology which is determined by the architecture of the underlying gene regulatory networks (Prusinkiewicz *et al*., 2007). Sorghum, that shows tremendous variations in panicle architecture, has been little studied (Brown *et al*., 2006). This may be because of its complex structure than other crops, more diverse and therefore difficult to phenotype. Variation in sorghum panicle architecture is particularly pronounced for the length of the primary branches, which are generally much longer at the base and shorter at the top of the panicle; the position of the node bearing the longest primary branches; the number of primary and secondary branches; rachis diameter, which is mostly bigger at the base of the panicle; number of grain per primary branch that becomes fewer from the base to the top of the panicle and the number and length of the internodes.

Unfortunately, the genetic basis for this variation is little understood. Brown *et al*. (2006) reported that variation in sorghum panicle architecture results from differences in branching, elongation and branch abortion and indicated that the number of branches at each order of branching varies. The need to improve the understanding of the genetic basis of this striking variation in panicle architecture is necessary to better guide breeding strategies directed towards improving sorghum grain yield.

Breeding efforts in Mali have been primarily for higher grain yield potential through conventional breeding; hybrid development using male sterility brought some progress in grain yield improvement. Sorghum yield potential, however, compared to other cereals such as maize and rice still remains consistent and low in Mali. Lately the integration of molecular markers into breeding programs has offered promising alternatives to improve the potential yield using approaches such as Marker Assisted Recurrent Selection (MARS) in populations derived from elite by elite crosses. Molecular markers are useful tools to dissect quantitative variation of highly polygenic traits such as panicle architecture traits and grain yield into Quantitative Traits Loci (QTL). The QTLs/markers information can be manipulated in breeding programs as Mendelian factors providing a way to improve breeding efficiency for those traits. QTL mapping provides estimates for number, position, effects, and interactions between QTLs. Therefore, it gives more information about the genetic architecture of quantitative traits. For sorghum panicle architecture traits, such information should help in designing breeding strategies to use for targeting valuable traits in breeding programs. The identification of candidate genes based on protein sequence similarity in related species is the first step in deciphering gene function in crops. Such approach can be used in sorghum by using rice and maize genome databases to propose a list of genes involved in the molecular control of sorghum inflorescence architecture.

Very few studies have been done on sorghum panicle architecture. These studies focused on the identification of QTLs for panicle traits, genetic correlation and broad sense heritability estimation (Pereira *et al.*, 1995; Hart *et al.*, 2001; Brown *et al.*, 2006; Srinivas *et al.*, 2009). The research questions, however, are to address the relationship between panicle architecture and grain yield; the determination of the pattern of panicle branching and the number of grains at different positions (base, medium and top) on the primary branches and candidate genes controlling sorghum panicle architecture. The detailed dissection of panicle traits in relation to grain yield was an important starting point. The genetic control of panicle architecture and the relationships between panicle architecture in sorghum and related cereal crops for candidate genes should be an innovative investigation.

The objectives of this study were to:

1.) assess the extent of genetic diversity in panicle architecture and yield in an $F_4$ population of sorghum;

2.) identify and map QTLs associated with panicle architecture and grain yield in sorghum; and

3.) identify sorghum candidate genes involved in inflorescence architecture using previously published evidences in rice and maize.

# CHAPTER TWO

## 2.0. LITERATURE REVIEW

## 2.1. Sorghum

### 2.1.1. Economic importance

Sorghum (*Sorghum bicolor* (L). Moench) is the fifth most important cereal grain crop in the world after wheat, rice, maize and barley. Its origin is diversely discussed; but Haussmann *et al*. (2002) claimed that sorghum originated from West Africa and is used as staple food in the semi-arid tropics of Africa and Asia. Sorghum is cultivated under rain fed and irrigated conditions in Africa, Asia, South America, and the United States (House, 1985). In Mali, based on tonnage, sorghum is the second most important cereal after millet. Sorghum is used traditionally but lately its industrial utilization has become important. Sorghum is processed into food and also used in the brewing industry for making beers. It is also used as a fodder in the green or dry forms and feed crop. Sorghum constitutes raw material for biofuel, starch, dextrose syrup, fiber, alcohol, soft porridge and malt extract production using wet-milling processes at industrial scale (Liang and Gao, 2001). Therefore, sorghum presents many advantages to economic development and constitutes the cornerstone of food security for millions of poor people in the arid and semi-arid areas of the world. Breeding objectives depend on the socioeconomic and production contexts. In industrialized countries such as the United States, Europe, Argentina, and Brazil, the production of sorghum is mainly obtained from hybrids and destined for animal feed. The principal breeding criteria focus on adaptation to mechanized cultivation and high yield performance. In tropical agriculture zones, countries of West, Central and East Africa and India, sorghum cultivation destined for human consumption is mainly achieved using pure lines as varieties. Selection objectives concern the exploitation of local varieties or introduced materials. According to House (1985), a number of sorghum traits were changed through breeding processes. These changes concerned the decrease in rachis internode length and mainly the increase in number of primary, secondary branches per panicle and seed size. Also breeding goals for sorghum shifted from wide adaptability to specific regional adaptation.

In Africa, the focus was on breeding for high yielding material for good grain quality and/ or forage by developing multi-purpose varieties. Photoperiod sensitivity in sorghum plays important role in its adaptation and affect the production. Drought, high temperature and nitrogen deficiency are the major abiotic constraints. Diseases such as grain mold, rust, smut, anthracnose, ergot and bacterial streak, insect pests such as the shootfly and stalk borer; parasitic weeds such as *Striga* are the main biotic constraints. Therefore, breeding objective for introduced material, resistance to *Striga*, diseases and insect pests were addressed. Later on, the development of early maturing material, improved grain quality and adaptability to drought-prone environments were of interest. Lately breeding efforts have been driven by the integration of molecular tools that speed up selection potential at early generation and the development of improved varieties. Therefore, the main variety types developed by breeding programs are breeding lines, although photoperiod sensitive hybrids are also being developed.

### 2.1.2. Different types of sorghum and varietal assortment

Sorghum [*Sorghum bicolor* (L.) Moench] belongs to the tribe Andropogoneae; group Sorghastrae; and genus Sorghum (Doggett, 1988). It has been classified into 31 species by Snowden (1936). De Wet and Harlan (1971), Harlan and De Wet (1972) and De Wet (1978) simplified the classification by defining three species: *Sorghum halepense* which is a perennial tetraploid; *Sorghum propinquum,* a perennial diploid; and *Sorghum bicolor* that is an annual diploid with haploid number of 10 chromosomes. *Sorghum bicolor* is composed of three subspecies: *bicolor,* *arundinaceum* and *drummondii*. Cultivated sorghum belongs exclusively to the subspecies bicolor and spikelet, inflorescence and plant characteristics were traditionally used to classify this subspecies. Harlan and de Wet (1972) proposed the classification of *Sorghum bicolor* into five basic races named bicolor, guinea, caudatum, kaffir and durra and 10 intermediate races obtained from the combination of the different basic races. Sorghum panicle types (Figure 2.1) show correlation with spikelet morphology. Therefore, the races bicolor and guinea spikelets were found generally on the more open panicles (types 2, 3, and 4); kafir and durra spikelets were associated with more compact panicle types (types 5, 6, and 7). Caudatum spikelets were observed in wide range of panicle types.

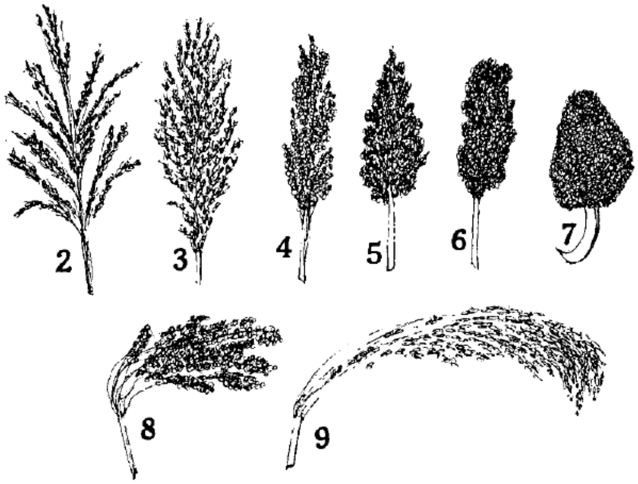


*Figure 2.1: Head types of cultivated sorghum. Type I consists for wild races and is considerably more diffuse than type 2 [Original picture from Harlan and de wet, (1972)]*

The bicolor race has long, clasping glumes, elongate seed, and open panicles. Long glumes and open panicles are noted in the guinea race; this race is more evolved than the bicolor race. Turtle-backed grains is the characteristic of the caudatum race. Intermediate races involving caudatum race are in general high yielding. Therefore many derivative hybrids from caudatum race were developed in Africa, particularly in Mali. The spikelet characteristics of kaffir race show less specialization than those of the guinea, caudatum, and durra. However, kaffir panicle is generally semi-compact to compact. The durra race exhibit specialization for panicle morphology than all the cultivated sorghums.

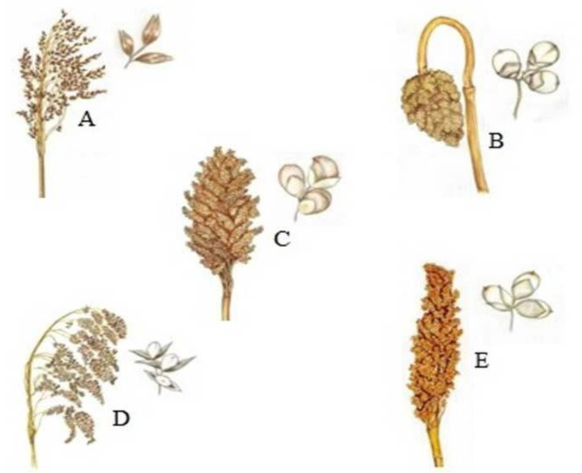


*Figure 2.2: Sorghum races A = bicolor, B = durra, C = caudatum, D = guinea and E = kafir (Picture adapted from Harlan and de wet, 1972).*

### 2.1.3. Types of panicles in sorghum

Rangaswani Ayyangar and Rajabhooshanam (1938) defined four types of sorghum panicles. The type I comprises conical panicles characterized by a steady decrease of the primary branches from the first to the last node at the top. An example is the race guinea. The type II comprises ovoid, ellipsoid and obovate panicles in which primary branches increase in length in the first few nodes and then decrease and an example is the race durra. The type III comprises cylindrical panicles where the length of the primary branch fluctuates within narrow limits from node to node; an example is the race caudatum. The type IV comprises truncate and corymbiform panicles. The length of the primary branches increase steadily from the first to the last node and an example is the broom-corn. Sorghum descriptor that includes twelve types of panicles based on the shape and the compactness of the panicle.

The characteristics of the different panicle types are: very lax panicle (typical of wild sorghum); very loose erect primary branches; very loose drooping primary branches; loose erect primary branches; loose drooping primary branches; semis-loose erect primary branches; semis-loose drooping primary branches; semi-compact elliptic; compact elliptic; compact oval; half broom corn and broom corn (IBPGR & ICRISAT, 1993).

### 2.1.4. Sorghum genome and relationship with members of the *Poaceae* family

Paterson *et al*. (2009) studied the sorghum genome in relation to other members of the *Poaceae* family showed that *Sorghum bicolor* (L.) Moench genome physical size is 730 megabases (Mb). It is essentially composed of heterochromatin that accounts for 62% (460 Mb) of the genome while in rice, heterochromatin occupies 15% (63 Mb) of the genome. This difference between sorghum and rice involved long terminal repeat (LTR) retro transposons. The proportion of DNA transposons in the genome account for 7.5% in sorghum, 2.7% in maize and 13.7% in rice. Sorghum genome exhibits intermediate content of retro transposons compared to those of maize and rice. Sorghum shows about 55%, maize 79% and rice twenty six percent 26% of retro transposons. Sorghum resembles rice in having a higher ratio of gypsy-like to copia-like elements than maize.

Most paralogues in sorghum are proximally duplicated, including 5,303 genes in 1,947 families of genes. The number and sizes of sorghum gene families are similar to those of rice (Figure 2.2). About 24% of gene families belong to sorghum and rice. Seven percent of gene families are unique to sorghum.

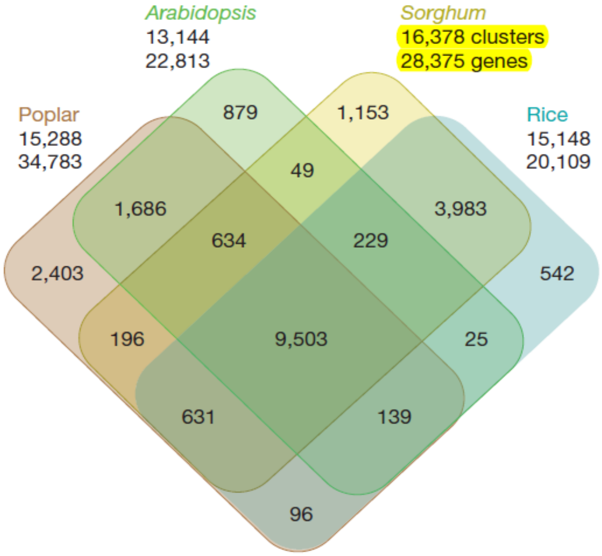


*Figure 2.3: Orthologous gene families between sorghum, Arabidopsis, Rice and poplar. [Original picture from Paterson et al. (2009)]. The numbers of gene families (clusters) and the total numbers of clustered genes are indicated for each species and species intersection*.

## 2.2. Flowering in sorghum

### 2.2.1. Floral biology: inflorescence biology and morphology

Singh *et al*. (1997) described the process of sorghum panicle formation, development and the different floral organs. Sorghum panicle is formed by the gradual differentiation of the vegetative primordium or the growing tip into the reproductive primordium (Figure 2.4).

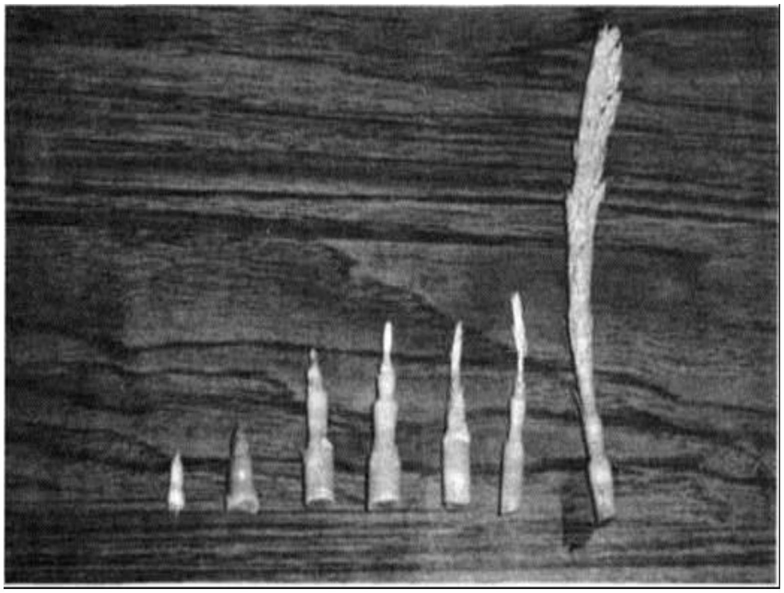


*Figure 2.4: Gradual primordial differentiation into sorghum inflorescence. [Original picture from Singh et al. (1997)]*

Sorghum panicle development starts by the elongation of the shoot apex into the rachis of the panicle. The rachis tapers off towards the top and is grooved longitudinally. The rachis elongates after increasing in dimension and forms branches and branchlets through the formation of primary, secondary and tertiary branch primordia. Two paired spikelet primordia are formed from the development of the tips of the tertiary branches primordium. One of the paired spikelet is hermaphrodite and the other staminate. Often, one hermaphrodite and two staminate spikelets can be formed. Spikelets and florets development in the panicle continue to be covered by the flag leaf. According to House (1985), the primordial differentiation into floral parts may take about 30 days after sowing.

Sorghum panicles present striking variation in morphology. They can be compact, semi-compact to open. Singh *et al*. (1997) describe the following: the peduncle elongation forces the panicle out of the leaf sheath after flag leaf unfolding. Variation in shape, size, and length of sorghum panicles is related to the variation in rachis, branch and internode length associated with the angle of branching. Sorghum spikelet development is basipetal meaning that the upper region of the panicle develops earlier than those in the lower. A raceme consists of one or several spikelets; one spikelet of a raceme is always sessile and the other pedicellate.

The length of the raceme varies according to the number of nodes and the length of the internodes. The shape of sessile spikelets ranges from lanceolate to almost round or ovate. There are two glumes which vary from hairy to non-hairy. The glumes are hard and tough with nerves, and are obscure except near the tip. The lower glume is enclosed by the upper glume with its membranous margin. The seed may be enclosed by the glume or may protrude from it either partially or completely. The number of sessile spikelets per panicle in cultivated sorghum varies from 2000 to 4000 (House, 1985).

### 2.2.2. Flower emergence: from floral induction to flower development

The Shoot Apical Meristem (SAM) undergoes several changes (floral induction, initiation and differentiation) to become an inflorescence meristem which is composed of several meristems (BM, branch meristem; FM, floral meristem; SM, spikelet meristem; SPM, spikelet pair meristem) (Barazesh and McSteen, 2008).

#### 2.2.2.1. Floral induction

Floral induction is a qualitative change where the transition of meristem development from vegetative to reproductive phase occurs. Floral induction is a separate event from floral development in most of cultivated sorghum, it requires a short-day signal from the leaves (Morgan and Quinby, 1987) for sorghum sensitive to day length.

#### 2.2.2.2. Floral initiation

Verbeke and Heupel. (1990) studied sorghum floral initiation and floral differentiation and found that sorghum is useful for the study of floral initiation because it exhibits a determinate growth habit in which the single apical meristem first produces leaves and then is completely converted to the production of floral primordia. Morphological changes that occur at the shoot apex in sorghum correspond to an increased mitotic activity in the apical meristem that initiate changes from the vegetative to the floral state (Figure 2.5). The apical meristem appears as a dome covered by leaf initials in the vegetative state (fig. 2.5a, 2.5b). Leaf primordia start as a series of single ridges (Fig. 2.5a) which continue to be deposited by the meristem (Fig. 2.5b) until a floral bud forms (Fig. 2.5c-2.5g). The stage shown in Figure 2.5c is equivalent to the stage 2 described by Lane. (1963). This scale has been used to indicate the stage of apical development in a wide variety of cereal crops (Large, 1954; Lane, 1963; Williams and Morgan, 1979). Floral initiation is the culmination of meristematic growth in sorghum. The first microscopically visible sign of floral initiation is a smooth apical dome not covered by leaf initials (Fig. 2.5c). In sorghum, initiation and floral development are separate events (Morgan and Quinby, 1987).

#### 2.2.2.3. Floral differentiation

Floral differentiation starts with morphological changes of meristems with the formation of the apex dome (Fig 2.5e to 2.5g) and continues until the formation of floral buds. Floral primordial appears in the meristem and then advance progressively. The floral differentiation ends with the development of the primordia of floral organs (Verbeke and Heupel., 1990).

Floral differentiation in sorghum terminates leaf differentiation and thereby regulates plant size (Pao and Morgan, 1986). Almost all internodal elongation in sorghum occurs during floral differentiation (Morgan *et al*., 1977). This process leads to the formation of the different parts of the panicle architecture. However, at this stage one cannot tell which form (open or compact) of the panicle will be formed.

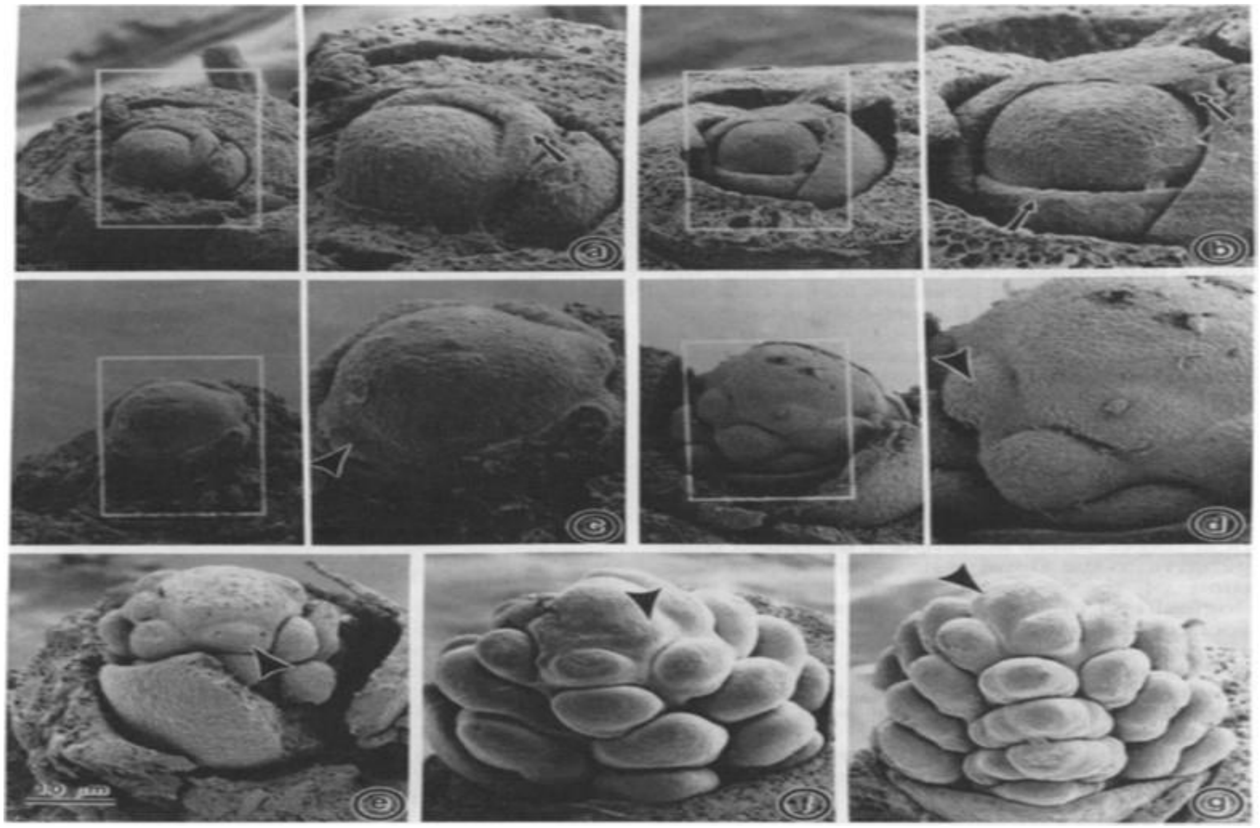


*Figure 2.5: Scanning electron micrographs of Sorghum bicolor apices showing the transition from vegetative to floral state. 1a and 1b are vegetative; leaf primordial are evident at the arrow. 1c through 1g are floral, with floral primordial evident at the arrow heads. 1a through 1g are all printed at same magnification, with magnification bar shown in 1e. 1a through 1d boxed areas on left enlarged 2x on right (Original picture from Verbeke et al., 1990).*

## 2.3. Panicle architecture

The sorghum panicle is composed mainly of the rachis, branches and spikelet pairs (Figure 2.2). The rachis has several nodes spaced all along the rachis. The space between two nodes is called internode. The diameter of the rachis become progressively slender from the base to the top. Primary branches developed directly on the rachis are composed of two parts: the first part is the sterile portion corresponding to the distance to the first secondary branch and the second part is the fertile portion that bear secondary, tertiary branches and spikelet pairs. The position of the longest primary branch is variable and the spikelet pair on which the sorghum grain is formed can be borne on the rachis, primary, secondary and tertiary branches.

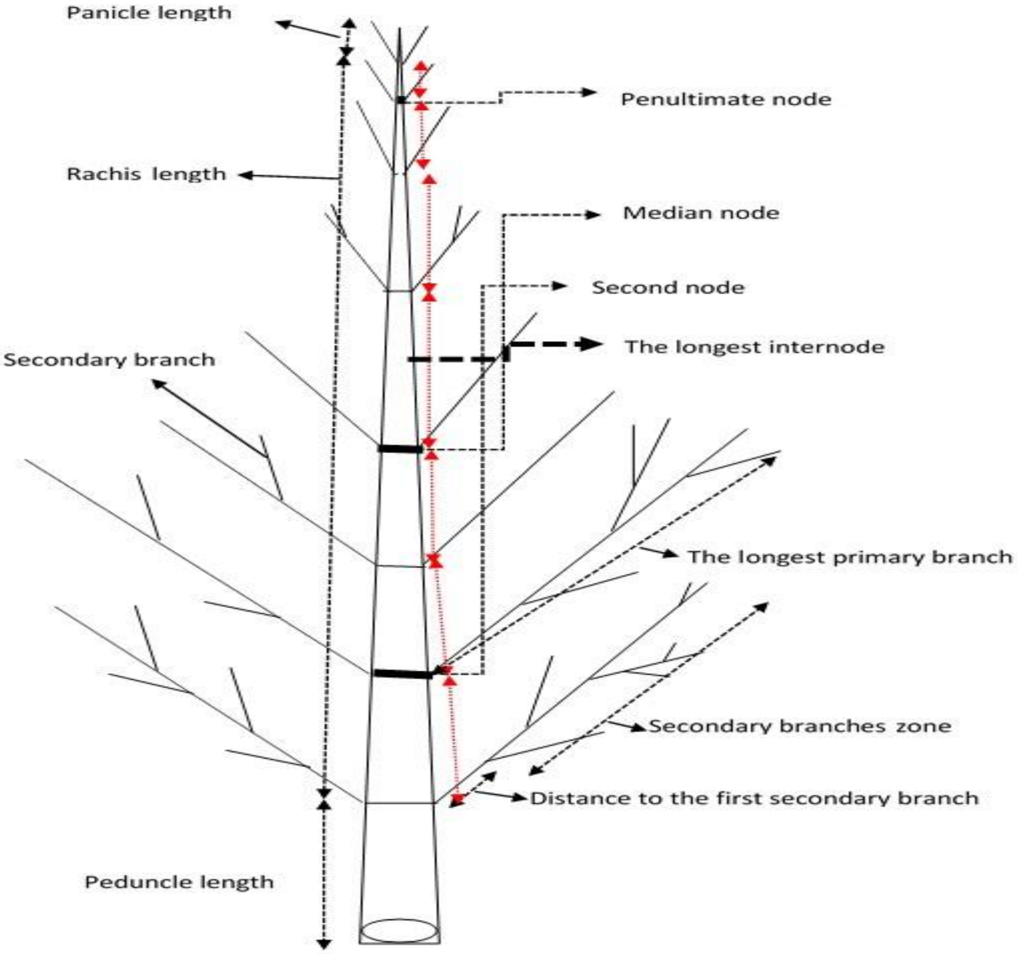


*Figure 2.6: Components of sorghum panicle architecture traits investigated (modified, Witt-Hmon et al., 2013).*

### 2.3.1. Principles of plant architecture: growth, elongation, branching

Plants display a variety of architecture defined by the degree of branching, internodal elongation and shoot determinacy (Wang and Li., 2006).

#### 2.3.1.1. Growth

The shoot apical meristem generates plant components such as leaves, stems and branches in an indeterminate growth and differentiation manner. Therefore, the main activities of the shoot apical meristem during plant development is to maintain the pluripotent stem cells, to form lateral organs and stems that determine different plant species architecture (Foucher *et al*., 2003). Axillary meristems are major determinants of plant architecture. They are involved in changes in cell proliferation and growth. This process is under gene expression and hormonal control (Tanaka *et al*., 2013). Branch formation involves two steps: initiation of new axillary meristem followed by their subsequent growth and development.

Phytohormone biosynthesis and response play an important role in growth of the inflorescence shoot. Mutations like *AUXIN RESISTANT1 (AXR1)* affect branching of the inflorescence shoot (Rouse *et al*., 1998; Stirnberg *et al*., 1999), while gibberrellic acid biosynthesis and response mutants, like *ga5* and *GIBBERELLIC ACID INSENSITIVE1 (GAI)*, affect floral internode growth (Kobayashi *et al*., 1994; Peng *et al*., 1997).

#### 2.3.1.2. Elongation

Stem elongation determines plant height and affects plant architecture and grain yield. The developmental transition from vegetative to reproductive stage induces internode elongation. The elongated internodes are the response to the growth and development of intercalary meristems in the internodes when the transition begins. Gibberellins belong to the family of tetracyclic diterpenoids; they play important roles in plant growth and developmental processes, especially in stem elongation (Hooley, 1994; Swain and Olszewski., 1996). Brassinosteroids (BRs) are natural plant growth hormones that promote compounds similar to steroids. They affect plant growth and development at very low concentrations (Clouse, 1996; Clouse and Sasse., 1998). The role of brassinosteroids was confirmed in rice stem elongation (Yamamuro *et al*., 2000). However, the role of such natural hormone is not reported in sorghum.

#### 2.3.1.3. Branching

Caraglio and Barthélémy (1997) described plant architectural variation in terms of the orientation of branches (orthotropic or plagiotropic); type of branching (monopodial or sympodial); degree of lateral shoot development as a function of their position on the mother branch (acrotony, mesotony or basitony); type of meristematic activity (rhythmic or continuous); number of internodes per growth unit; leaf arrangement (phyllotaxis) and position of reproductive organs on the branches (terminal or lateral). Plant architecture is a dynamic concept reflecting plant development over time. Halle *et al*. (1978) claim that branching patterns such as the position, the form resulting from the expression of meristematic activities contribute to plant architecture. Similarly grass inflorescence architecture is determined mainly by the branching pattern, from which the spikelet meristems initiate (Tanaka *et al*., 2013).

### 2.3.2. Application of plant architecture principles on sorghum panicles

Doust and Kellogg (2002) have demonstrated that meristem and its activities play a central role in the generation of varied inflorescence architecture of plants. Plant architecture modeling proposed by Barthélémy and Caraglio, (2007) can be transposed to inflorescences (Jabbour and Citerne, 2010). In sorghum, the description of branching topology refers to the arrangement of branches on the rachis and their positions. Description of the geometry of sorghum panicles includes the lengths of internodes and branches; rachis diameter and the magnitudes of the branching angles.

Singh *et al*. (1997) found that the grand growth stage in sorghum is due to cell elongation and growth during the vegetative phase. They also indicated that floral initiation leading to inflorescence development involves:

- elongation of the apical meristem
- differentiation of primary branches primordia on the floral apex
- differentiation of secondary branches primordia
- development of secondary and tertiary branches primordia
- elongation of the panicle
- formation of the panicle branches
- formation of fertile (sessile) and sterile (pedicellate) spikelet on the branches.

## 2.4. Genetic control of panicle development

### 2.4.1. Genes involved in panicle development

#### 2.4.1.1. Inflorescence meristem development

The transition from vegetative to reproductive state is the first step in inflorescence development. LEAFY (*LFY)* act upstream in a transcriptional framework by regulating the transition to flowering in the expression pattern of the floral homeotic ABC genes (Weigel *et al*., 1992). It also acts on the expression pattern in spikelet pair meristem and floral meristem in the developing floral organ primordia. In rice, LEAFY HULL STERILE1 (LHS1)/OsMADS1 is involved in spikelet meristem identity, because loss-of-function mutants showed defected identity of the lemma/palea (Jeon *et al*., 2000). A putative orthologous of LEAFY was mapped on chromosome 6 in sorghum by Brown *et al*. (2006).

### 2.4.1.2. Spikelet pair and branch meristem development

The gene BARREN STALK1 (*BA1*) in maize controls the early developmental switch that is involved in the initiation of the primary branches. The *BA1* gene in maize encodes for orthologous basic helix–loop–helix transcription factors. It is expressed between pre-existing and newly initiated meristems; for instance between the shoot apical and axillary meristems, inflorescence meristem and primary branch meristem, primary branch meristem and spikelet pair meristem, upper floral meristem and lower floral meristem (Komatsu *et al*., 2003a). Ramosa 1 (*ra1*) and ramosa 2 (*ra2*) play important roles in branch determinacy; only the expression pattern of ramosa 2 (*ra2*) is conserved in sorghum, rice, barley and maize (Bortiri *et al.*, 2006).

### 2.4.1.3. Spikelet meristem development

Branch meristems induced by genes such as *BIF2*, *BA1* and *LAX* acquire new identities and initiate spikelet meristems. FRIZZY PANICLE (*FZP*) in rice and BRANCHED SILKLESS1 (*BD1*) in maize and sorghum act to regulate meristem identity during the transition from spikelet meristem to floral meristem (Colombo *et al*., 1998; Chuck *et al*., 2002; Komatsu *et al*., 2003b). *FZP* and *BD1* are orthologs; they encode an ethylene-responsive element-binding factor. They are expressed in analogous patterns at the junction of spikelet meristem and rudimentary glumes in rice, and inner/outer glumes in maize. Other genes regulating spikelet meristem determinacy include maize and sorghum INDETERMINATE SPIKELET1 (*IDS1*) genes (Chuck *et al*., 1998; Brown *et al.*, 2006). The spikelet meristem first initiates a lateral floral meristem, and then converts it into the second floral meristem (Irish, 1997a; 1997b; 1998). Comparative analyses of *BD1* and *FZP* indicate that the genetic control of spikelet determinacy is similar in rice, sorghum and maize (Chuck *et al*., 2002; Komatsu *et al*., 2003b). Similarly the analyses of *BA1* and *LAX* suggest that their gene functions are conserved in branch meristem initiation. Therefore, spikelets that bear sorghum grain formation are affected by the branching.

### 2.4.2. Hormonal control of inflorescence development

The control of branching by axillary meristems is under hormonal, environmental, developmental, and genetic control. Hormones play a critical role in regulating branching (McSteen and Leyser, 2005; Beveridge, 2006; Ongaro and Leyser, 2008). Auxin is required for branch meristem initiation during both vegetative and inflorescence development (Benjamins and Scheres, 2008). Cytokinin regulates meristem size and hence indirectly affects branching (Shani *et al*., 2006; Kyozuka, 2007). Plants are able to produce organs throughout their lifetime because the meristem reserves a pool of undifferentiated cells to maintain the meristem as lateral organ primordia are produced and the balance between meristem maintenance and differentiation controls meristem size (Barazesh and McSteen, 2008). In sorghum, there is evidence for developmental regulation of the amounts of sterols and pentacyclic triterpenes in leaves which may be correlated with floral initiation (Heupel *et al*., 1987).

## 2.5. Genetic maps and QTL mapping in sorghum

### 2.5.1. Molecular markers

In sorghum, the commonly used marker systems include Simple Sequence Repeats (SSRs) or microsatellites, Diversity Array Technology (DArTs) and Single Nucleotide Polymorphism (SNPs). Now SNP are largely preferred to SSR. They can be automated for large datasets, such as Genotyping by Sequencing (GBS), multiplexing. Molecular techniques have been widely used to monitor differences in DNA sequence. SSRs were the most popularly used technique due to the ease in detection and automation. However, the adoption of the SNP marker system is now highly preferred due to the amount of sequence information. A good molecular marker must be:

- Co-dominant: it is possible to discriminate between heterozygous and homozygous;
- Highly polymorphic: reflect variability among genotypes of a cross;
- Specific to a locus
- Neutral: allelic substitution don't have any selective effect on other genetic marker;
- Abundant: they are found in great number in the genomes of plants, both in non-coding and coding regions.

Genetic markers have great potential in studying complex traits. They reveal allelic polymorphisms and act as signals for target genes between genotypes (Collard *et al*., 2005). The following factors are important in marker selection: i) reliability and reproducibility, ii) easy technical procedure, iii) cost, iv) level of polymorphism, and v) required DNA quality and quantity. Progress has been achieved by gathering information on the number of loci involved in trait expression, their location along the chromosome, and the relative contribution to trait expression of each locus (Maradiaga, 2003).

### Sorghum genetic maps

Sorghum is a diploid grass with 10 haploid chromosomes (2n=2x=20). Genetic maps provide the understanding of evolution, genetic diversity and phylogeny relevant in revealing the locations of QTLs influencing all measurable traits (Paterson, 1995). In sorghum, several linkage maps have been developed. The first genetic map of sorghum was created by Hulbert *et al*. (1990) using 55 $F_2$ plants and maize DNA probes. Ragab *et al*. (1994) constructed a linkage map with 15 linkage groups, with a length of 633 cM and average distance of 8.9 cM. Pereira *et al*. (1994) developed a sorghum linkage map with 10 complete linkage groups using maize and sorghum probes. Subudhi and Nguyen (2000) aligned the 10 linkage sorghum groups using information generated from a RIL population, sorghum and maize probes, as well as cereal anchors from three different linkage maps (Chittenden *et al*., 1994; Ragab *et al*., 1994; Xu *et al*., 1994). Menz *et al*. (2002) constructed a 1713 cM high-density map using 2454 AFLPs, 136 SSRs previously mapped in sorghum, and 203 cDNAs and genomic clones from rice, barley, oat, and maize. Wu and Huang (2006) developed a sorghum genetic map using SSR markers. The number of linkage groups in sorghum can be visualized by FISH markers (Kim *et al*., 2005), where the designation of the chromosomes can be LG for linkage groups (Zhin-Ben *et al*., 2006) or SB for *Sorghum bicolor* (Kim *et al*., 2005) followed by either A, B,C, D or 1, 2, 3, 4. The arrangements of the 10 LGs or SBs, depend on chromosome length, ranging from 5.11 to 2.94 µm with an estimated DNA content ranging from 119.3 to 68.6 Mbp (Kim *et al*., 2005). Gloria *et al*. (2008) developed a molecular map for the bloom cuticle locus and fill the gap in sorghum chromosome 10. Sorghum consensus map developed by integrating the colinearity of six independent sorghum component maps into a single reference resource that contains SSRs, AFLPs, and high-throughput Diversity Array Technology (DArT) markers. This consensus map spanning 1603.5 cM, consists of 2029 unique loci (1190 DArT loci and 839 other loci) (Mace *et al*., 2009). An expanded consensus map was developed through the addition of 1243 markers comprising additional 888 DArTs, 229 SSRs, 81 RFLPs, and the position of 45 genes were established on the map (Mace and Jordan, 2011). The sequencing of the sorghum genome using the accession BTx623 has provided the opportunity for whole-genome sequencing approaches (Paterson *et al*., 2009). Therefore, an ultra-high-quality SNP map was constructed and used for QTL analysis of eight agronomically important traits under two contrasting photoperiods (Zou *et al*., 2012).

### 2.5.3. Principle of QTL mapping

QTL mapping is a statistical association between phenotypes of interest and molecular markers in a segregating population. It is a linkage-based method for QTL detection in the population derived from cross between two parents (Dudley, 1993). The objective of QTL mapping is to identify neutrally inherited markers that are close to the genetic causatives or genes controlling complex quantitative traits (Ross-Ibarra *et al*., 2007). QTL mapping requires population development such as $F_2$ or other generation of segregation, back cross (BC), double haploid (DH), recombinant inbred line (RIL) and near isogenic line (NIL) populations, derived from the genetic hybridization of two parental genotypes with an alternative trait of interest.

It also requires phenotyping of a large number of progenies or lines that are segregating for a trait of interest under different environmental conditions. Finally genotyping of the population using a set of polymorphic DNA markers that differentiate the parental genotypes and segregate among the progenies in a mapping population must be done. The two parental genotypes are screened with markers for polymorphism. The selected polymorphic markers are then used to screen the progenies of the two parents. The genotypic data generated are used to construct the genetic map of the population representing the order and position of the different markers along the chromosomes. Genetic map construction is based on the assessment of the recombination rates between marker loci so that markers along the same chromosome are statistically correlated with phenotypic characteristics of the progenies in the population. QTL detection is based on the association of genotypic and phenotypic scores of the progenies in the population. Therefore, markers that are genetically linked to a QTL influencing the trait of interest will segregate more frequently with trait values. The precision of QTL mapping depends on the genetic background, the size of the mapping population and a number of marker loci used (Ibrokhim *et al*., 2008). QTL mapping provides information on the genetic architecture of complex traits such as location, the number of QTLs and magnitude of their estimated additive, dominance and epistasis effects (Holland, 2007). A number of QTL mapping methods have been developed; they range from the simplest method of single-marker analysis (Sax, 1923) to more sophisticated methods such as interval mapping (Haley and Knott, 1992), multiple regression (Whittaker *et al*., 1996), and composite interval mapping (Zeng, 1994). Software packages for QTL mapping include MAPMAKER/QTL (Lincoln *et al*., 1993), QTL Cartographer (Basten *et al*., 1994), PLABQTL (Utz and Melchinger, 1996), QGene (Nelson, 1997), TASSEL (Buckler, 2007) and R/QTL (Broman and Sen, 2009).

### 2.5.4. Review of QTL in sorghum

Many QTLs studies have been undertaken in sorghum on different traits including grain and panicle traits (Pereira *et al*., 1995; Rami *et al*., 1998; Hart *et al*., 2001; Brown *et al*., 2006; Feltus *et al*., 2006; Murray *et al*., 2008; Srinivas *et al*., 2009; Zou *et al*., 2012; Morris *et al*., 2013; Nagaraja *et al*., 2013). However, the relation between panicle traits and grain yield as well as the branching pattern were not clearly addressed in these studies. Also very few candidate genes involved in sorghum panicle architecture have been identified in these studies. Therefore, the need to extend QTLs studies to these gaps should be of interest. Other QTLs studies were performed in sorghum with focus on stem morphology (Lin *et al*., 1995; Pereira *et al*., 1995; Hart *et al*., 2001; Feltus *et al*., 2006; Brown *et al*., 2008; Murray *et al*., 2008; Shiringani, 2009), leaf morphology (Hart *et al*., 2001; Feltus *et al*., 2006), maturity (Crasta *et al*., 1999; Chantereau *et al*., 2004; Hart *et al*., 2001; Brown *et al*., 2006), stem composition (Murray *et al*., 2008; Ritter *et al*., 2008; Shiringani, 2009), stay-green drought tolerance (Tuinstra *et al*., 1996; Crasta *et al*., 1999; Subudhi and Nguyen, 2000; Tao *et al*., 2000; Kebede *et al*., 2001; Sanchez *et al*., 2002 ; Hausmann *et al*., 2002; Harris *et al*., 2007), fertility restoration (Klein *et al*., 2001; Jordan *et al*., 2010), aluminium tolerance (Magalhaes *et al*., 2004), and biotic stress resistance (Klein *et al*., 2001; Tao *et al*., 2003; Mohan *et al*., 2009; Perumal *et al*., 2009) and photoperiod (Chantereau *et al*., 2004; Murphy *et al*., 2011).

### 2.5.5. QTL mapping for inflorescence traits in cereals

#### 2.5.5.1. Sorghum

Much of the natural variation in inflorescence shape observed are actually due to the cumulative effect of several loci. The study of quantitative trait loci (QTL) is an important field of cereal genetics aimed at yield improvement. Quantitative studies have been energized recently by the advancement of genomic tools. Several QTLs associated with panicle traits such as panicle length, seed branch length, length of sterile portion of seed branch, number of seed branches per panicle, and 100-seed weight were identified by Pereira *et al.* (1995). Rami *et al*. (1998) reported QTLs for panicle length. Hart *et al*. (2001), on the basis of the map positions, mapped the same QTL for panicle length mapped by Pereira *et al*. (1995). Using two sorghum inbred lines with different inflorescences, Brown *et al*. (2006) mapped QTLs for number of primary, secondary and tertiary branches, branch length, and rachis diameter. Srinivas *et al*. (2009) reported QTLs for grain yield, panicle length, panicle weight, seed weight and number of primary branches. The QTLs for number of branches identified by Brown *et al*. (2006) and those of Srinivas *et al*. (2009) were not related. Primary, secondary, and tertiary branching in sorghum are largely under separate genetic control. There is no overlap between QTL detected for primary and secondary branch number (Bommert *et al*., 2005). Morris *et al.* (2013) found in sorghum co-localization between QTLs for panicle length and candidate genes INDETERMINATE 1 (ID1) and ABERRANT PANICLE ORGANIZATION 1 (APO1). Very little information on sorghum panicle branching pattern was addressed in those previous studies. However, many architectural QTLs were detected and this information will be used in the present study to assess the consistency of QTLs for panicle traits in sorghum. The results of this study will hopefully fill the gap by extending the knowledge on the genetic control of the branching pattern in sorghum.

#### 2.5.5.2. Rice

Rice panicle characteristics such as the number of primary and secondary branches influence spikelet number per panicle (Lin *et al*., 1996; Wu *et al*., 1996; Xiao *et al*., 1996). QTL mapping is the first step to dissect Mendelian factors underlying rice panicle architecture (Yano and Sasaki, 1997). Many studies on QTL mapping for spikelet number per panicle (Lin *et al*., 1996; Lu *et al*., 1996; Xiao *et al*., 1996; Zhuang *et al*., 1997; Redona and Mackill, 1998; Yagi *et al*., 2001 and Fujita *et al*., 2012) and for the number of primary branches per panicle (Lin *et al*., 1996; Wu *et al*., 1996; Sasahara *et al*., 1999) have been conducted on rice using mapping populations derived from inter-specific crosses, indica-japonica inter-subspecific crosses and indica-indica crosses. Yamagishi *et al*. (2002), detected four and two QTLs respectively for the number of primary and secondary branches per panicle. Also, they detected one QTL for average number of spikelets on one secondary branch. Yamamoto *et al*. (2007), identified QTLs for five morphological components of panicle architecture including number of spikelets per panicle, number of primary branches per panicle, average number of secondary branches per primary branch, panicle length, and primary branch average length. Guo and Hong. (2010) detected QTLs for the number of primary branches per panicle, number of secondary branches per panicle, secondary branch distribution density, number of spikelets per panicle and spikelet density. The QTLs studies on rice panicle may serve as a guideline in defining architectural traits in sorghum.

#### 2.5.5.3. Maize

Maize tassel architecture is relevant to hybrid seed production and an amenable system for QTL analysis. Maize inbreds vary considerably for tassel branch number, branching pattern and spikelet density. Using maize tassels, Upadyayula *et al*. (2006) detected two QTLs for tassel branch number, five for spikelet pair density on the central spike and two for spikelet pair density on the branches. Mayor (2008) detected QTLs for tassel branch number, tassel length, central spike length and branching zone length. Kamelmanesh *et al*. (2012) detected six QTLs for tassel branch number. Therefore, branching pattern in maize tassel characterization may be transposed onto sorghum panicles.

### 2.5.6. Candidate genes

In sorghum, Brown *et al*. (2006) mapped QTL clusters for plant height, rachis length, and branch length close to the candidate gene *Dw3* and QTL for primary branch number in the region of the ramose 2 (*ra2*) gene. The *Dw3* gene, encodes for a P-glyocoprotein responsible for auxin transport (Multani *et al*., 2003) and ramosa 2 gene regulates the pattern of branching. In maize tassel, ramosa 1 (*ra1*) was mapped closely to a QTL for branch number, thick tassel dwarf1 (*td1*), which is in the same region as the QTL that controls ear weight, tassel branch angle, and spikelet pair density on primary branches, *ra2* which maps to the region with a QTL for kernel number per row, and fasciated ear2 (*fea2*), which localizes to a region with a QTL for branch number (Upadyayula *et al*., 2006). Ramosa 2 (*ra2*) expression pattern is conserved in sorghum, rice, maize and barley suggesting its important role in grass inflorescence architecture (Bortiri *et al*., 2006). In rice, FRIZZY PANICLE (*FZP)* acts as a positive regulator of floral meristem identity to suppress the formation of axillary meristems of rice spikelet. The *LAX* gene encodes a putative transcriptional regulator that contains a bHLH domain, and has been identified as a regulator controlling the rachis-branch meristem initiation and/or maintenance during rice reproductive development (Komatsu *et al*., 2003a). The rice cytokinin oxidase, *OsCKX2*, regulates the development of inflorescence meristems by affecting the cytokinin contents in the rice vascular system of developing culms, suggesting the important role of cytokinin in forming the inflorescence architecture (Ashikari *et al*., 2005). Together, *RA1*, *RA2*, and *RA3* coordinate to regulate meristem identity and determinacy in the maize inflorescence. Therefore, inflorescence architecture genes that map in the vicinity of QTL that harbor beneficial traits are of significant importance in crop improvement programs. Most of the studies on genes function involved in inflorescence architecture were on rice and maize. Therefore, genes playing important roles in rice or maize inflorescence architecture may be used in sorghum as candidate genes to access their presence and suggest a possible function in sorghum.

# CHAPTER THREE

## 3.0. PHENOTYPING FOR SORGHUM PANICLE ARCHITECTURE AND YIELD IN TIANDOUGOU/LATA-3 $F_4$ POPULATION

### 3.1. Introduction

Sorghum **(***Sorghum bicolor*) is an important food and fodder crop. It is a traditional staple cereal crop in Mali. Variation in sorghum panicle architecture can be used to establish linkages with grain yield potential, and indirect selection may be targeted if panicle traits are found to be associated with grain yield. Therefore, comprehensive knowledge of panicle architecture diversity and genetic relationships between panicle traits and grain yield are invaluable aid in crop improvement strategies for current and future breeding programs. According to Grenieret *et al*. (2001), measurement of morphological variation is the most easily obtained indicator of genetic diversity. Morphological characters are ecologically adaptive and good indicators of genetic variation, local differentiation, or ecotypes and can be used to classify the phenotypic diversity. Phenotyping of sorghum panicle architecture traits involves a methodological dissection of the panicle into its structural units such as branching pattern, rachis elongation and grain distribution. This should provide better understanding and elucidate relationships between panicle architecture and grain yield. Phenotyping sorghum panicle architecture also examine the heritability, correlation and principal component contribution traits into panicle architecture variability. Heritability is a useful quantitative parameter, which considers the role of heredity and environment in determining the expression of a character (Kukadia *et al*., 1983). Grain yield is a complex trait, dependent on many characters. Yield potential including a desirable combination of traits has always been the major objective of sorghum breeding programs (El-Din *et al*., 2012). The ability of the breeder to identify superior genotypes within segregating populations is the key to the success in any plant breeding program. Trait heritability estimation is a first step towards assessing the amount of genetic variation present in a breeding population. Regardless of the type of heritability estimate, heritability broadly defined, is the proportion of observable field variation that is due to genetic factors (Nyquist, 1991). Several studies have been conducted on sorghum populations to estimate heritability, as well as phenotypic and genotypic correlations among traits (Sanchez-Gomez, 2002; Tariq *et al*., 2007; Warkad *et al*., 2008; Ayala, 2011, Ali *et al*., 2012; Nagaraja *et al*., 2013). Discrepancies in heritability estimates for grain yield may be due to factors such as different estimation methodologies, type of population (wide crosses), diversity of environments, and generation of evaluation. Broad-sense heritability for some panicle architecture traits such as number of primary and secondary branches, rachis diameter, and primary branch length were estimated in different sorghum populations (Pereira *et al*., 1995; Brown *et al*., 2006 and Srinivas *et al*., 2009). Heritability in certain cases is similar to repeatability assessed by multiple measurements of the trait on each individual provided by temporal and spatial repetition (Falconer and Mackay., 1996). Genetic improvement for quantitative traits depends upon the nature and amount of variability present in the genetic stock and the extent to which the desirable traits are heritable (Chavan *et al*., 2010). In addition to heritability, the knowledge of genetic variability existing among different parameters contributing to yield is also an important criterion for yield enhancement. Although sorghum in Mali possesses a wide range of genetic variability, the improvement of landraces for yielding ability has not been substantial. Hybridization has boosted yield levels of sorghum, besides improving other characters like resistance to biotic and abiotic stresses.

Correlation analysis provides information on the interrelationships of important plant characters and leads to a directional model for direct and/or indirect improvement in grain yield (Khan *et al*., 2004). Principal component analysis (PCA) is used to reveal the pattern of character variation among individual accessions in a population. It involves a procedure that transforms total variation of original characters into a smaller number of uncorrelated new characters, so called principal components (Johnson, 1998). Phenotyping sorghum panicle architecture traits become more useful to address the variability, heritability, correlation among panicle traits to grain yield. Therefore, the objectives of this research were 1.) to assess genetic variability in sorghum panicle architecture traits 2.) to estimate broad sense heritability of panicle architecture traits in $F_4$ families, 3.) to determine correlations among panicle architecture traits in relation to grain yield and 4.) to estimate grain yield using highly heritable and easily measured panicle architecture traits.

## 3.2. Materials and Methods

Phenotyping of sorghum panicle architecture traits was conducted at the research station of Sotuba (12°39’47’’North 7°54’50’’ West), located at Bamako, Mali in 2011 and 2012. Two sowing dates corresponding to different conditions of photoperiod were used in 2011 to phenotype and characterize the effect of delaying sowing date on panicle architecture and grain yield since sorghum reacts to photoperiod. The first sowing date coded (SB1) was performed at the beginning of the rainy season (mid- June) and the second sowing date coded (SB2) was performed 26 days after the first sowing at mid-July.

In 2012, one sowing date was performed at the same sowing date as the first sowing date of 2011. Therefore, because of this similarity with SB1, the first sowing date of 2012 was coded (SB1’).

### 3.2.1. Plant Materials

A bi-parental population was obtained from the cross between two contrasting parents (Tiandougou and Lata-3) for panicle architecture traits. The female parent, Tiandougou, is a short caudatum-guinea type from the IER breeding programs (Plate 3.1a). It is a dual-purpose variety with compact panicle and high yield. Lata-3 (Plate 3.1b) used as a male is a Guinea type, with open and drooping primary branches panicles also high yielding and intermediate height from ICRISAT, Mali with open and drooping primary branches panicles also high yielding and intermediate height. The population was advanced to the $F_3$ generation by single seed descent. Each single $F_3$ plant was selfed and the corresponding $F_4$ panicle harvested. In order to constitute a stock of seeds representative of each the original $F_3$ plant, 10 $F_4$ plants were grown for each $F_4$ family, self-pollinated and $F_5$ seeds harvested as a bulk ($F_{3:5}$). This multiplication was conducted in 2010 during the rainy season. A mapping population composed of 401 $F_4$ families was used for phenotyping sorghum panicle architecture traits and grain yield.

*Plate 3.1a: Compact panicle of the Female parent (Tiandougou)*

*Plate 3.1b: Open and drooping primary branches panicle of the Male parent panicle (Lata-3)*

### 3.2.2. Experimental Design

The experimental design was an augmented design with 29 blocks and 16 entries composed of 14 test genotypes and 2 checks per block. A total of 464 entries included 404 unique $F_4$ families and 60 checks that were composed of 30 of each parent Tiandougou and Lata-3. The $F_4$ families were randomized and the two parents (checks) were maintained in each block. Six grams of seed for each of the 464 entries were weighed and treated with Calthio C against pest before planting. The seedbed was plowed and compound fertilizer (DAP) applied at 150 kg/ha before ridges formation. Each entry was sown on two rows with 10 hills per row. Spacing between rows was 0.75 m and between hills on the row was 0.40 m. Standard cultural practices, including fertilization, weed and insect control, were followed at each sowing date to minimize exogenous variability that would otherwise mask variability due to genetic differences of the germplasm evaluated.

### 3.2.3. Data collection

One representative panicle for each of the 404 $F_4$ families and for the 60 checks were harvested, sun dried and their traits measured. Panicle architecture complexity was disaggregated into geometrical and topological variables adapted from the model used by Segura *et al.* (2006).

In addition, traits related to grain yield such as number of grains were collected. For the strategy of phenotyping, three organization levels were considered (Figure 3.1): first, different types of axes (peduncle, panicle, rachis, primary and secondary branches and internodes); second, different places in the panicle (secondary, median and penultimate nodes and longest primary branch) and third, different types of measured variables (geometry, topology and grain yield and its components). Geometrical variables were based on the length and the diameter of different axes. Topological variables concerned number and position of internodes and branches as well as branching density. Grain yield components variables mainly concerned weight and number of grains. These measured variables were used to calculate other variables as close as possible to biological factors such as rachis conicity, slenderness and volume; primary and secondary branch density and number of grains per panicle.

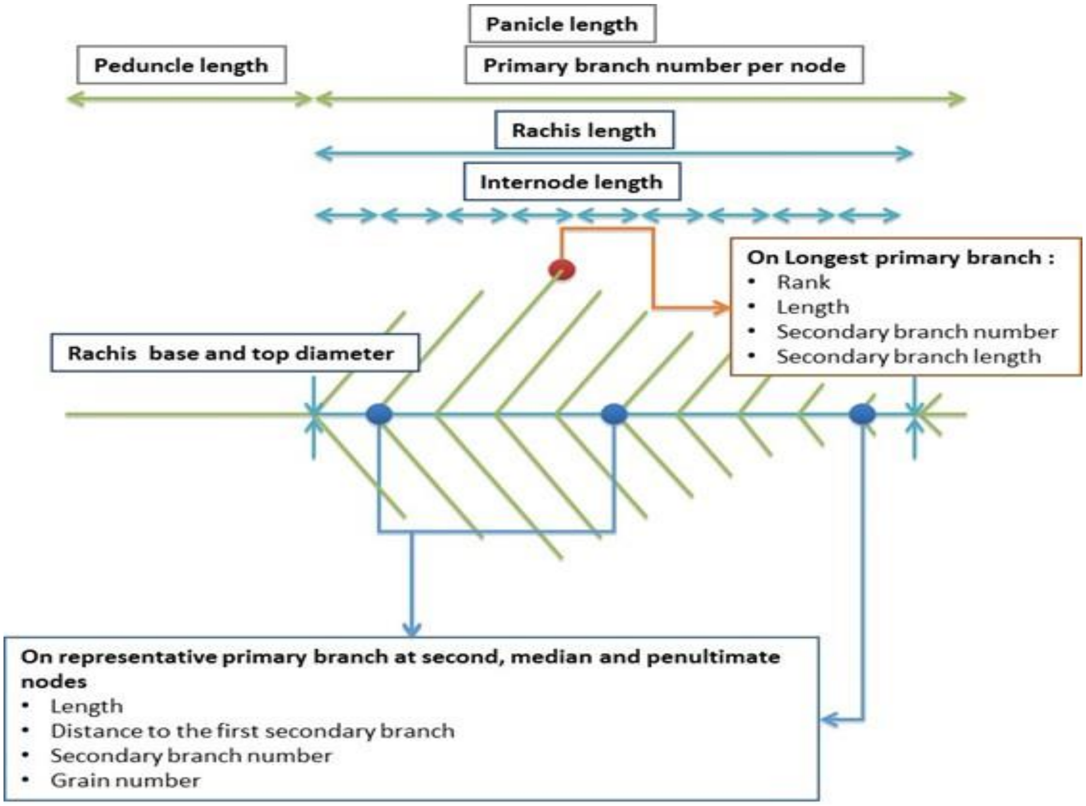


*Figure 3.1: Methodological approach for measuring panicle architecture traits*

A total of 48 panicle architecture traits were evaluated during the first and second phenotyping seasons in 2011 (SB1 and SB2) and 2012 (SB1'). The data collected were structured according to the model used by Segura *et al*. (2006). Geometrical variables that account for 24 variables composed of 16 measured and eight calculated variables. Topological variables were 16 with nine calculated traits. Grain yield and its components account for eight including two calculated variables (Table 3.1).

Table 3.1: Data on sorghum panicle architecture variables with code collected in 2011 (SB1 and SB2) and 2012 (SB1') at Sotuba research station in Mali (modified Segura *et al*., 2006)

| **Variables** | | **Formula** | **SB1** | **SB2** | **SB1'** |
|---|---|---|---|---|---|
| **Geometry** | | | | | |
| Plant height (mm) | PH | | x | x | x |
| Panicle length (mm) | PA_L | | x | x | x |
| Peduncle length (mm) | PE_L | | x | x | x |
| Rachis length (mm) | RA_L | | x | x | x |
| Mean internode length (mm) | IN_L | RA_L/PA_IN_N | x | x | x |
| Maximum internode length (mm) | IN_L_max | | x | x | x |
| Rachis base diameter (at first internode) (mm) | RA_B_Dia | | x | x | x |
| Rachis top diameter (at last internode) (mm) | RA_T_Dia | | x | x | x |
| Rachis mean diameter (mm) | RA_Dia | (RA_B_Dia + RA_T_Dia) / 2 | x | x | x |
| Rachis conicity | RA_coni | (RA_B_Dia - RA_T_Dia) / RA_L | x | x | x |
| Rachis slenderness | RA_slend | RA_L / RA_Dia | x | x | x |
| Rachis volume ($mm^3$) | RA_Vol | RA_L(B_area*+T_area*)/2 | x | x | x |
| Primary branch length at second node (mm) | PB_L_SN | | x | x | x |
| Primary branch length at median node (mm) | PB_L_MN | | x | x | x |
| Primary branch length at penultimate node (mm) | PB_L_PN | | x | x | x |
| Length of the longest Primary Branch (mm) | PB_L_max | | x | x | x |
| Secondary branch length (mm) | SB_L | | x | x | |
| Length of the longest secondary branch (mm) | SB_L_max | | x | x | |
| Distance to the first Secondary Branch at second node (mm) | SB_DF_SN | | x | x | x |
| Distance to the first Secondary Branch at median node (mm) | SB_DF_MN | | x | x | x |
| Distance to the first Secondary Branch at penultimate node (mm) | SB_DF_PN | | x | x | x |
| Primary branch branching zone at second node (mm) | PB_BZ_SN | PB_L_SN - SB_DF_SN | x | x | x |
| Primary branch branching zone at median node (mm) | PB_BZ_MN | PB_L_MN - SB_DF_MN | x | x | x |
| Primary branch branching zone at penultimate node (mm) | PB_BZ_PN | PB_L_PN - SB_DF_PN | x | x | x |
| **Topology** | | | | | |
| Number of internodes per panicle | PA_IN_N | | x | x | x |
| Maximum number of primary branch per node | Nb_PB_max | | x | x | x |
| Number of primary branches per panicle | PA_NPB | | x | x | x |
| Average number of primary branches per node | av_NPB | PA_NPB / PA_IN_N | x | x | x |
| Primary branch density per panicle | PB_Dens | PA_NPB / RA_L | x | x | x |
| Number of secondary branches on the longest primary branch | Nb_SB_LPB | | x | x | |
| Number of Secondary Branches at second node | Nb_SB_SN | | x | x | x |
| Number of Secondary Branches at median node | Nb_SB_MN | | x | x | x |
| Number of Secondary Branches at penultimate node | Nb_SB_PN | | x | x | x |
| Secondary branches density at second node | SB_Dens_SN | Nb_SB_SN / PB_BZ_SN | x | x | x |
| Secondary branches density at median node | SB_Dens_MN | Nb_SB_MN / PB_BZ_MN | x | x | x |
| Secondary branches density at penultimate node | SB_Dens_PN | Nb_SB_PN / PB_BZ_PN | x | x | x |
| Relative position of the longest internode on the rachis | Rel_Pos_IN_L_max | Pos_IN_L_max / PA_IN_N | x | x | x |
| Relative position of the node with the maximum number of primary branches | Rel_Pos_NPB_max | Pos_NPB_max / PA_IN_N | x | x | x |
| Relative position of the longest primary branch | Rel_Pos_PB_L_max | Pos_PB_L_max / PA_IN_N | x | x | x |
| Relative position of the longest secondary branch on the longest primary branch | Rel_Pos_SB_L_max | Pos_SB_L_max / Nb_SB_LPB | x | x | |
| **Yield** | | | | | |
| Panicle grain yield (g) | PA_GY | | x | x | x |
| Panicle weight (g) | PA_W | | x | x | x |
| Panicle harvest index | PA_HI | PA_GY / PA_W | x | x | x |
| Thousand Grain Weigth | TGW | | x | | x |
| Number of grains per panicle | NG_PA | PA_GY / (TGW/1000) | x | | x |
| Number of grains at second node | NG_SN | | x | x | x |
| Number of grains at median node | NG_MN | | x | x | x |
| Number of grains at penultimate node | NG_PN | | x | x | x |

Formula are detailed for calculated variables

* B_area = $\pi$ (RA_B_Dia / 2)²; T_area = $\pi$ (RA_T_Dia / 2)²

### 3.2.4. Data analysis

The objectives of these analyze were to determine variance components due to genotype and blocks and to test the significance of the different panicle traits in the three sowing dates in order to generate adjusted means for each trait to be used for QTLs detection. Patterson and Thompson, (1971) proposed the mixed model, REML (Residual Maximum Likelihood) to perform analysis of variance for incomplete block design such as augmented design. The principle relies on random and fixed term. Therefore, variance component analysis was used in Genstat software to establish statistical differences between the $F_4$ families. Two models were automatically fitted. The first model was used to estimate variance components in which the $F_4$ families were fitted as random term (model 1). A second model was fitted in which the $F_4$ families were used as fixed terms (model 2). This was to produce the adjusted means for each phenotypic trait and to test the significance of differences among $F_4$ families underlying on the statistic proposed by Wald (1943) and F statistic for fixed effects. The Student T Test was performed to compare the mean of the parents to the means of the $F_4$ progenies using the software MINITAB version 16.

The linear model for augmented design was as follows:

$$y_{ij} = \mu + \alpha_i + \beta_j + \varphi_k + \beta\varphi_{jk} + \varepsilon_{ijk}$$

Where

Yij is the phenotypic value

$\mu$ is the population mean;

$\alpha_i$ is the effect due to the *i*-th genotype test;

$\beta_j$ is the effect due to the *j*-th genotype check;

$\varphi_k$ is the effect due to the *k*-th block;

$\beta\varphi_{jk}$ is the effect due to the interaction of the *i*-th genotype check with the *j*-th block;

$\varepsilon_{ijk}$ is the error associated with the *ij*-th individual observations.

### 3.2.5. Correlation, Principal Component Analysis and ANOVA for panicle traits

The adjusted means of data for the $F_4$ families was used to perform all other analysis such as Pearson correlation to determine association among traits, and Principal Component Analysis (PCA) to evaluate contribution of traits.

Pearson correlation with significant test was computed on the phenotypic and genotypic data for all the panicle architecture traits and individually on each subgroup of traits such as geometry, topology and yield to generate the different correlation matrices. PCA based on the correlation coefficient was computed first for all traits and for geometry, topology and yield. Shapiro test of normality (Shapiro and Wilk, 1965) prior to the linear model of ANOVA (type III) was conducted on the phenotypic data.

The linear model is: $Y_{ij} = \mu + G_i + D_j$

Where:

$Yij$ = phenotypic value

$\mu$ = population mean

$G_i$ = genotypic factor

$D_j$ = sowing date factor

### 3.2.6. Calculation of Broad sense heritability

According to Knapp *et al*. (1985), broad-sense heritability of phenotypic means can, in each case, be expressed as follows: $H^2 = 1 - (1/F)$

Where F is the Fisher statistic for the genotypic effect of the considered model in the ANOVA.

### 3.2.7. Construction of linear model and BLUP extraction

A mixed model was fitted with genotype as random effects and sowing dates as fixed effects, the residual was analyzed and the Shapiro normality test applied prior to the Best Linear Unbiased Predictor (BLUP) extraction for each trait.

### 3.2.8. Analysis on BLUP

Pearson correlation with a significant test was computed on the BLUP data to determine the genotypic correlation matrix using the Rcorr function of the Hmisc R package. Principal Component Analysis (PCA) was computed using the PCA function of the FactoMineR R package.

### 3.2.9. Contribution of panicle traits to panicle grain yield

A multivariate regression was computed to establish the contribution of panicle architecture traits to the grain yield according to a linear model.

## 3.3. Results

### 3.3.2. Experimental design data analysis

REML component analysis revealed significant differences between the 401 $F_4$ families for a great number of traits (Table 3.2).

Table 3.2: F probability (Fpr) of REML analysis on 401 $F_4$ families across three sowing dates at Sotuba research station, Mali, 2011 and 2012

| Panicles traits | FprSB1 | FprSB2 | FprSB1' |
|---|---|---|---|
| Plant height (cm) | < 0.001 | < 0.001 | <0.001 |
| Peduncle length (cm) | < 0.001 | < 0.001 | <0.001 |
| Panicle length (cm) | < 0.001 | < 0.001 | <0.001 |
| Rachis length | < 0.001 | < 0.001 | <0.001 |
| Length of the longest Primary Branch | 0.062 | 0.021* | 0.002** |
| Average primary branch length | 0.007** | < 0.001 | 0.002** |
| Length of Primary Branch at second node | 0.153 | 0.001** | 0.051 |
| Length of Primary Branch at median node | < 0.001 | 0.497 | 0.056 |
| Length of Primary Branch at penultimate node | < 0.001 | < 0.001 | <0.001 |
| Average internode length | 0.004** | < 0.001 | 0.020* |
| Rachis base Diameter | 0.999 | 0.131 | <0.001 |
| Rachis top Diameter | 0.210 | 0.563 | <0.001 |
| Number of Primary Branches per panicle | < 0.001 | < 0.001 | <0.001 |
| Average primary branches per node | < 0.001 | < 0.001 | <0.001 |
| Number of internodes per panicle | 0.005** | 0.005** | <0.001 |
| Number of Secondary Branches at second node | 0.483 | 0.014* | 0.099 |
| Number of Secondary Branches at median node | 0.050 | 0.002** | 0.077 |
| Number of Secondary Branches at penultimate node | < 0.001 | < 0.001 | 0.009** |
| Average secondary branch per primary branch | 0.091 | 0.026* | 0.049* |
| Number of grains at second node | < 0.001 | 0.012* | <0.001 |
| Number of grains at median node | < 0.001 | < 0.001 | 0.046* |
| Number of grains at penultimate node | < 0.001 | < 0.001 | <0.001 |
| Average number of grains per primary branch | < 0.001 | < 0.001 | <0.001 |
| Grain yield per panicle (g) | 0.047* | 0.147 | 0.005** |
| Panicle weight (g) | 0.122 | 0.356 | 0.017* |
| Panicle Harvest index | 0.081 | 0.053 | <0.001 |

NB: Fpr = F probability of REML analysis; SB1 = first sowing date of 2011; SB2 = second sowing date of 2011; SB1' = first sowing date of 2012; <0.001 = highly significant at P<0.001; ** = highly significant at P< 0.01; * = significant at P< 0.05.

In the second sowing date of 2011, grain yield per panicle did not show significant differences among the progenies. However, number of secondary branches at second and median nodes as well as the length of the primary branches at the second node show significant differences among the progenies compare to the first sowing dates in 2011 and 2012.

### 3.3.3. Performance of the $F_4$ families and their parents

The Student T Test revealed that grain yield per panicle and length of primary branch at median node were reduced compared to the two parents in the second sowing date in 2011. The $F_4$ families performed better than the parent Lata-3 in all the sowing dates for number of grains and the length of the primary branch at the penultimate node. Highly significant differences ($P < 0.001$) between the mean of the parent Tiandougou and the mean of the $F_4$ families were observed for the length of the longest primary branch, number of secondary and grain on primary branch at second node as well as rachis length across the different sowing dates. Peduncle length, number of internode and rachis top diameter were reduced in the $F_4$ families compared to the parent Tiandougou in the second sowing date of 2011 (Table 3.3).

Table 3.3: Mean comparison of the parents and $F_4$ progenies, T test probability of the parents Lata-3 and Tiandougou vs progenies across the three sowing dates at Sotuba research station, Mali

| Traits | Mean $F_4$ | PSB1 Lata | PSB2 Lata | PSB1' Lata | PSB1 Tiand | PSB2 Tiand | PSB1' Tiand |
|---|---|---|---|---|---|---|---|
| Plant height (cm) | 172.4 | 1 | 1 | 0.836 | 0.000 | 0.000 | 0.138 |
| Peduncle length (cm) | 37.0 | 1 | 1 | 1 | 0.000 | 0.086 | 0.000 |
| Panicle length (cm) | 33.2 | 1 | 1 | 1 | 0.158 | 0.000 | 0.000 |
| Rachis length | 30.2 | 1 | 1 | 1 | 0.000 | 0.000 | 0.000 |
| Length of the longest Primary Branch | 10.0 | 1 | 1 | 1 | 0.000 | 0.000 | 0.000 |
| Length of Primary Branch at second node | 8.5 | 0.000 | 1 | 1 | 0.14 | 0.000 | 0.000 |
| Length of Primary Branch at median node | 7.8 | 1 | 0.000 | 1 | 1 | 0.000 | 1 |
| Length of Primary Branch at penultimate node | 3.8 | 0.000 | 0.000 | 0.000 | 0.376 | 1 | 1 |
| Rachis base Diameter | 7.1 | 1 | 1 | 0.000 | 1 | 0.018 | 1 |
| Rachis top Diameter | 0.8 | 0.001 | 0.675 | 0.197 | 1 | 0.000 | 0.68 |
| Number of internode per panicle | 15.5 | 1 | 1 | 1 | 0.000 | 1 | 0.000 |
| Number of Primary Branches per panicle | 100.2 | 0.943 | 0.000 | 0.994 | 0.537 | 0.989 | 0.000 |
| Number of Secondary Branches at second node | 7.9 | 1 | 1 | 0.955 | 0.000 | 0.833 | 0.000 |
| Number of Secondary Branches at median node | 5.8 | 0.026 | 0.000 | 1 | 0.026 | 0.000 | 0.000 |
| Secondary Branches at penultimate node | 2.7 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 1 |
| Number of grain at second node | 66.5 | 0.000 | 1 | 0.000 | 0.000 | 0.000 | 0.000 |
| Number of grain at median node | 37.7 | 0.000 | 0.000 | 1 | 0.928 | 0.529 | 1 |
| Number of grain at penultimate node | 9.9 | 0.000 | 0.000 | 0.000 | 1 | 1 | 1 |
| Grain yield per panicle (g) | 69.0 | 1 | 0.039 | 1 | 1 | 0.000 | 1 |
| Panicle weight (g) | 92.2 | 1 | 0.000 | 1 | 1 | 1 | 0.743 |
| Panicle Harvest index | 0.8 | 1 | 1 | 0.000 | 0.000 | 0.000 | 0.000 |

PSB1 Lata = T test probability for Lata-3 vs progenies in the first sowing date in 2011; PSB2 Lata = T test probability for Lata-3 vs progenies in the second sowing date in 2011; PSB1'Lata = T test probability for Lata-3 vs progenies in the first sowing date in 2012; PSB1 Tiand = T test probability for Tiandougou vs progenies in the first sowing date in 2011; PSB2 Tiand = T test probability for Tiandougou vs progenies in the second sowing date in 2011; PSB1'Tiand = T test probability for Tiandougou vs progenies in the first sowing date in 2012.

### 3.3.4. Analysis of Variance (ANOVA)

Analysis of variance showed significant differences between the genotypes and between the sowing dates for most of the sorghum panicle architecture and grain yield related traits (Table 3.4).

Table 3.4: ANOVA for sorghum panicle traits and grain yield and its components

| Traits | Factors | Sum Sq | Df | F value | Pr(>F) | Significance |
|---|---|---|---|---|---|---|
| PH | Genotype | 130295324 | 400 | 10.696 | < 2.20E-16 | *** |
| | Date | 29257835 | 2 | 480.37 | < 2.20E-16 | *** |
| PA_L | Genotype | 1312511 | 400 | 3.4362 | < 2.20E-16 | *** |
| | Date | 85253 | 2 | 44.638 | < 2.20E-16 | *** |
| IN_L | Genotype | 8615 | 400 | 2.3151 | < 2.20E-16 | *** |
| | Date | 1348.3 | 2 | 72.467 | < 2.20E-16 | *** |
| PB_L_SN | Genotype | 199210 | 400 | 2.1354 | < 2.20E-16 | *** |
| | Date | 9822 | 2 | 21.057 | 1.232E-09 | *** |
| PB_L_MN | Genotype | 131922 | 400 | 1.7239 | 6.205E-11 | *** |
| | Date | 5808 | 2 | 15.18 | 3.397E-07 | *** |
| PB_L_PN | Genotype | 122931 | 400 | 1.6182 | 6.994E-09 | *** |
| | Date | 10378 | 2 | 27.321 | 3.374E-12 | *** |
| SB_L | Genotype | 10257.6 | 400 | 1.7033 | 6.481E-08 | *** |
| | Date | 12.5 | 1 | 0.8301 | 0.3628 | not |
| SB_DF_SN | Genotype | 23831 | 400 | 1.453 | 5.68E-06 | *** |
| | Date | 238 | 2 | 2.8976 | 0.05575 | not |
| SB_DF_MN | Genotype | 21548.4 | 400 | 1.5094 | 6.386E-07 | *** |
| | Date | 59.2 | 2 | 0.8293 | 0.4368 | not |
| SB_DF_PN | Genotype | 10598.8 | 400 | 1.1519 | 0.049613 | * |
| | Date | 285.5 | 2 | 6.2059 | 0.002118 | ** |
| RA_B_Dia | Genotype | 844.76 | 400 | 2.132 | < 2.20E-16 | *** |
| | Date | 60.52 | 2 | 30.551 | 1.664E-13 | *** |
| RA_T_Dia | Genotype | 49.107 | 400 | 1.0288 | 0.3681 | not |
| | Date | 9.298 | 2 | 38.96 | < 2.20E-16 | *** |
| PA_IN_N | Genotype | 3898.3 | 400 | 2.5296 | < 2.20E-16 | *** |
| | Date | 24.1 | 2 | 3.1329 | 0.04413 | * |
| PA_NPB | Genotype | 698771 | 400 | 5.0133 | < 2.20E-16 | *** |
| | Date | 9822 | 2 | 14.093 | 9.696E-07 | *** |
| Nb_SB_MN | Genotype | 759.64 | 400 | 1.1226 | 0.08864 | not |
| | Date | 439.9 | 2 | 130.01 | < 2.20E-16 | *** |
| PA_GY | Genotype | 290136 | 400 | 1.8368 | 3.071E-13 | *** |
| | Date | 17225 | 2 | 21.81 | 6.037E-10 | *** |
| PA_W | Genotype | 481772 | 400 | 1.8352 | 3.364E-13 | *** |
| | Date | 72990 | 2 | 55.608 | < 2.20E-16 | *** |
| TGW | Genotype | 4235.3 | 400 | 1.6271 | 8.026E-07 | *** |
| | Date | 611.4 | 1 | 93.957 | < 2.20E-16 | *** |
| NG_PA | Genotype | 486873026 | 400 | 1.6671 | 2.446E-07 | *** |
| | Date | 1073313 | 1 | 1.47 | 0.2261 | not |

NB: Sum Sq = Sum of square; Df = degree of freedom; Pr(>F) = probability greater than F

In general, traits at the top of the panicle were not heritable; therefore, no need to phenotype them. The number of secondary branches at the basal part of the panicle appeared to be just informative. The traits secondary branch length, distance to the first secondary branch at second and median nodes, primary branch density and number of grains per panicle were not affected by the sowing date. Variables such as secondary branch zone at penultimate node and panicle harvest index did not appear to be under genetic control.

### 3.3.5. Broad sense heritability estimates

The Broad-sense heritability estimates ranged from 0.03 to 90.7 % (Figure 3.2). High heritability estimates were observed for the characters plant height (90.7 %), number of primary branches per panicle (80.1 %), primary branch density (80.5 %), peduncle length (79.9 %), panicle length (70.9%) and number of nodes per panicle (60.5%).

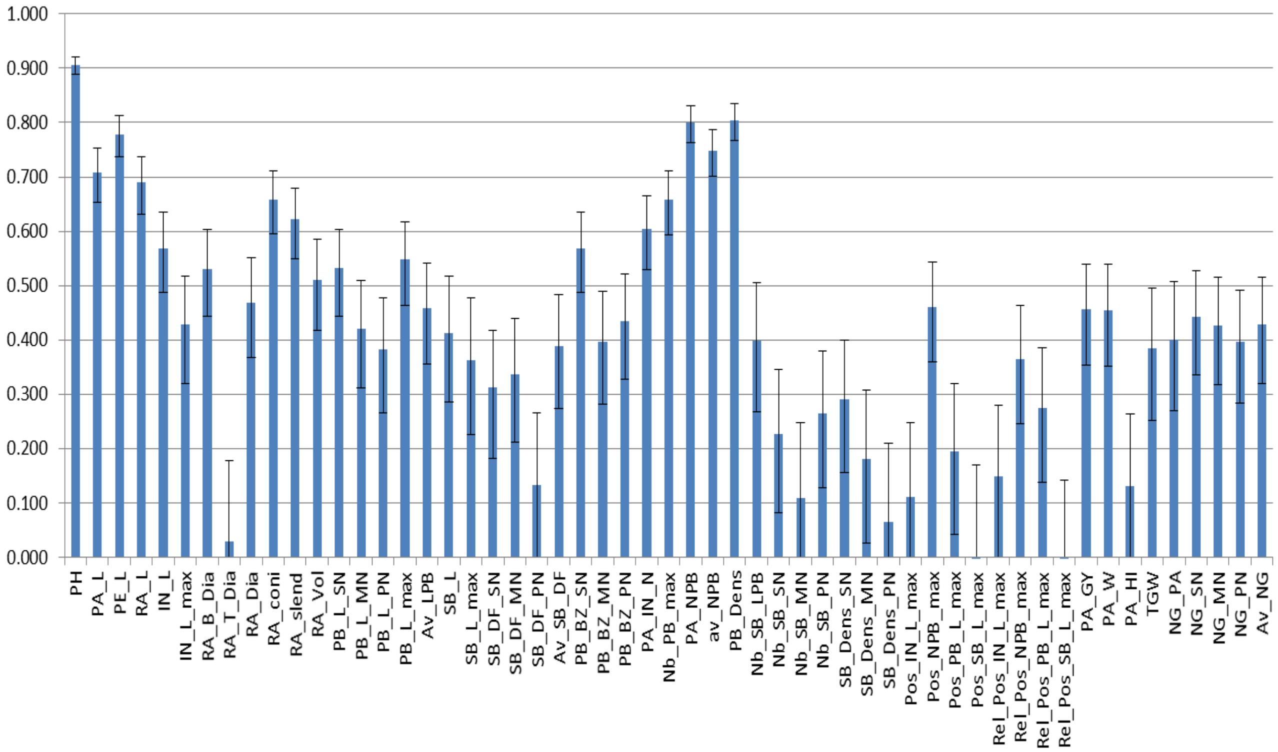


*Figure 3.2: Broad sense heritability estimates for panicle architecture traits and grain yield*

Moderate heritability estimates were exhibited for the traits: internode length (56.8%), rachis base diameter (53.1%), grain yield per panicle (45.6 %), panicle weight (45.5%), average number of grains per primary branch (42.8%), secondary branch length (41.3%), number of grains per panicle (40.0%) and 1000 grain weight (38.5%), whereas moderate to low heritability estimates were recorded for secondary branching variables such as number and position at median and penultimate nodes (22.7%). Very low heritability estimates were noted for secondary branch density at the penultimate node, distance to the first secondary branch at the penultimate node, panicle harvest index and rachis top diameter.

A gradient of genetic variability was observed for the length of  the different types of axes such as stem, peduncle, panicle, rachis, primary and secondary branches at different locations in the panicle like the rachis, internode, second, median and penultimate nodes (Fig 3.3). The same tendency was observed for the number of branches where heritability estimates decreased progressively for the following traits: number of primary branches per panicle, maximum number of primary branches per node, number of secondary branches on the longest primary branches and number of primary and secondary branches at second, median and penultimate nodes. Therefore, moderate heritability estimated at median node and low heritability estimated at penultimate node.

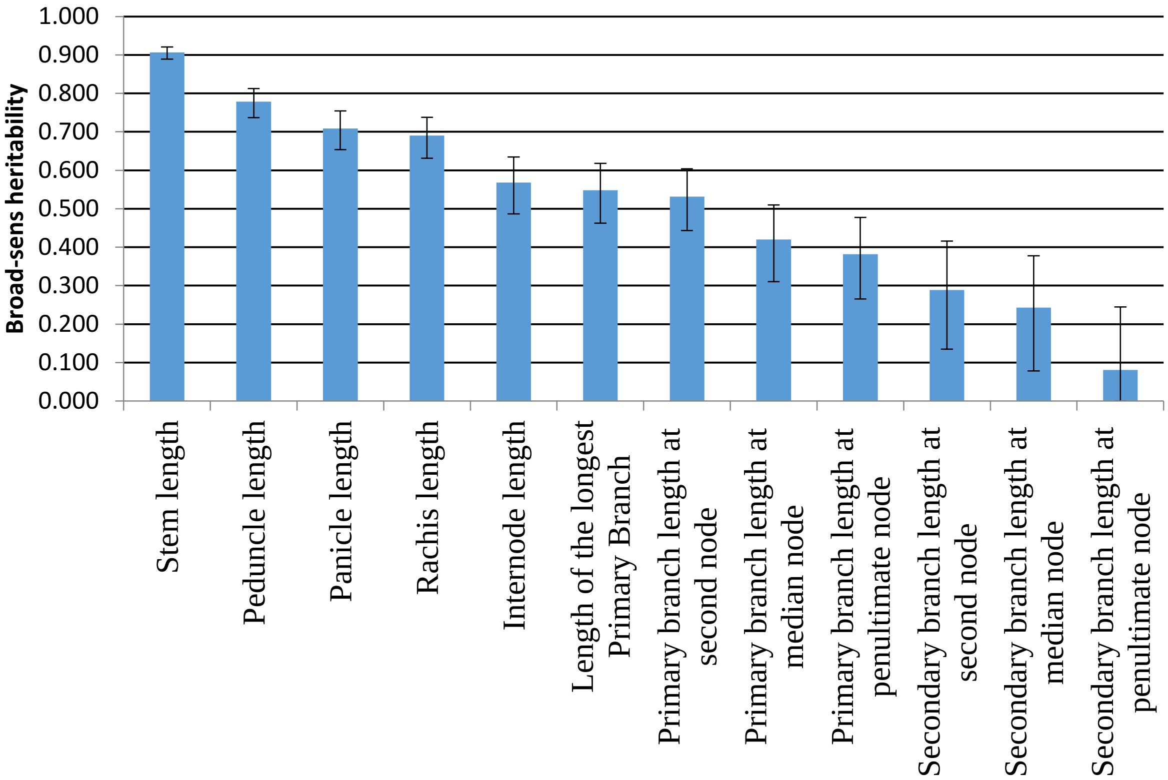


*Figure 3.3: Gradient in broad sense heritability for length of panicle traits*

### 3.3.6. Principal Component Analysis

Three dimensional (3D) observations of selected sorghum panicle architecture variables and grain yield per panicle indicated the contribution of each dimension. According to Sneath and Sokal, (1973) eigenvalues > 1 were considered significant, whereas Hair Jr *et al.* (1998) suggested that component loadings greater than 0.30 were considered to be meaningful. Therefore three axes with eigenvalues greater than 1 were considered in this study. The first dimension explained 29.83% (Figure 3.4)

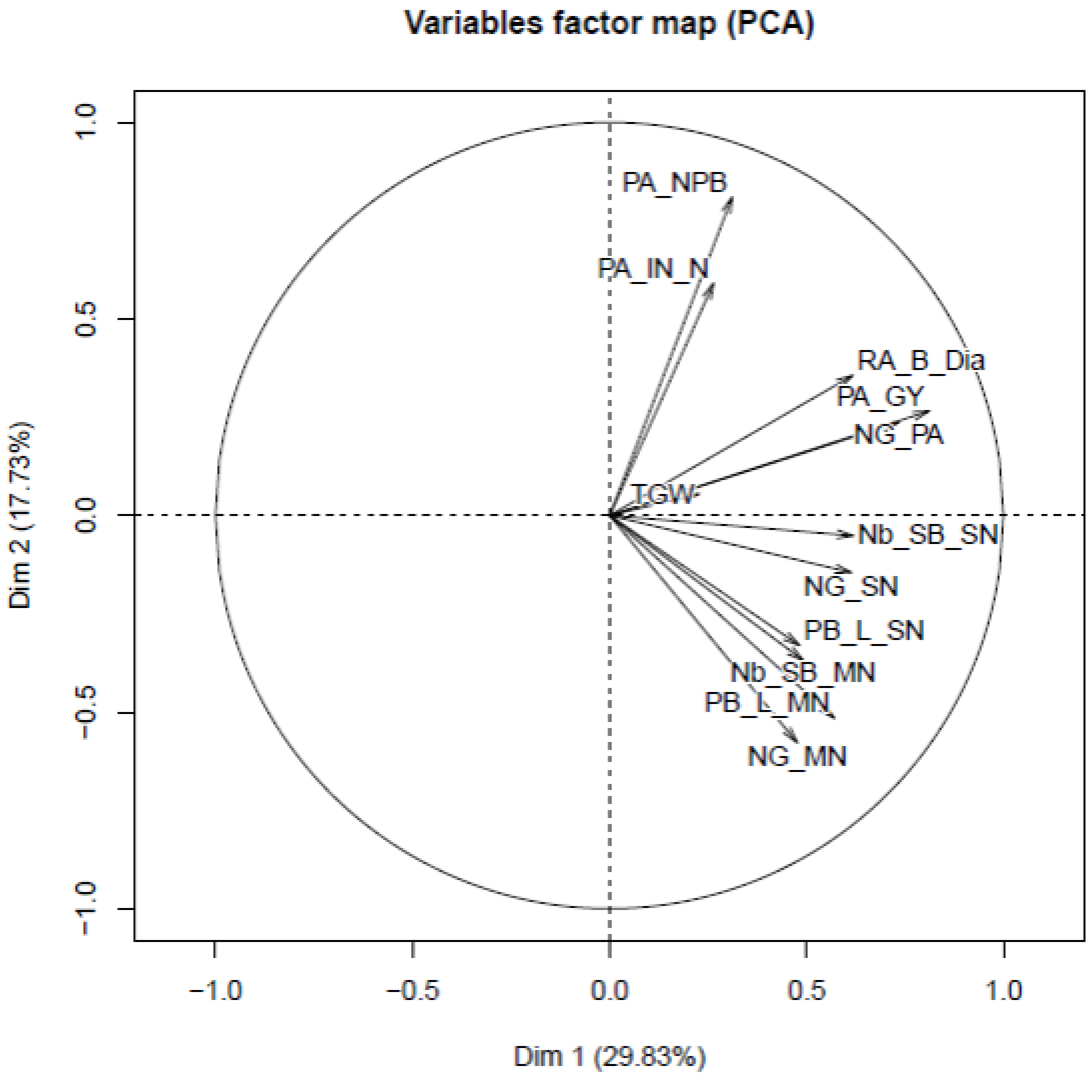


*Figure 3.4: Contribution of sorghum panicle architecture traits to the dimension 1 and 2*

The second one explained 17.73% while the third explained 10.08% (Figure 3.4). The following variables made a substantial contribution to the first dimension: number of grains on primary branches at median nodes and primary branch length at median node and number of secondary branches at second node. The variables with high contributions to the second dimension were the number of primary branches and internodes per panicle, rachis base diameter and panicle grain yield.

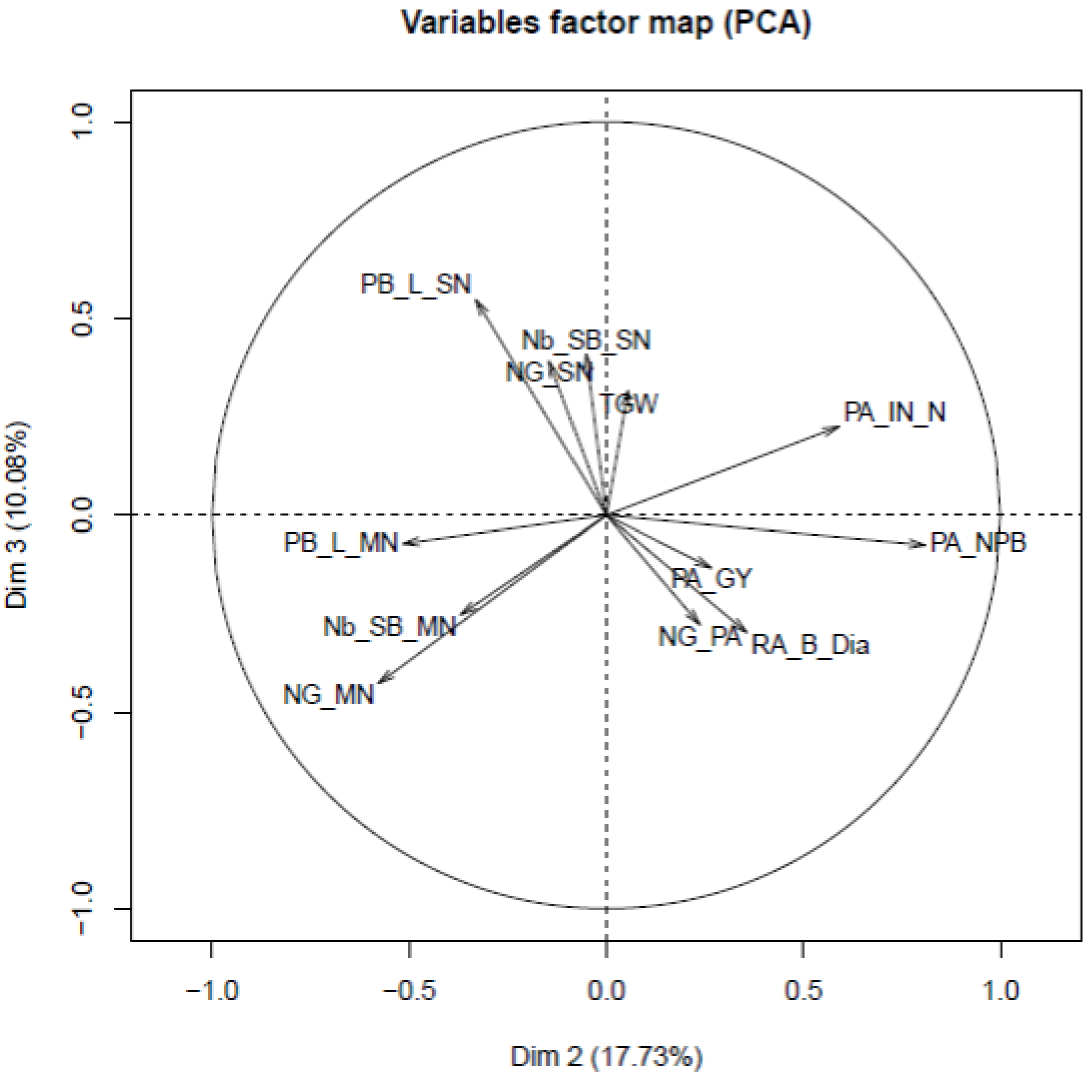


*Figure 3.5: Contribution of sorghum panicle architecture traits to the dimension 2 and 3*

The third dimension was influenced by the high contribution of primary branch length and number of grains at second node.

The PCA result indicated that the number of primary branches per panicle is determined by the number of internodes on the panicle, rachis basal diameter affect the grain yield per panicle. The number of grains per panicle is more relevant in grain yield per panicle than the thousand grain weight. The number of grains on a particular primary branch is mainly determined by the number of secondary branches rather than the length of the primary branch.

### 3.3.7. Correlation among traits

Highly significant positive correlations ranging from 0.31 and 0.70 were detected between panicle length and the length of primary branches, number of internodes per panicle, number of secondary branches and internodes length with high coefficient of correlation (Table 3.5).

The number of internodes per panicle was highly significant and positively correlated to the number of primary branches (r = 0.55) and panicle grain yield (r = 0.31).

The number of primary branches per panicle was highly significant and positively correlated with grain yield per panicle with (r = 0.49) and highly negatively correlated to the length of the internodes (- 0.39).

Rachis base diameter was highly significant and positively correlated to the number of primary branches per panicle and the grain yield per panicle with the coefficient of correlation (r = 0.56 and 0.53, respectively).

Three sorghum panicle architecture traits were highly significantly positively correlated to grain yield per panicle with the coefficient of correlation ranged from 0.30 to 0.53. Rachis base diameter presented the highest correlation followed by the number of primary branches per panicle and the number of internodes per panicle (Table 3.5).

Table 3.5: Correlation among sorghum panicle architecture traits and grain yield per panicle

| | PA_L | PB_L_max | PB_L_SN | IN_L | RA_B_Dia | PA_IN_N | PA_NPB | Nb_SB_LPB |
|---|---|---|---|---|---|---|---|---|
| PB_L_max | **0.706** | | | | | | | |
| | 0.000 | | | | | | | |
| PB_L_SN | **0.594** | **0.804** | | | | | | |
| | 0.000 | 0.000 | | | | | | |
| IN_L | **0.489** | **0.465** | **0.444** | | | | | |
| | 0.000 | 0.000 | 0.000 | | | | | |
| RA_B_Dia | 0.079 | 0.031 | -0.080 | -0.136 | | | | |
| | 0.116 | 0.535 | 0.110 | 0.006 | | | | |
| PA_IN_N | **0.350** | 0.159 | 0.097 | **-0.565** | 0.241 | | | |
| | 0.000 | 0.001 | 0.053 | 0.000 | 0.000 | | | |
| PA_NPB | 0.056 | -0.148 | -0.179 | **-0.391** | **0.566** | **0.551** | | |
| | 0.260 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | | |
| Nb_SB_LPB | **0.315** | **0.372** | **0.304** | 0.015 | 0.066 | 0.272 | 0.044 | |
| | 0.000 | 0.000 | 0.000 | 0.764 | 0.188 | 0.000 | 0.377 | |
| PA_GY | 0.149 | 0.184 | 0.056 | -0.120 | **0.533** | **0.307** | **0.440** | 0.224 |
| | 0.003 | 0.000 | 0.265 | 0.016 | 0.000 | 0.000 | 0.000 | 0.000 |

NB: first value in each cell indicates the coefficient of correlation and the second value is the probability. High coefficient of correlation are indicated in bold.

### 3.3.8. Transgressive segregation

Transgressive segregations were observed for most of the panicle architecture traits, grain yield and its related traits. The Figures 3.6 to 3.8 show the distribution for four traits in the three sowing dates.

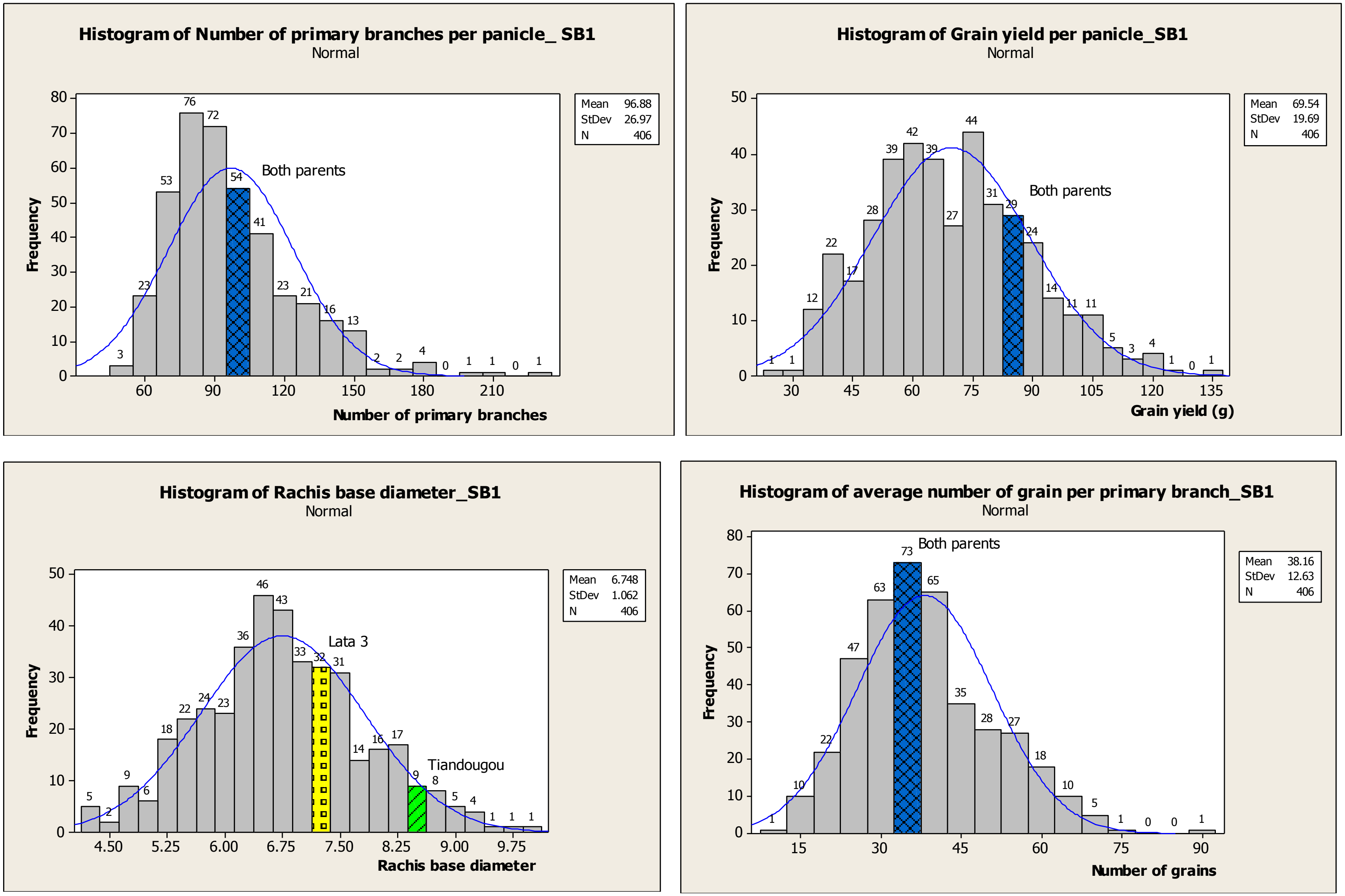


*Figure 3.6: Distribution of four panicle traits showing transgressive segregation in the first sowing date (SB1), 2011, Sotuba, Mali*

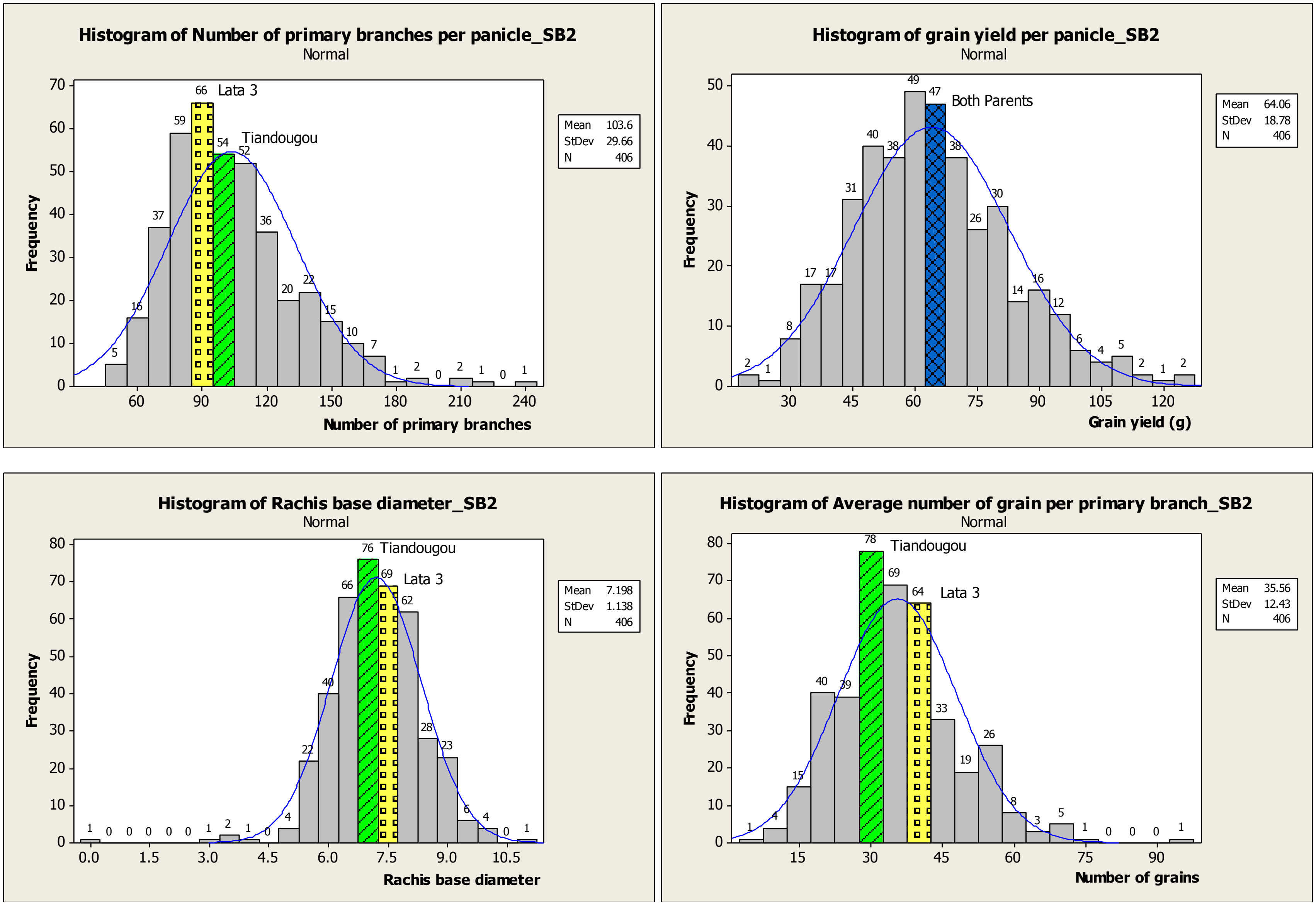
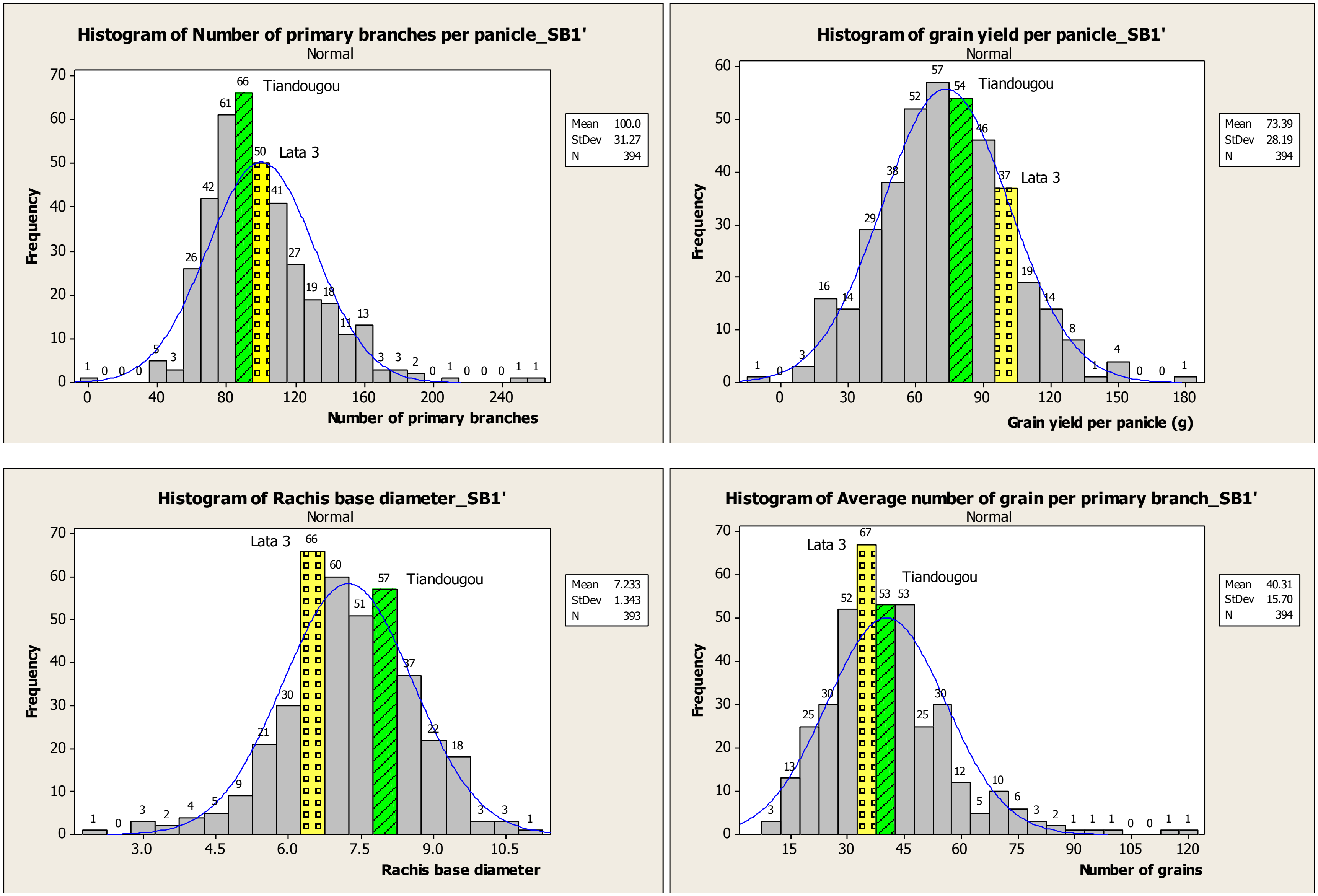


*Figure 3.7: Distribution of four panicle traits showing transgressive segregation in the second sowing date (SB2), 2011, Sotuba, Mali*


Histogram of Number of primary branches per panicle_SB1'

Histogram of grain yield per panicle_SB1'

Histogram of Rachis base diameter_SB1'

Histogram of Average number of grain per primary branch_SB1'

*Figure 3.8: Distribution of four panicle traits showing transgressive segregation in the first sowing date (SB1'), 2012, Sotuba, Mali*

### 3.3.9. Estimation of grain yield per panicle using panicle architecture traits

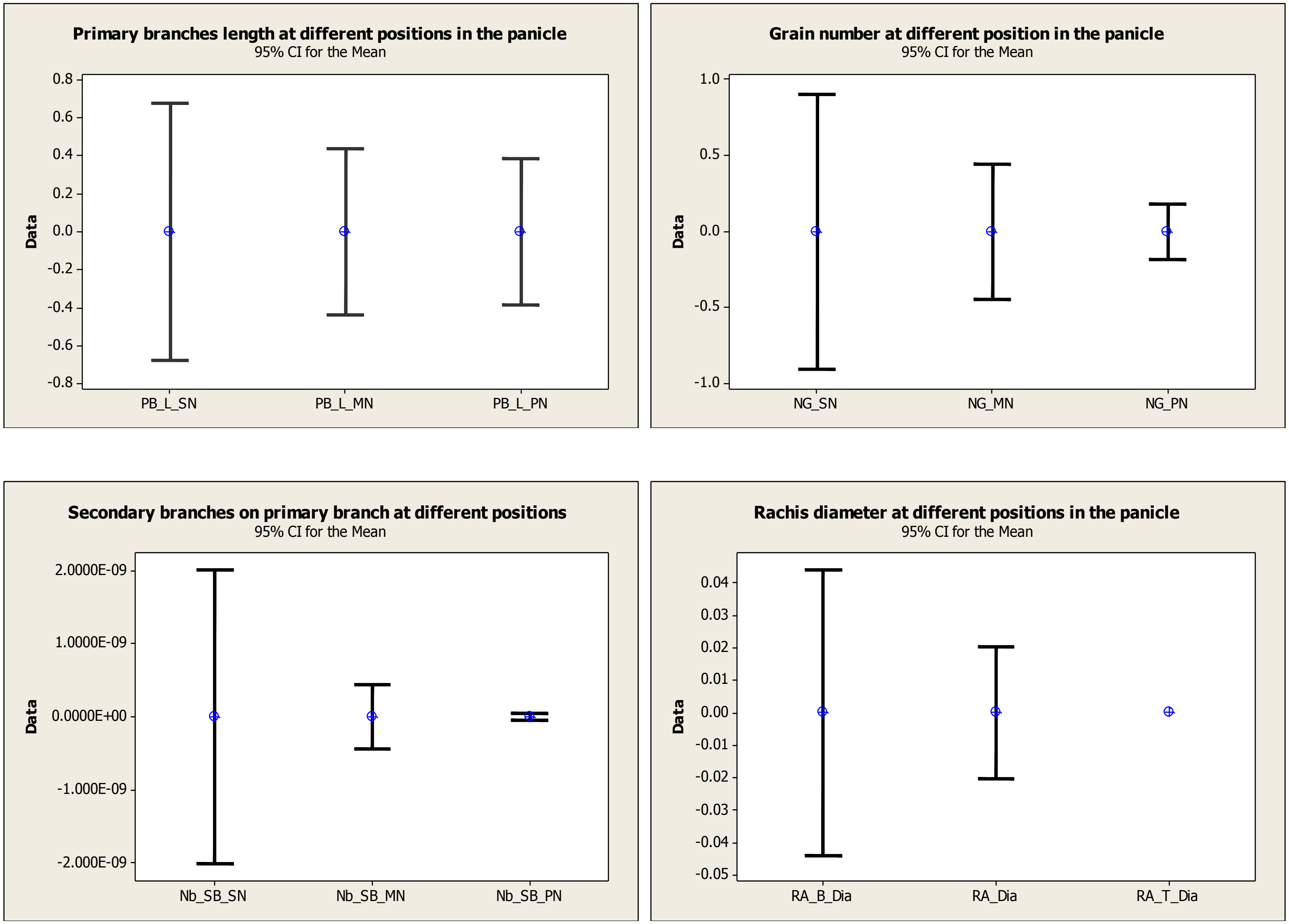
A multivariate regression, correlating panicle architecture traits with the grain yield per panicle, indicated that the number of internodes per panicle and rachis base diameter explained the grain yield per panicle with a determination coefficient ($R^2$) of 31.8%.

The regression equation was: PA_GY = - 0.000 + 7.76 RA_B_Dia + 1.23 PA_IN_N,

where: PA_GY was grain yield per panicle; RA_B_Dia was rachis base diameter and PA_IN_N was number of internodes per panicle. Therefore, it could be possible to have an estimate of the grain yield per panicle by measuring those easily measurable traits.

### 3.3.10. Branch length, grain number and rachis diameter at different positions on panicle

Primary and secondary branches, number of grains on primary branches and rachis diameter decrease from the base of the panicle toward the top of the panicle (Figure 3.7)



Primary branches length at different positions in the panicle

Grain number at different position in the panicle

Secondary branches on primary branch at different positions

Rachis diameter at different positions in the panicle

*Figure 3.9: Primary and secondary branches length; grain number and rachis diameter at second, median and penultimate nodes*

## 3.4. Discussion

Most of cultivated sorghum are photoperiod sensitive (Chantereau *et al*., 2004). The $F_4$ families performed better in the first sowing date than in the second one. Grain yield was reduced in the second sowing date in 2011 because of photoperiod that shortened the vegetative cycle and affected the potential yield. Consequently, highly significant differences were observed among the genotypes owing to the delay of the sowing date that resulted in high variability in the population. The reduction in grain yield resulted from the decrease in some panicle architecture traits such as the length of the primary branches at the median node, number of primary branches and nodes per panicle. The weight of the panicle which is one of the principal grain yield components was also affected negatively by the delay in the sowing date. A shorter vegetative cycle affects panicle elongation and branching. The ANOVA results confirmed the effect of the sowing date on most of sorghum panicle architecture traits.

Broad sense heritability for grain yield was moderate (46%) in this study. This is similar to the observation made by Addissu (2011) who reported 47% broad sense heritability for grain yield per plant. Brown *et al*. (2006), Murray *et al*. (2008) and Srinivas *et al*. (2009) reported moderate broad sense heritability estimates of 56%, 57% and 65%, respectively, for grain yield per panicle. However, Ali *et al*. (2012) reported high broad sense heritability estimates of (98%) for grain yield per plant. Warkad *et al*. (2008) also reported high broad sense heritability for grain yield per plant (86.0%), Godbharle *et al*. (2010) using elite ‘B’ and ‘R’ lines and Chavan *et al*. (2010) reported broad sense heritability estimates of up to (95 %) for grain yield per plant in $F_5$ families of sorghum. Therefore, this discrepancy in heritability estimates for grain yield per panicle may be explained by the interaction between the genotypes and their environments (GxE).

High heritability was observed for panicle length. This is in agreement with that obtained by Ali *et al*. (2012) who found, after the first cycle of selection, high broad sense heritability of 91% for panicle length. Chavan *et al*. (2010) reported 87% broad sense heritability in $F_5$ families in sorghum for panicle length. Rami *et al*. (1998) also reported a high broad sense heritability (83%) for panicle length. Moderate heritability was noted for panicle weight. Srinivas *et al*. (2009) also reported moderate (55%) broad sense heritability for panicle weight. Warkad *et al*. (2008) reported moderate broad sense heritability for number of nodes per panicle (34%). That is in agreement with the result obtained in this study.

High heritability observed for the number of primary branches per panicle in this study is in agreement with the finding of Godbharle *et al*. (2010), Chavan *et al*. (2010) and Brown *et al*. (2006) with broad sense heritability estimates of 96%, 93% and 94%, respectively for number of primary branches per panicle. However, Srinivas *et al*. (2009) reported relatively high (74%) broad sense heritability for the number of primary branches per panicle whereas Warkad *et al*. (2008) reported a moderate broad sense heritability estimate of 55% for the number of primary branches per panicle. These differences may be explained by the genetic background of the genotypes and the sowing dates. Number of branches was reduced in the late sowing date.

Moderate heritability estimates were recorded for the number of secondary branches in this study while Brown *et al*. (2006) found relatively high broad sense heritability (72%) for number of secondary branches. The number of grains per primary branch was influenced by other factors such as soil fertility. Godbharle *et al*. (2010) reported high broad sense heritability (95%) for number of grains per primary branch while moderate heritability was detected in this study. Brown *et al*. (2006) reported high broad sense heritability for rachis diameter (90%). However, in this study, rachis diameter showed moderate broad sense heritability.

In this study, principal component analysis revealed some important sorghum panicle architecture traits with positive contribution. The traits included panicle length, number of grains per panicle, the lengths of primary and secondary branches, number of secondary branches and grains per primary branch at median and penultimate node. Bucheyeki *et al*. (2009) also reported high loading of grain number per panicle that is consistent with the findings of this study. Principal component analysis displayed relationship between grain yield and some of the panicle architecture traits such as rachis base diameter, number of secondary branches on the basal primary branches and the number of primary branches per panicle. Emphasis should be placed on these traits in relation to their heritability and correlation for better understanding of the basic architectural principles such as branching pattern, elongation and relation to grain yield. This will provide the basis for developing breeding strategies to improve grain yield potential in sorghum.

Correlations among traits determine the genetic relatedness that provide the basis of breeding strategies for direct or indirect selection. The phenotype of a plant is the result of interaction of a large number of factors. Therefore, final yield is the total sum of several component characters. The phenotypic and genotypic correlation coefficients among different characters revealed that genotypic correlation coefficients were higher than phenotypic correlations. In general, the signs and magnitudes of correlations at both phenotypic and genotypic level are similar. Positive correlations were observed between grain yield and its components. Brown *et al*. (2006) found similar significant positive correlations between panicle grain yield and number of primary branches per panicle, plant height, 100 seed weight and seeds per panicle. The coefficient of correlation ranged from 0.3 to 0.82. Similar high positive correlation (0.82) was also found between panicle grain yield and grain number per panicle by Brown *et al*. (2006). Positive association of grain yield with panicle length, plant height, number of primary branches per panicle, and number of grains per panicle have been reported by Mahajan *et al*. (2011). Panicle length and number of grains per panicle had greater importance in increasing grain yield per panicle (El-din *et al*., 2012). For efficient selection in sorghum including panicle architecture traits, the following consideration should be kept in mind for instance, panicle length is proportional to the length of the rachis and affects rachis slenderness and volume. Longer panicles have long internodes, long primary and secondary branches and poorly correlated to grain yield per panicle. However, increasing the number of primary branches per panicle increases the number of grains and consequently grain yield per panicle. Increasing the number of internodes per panicle may increase the number of primary and secondary branches at the base and middle of the panicle and, subsequently, the panicle grain yield may be improved. The bigger the rachis base diameter, the more the primary and secondary branches the more the grains and subsequently high grain yield per panicle would result. Phenotyping specific axes and in specific parts on the panicle is important to fully account for genetic variability in sorghum panicle architecture; for instance, length data collected on primary branches at second node were much more informative than on secondary branches at the penultimate node. Number of primary branches per panicle was highly heritable compared to the dissected pattern of branching. However, the number of branches at the second node affects the number of grains more than the branch at the top of the panicle. This suggests that the length and the number of primary branches may play an important role in capturing panicle architecture and act as a good predictor to select for grain yield improvement. Therefore, a simple strategy of sorghum panicle architecture phenotyping should rely on the characterization of panicle elongation by measuring the length of the panicle, longest primary branch and basal primary branches.

Branching intensity is also an important trait to assess architectural variability in sorghum panicle by measuring the number of primary branches per panicle and the average number of primary branches per node. Rachis basal diameter influences the branching that plays a major role in grain setting and consequently the grain yield.

## 3.5. Conclusion

Delay in sowing date negatively affected panicle architecture trait and grain yield. Most of the sorghum panicle architecture traits were heritable in the $F_4$ families derived from Tiandougou/Lata-3. Panicle length, primary branches length at second and median nodes, secondary branch length, number of primary branches per panicle, number of secondary branches on primary branch at second nodes and position of the longest primary branch had positive contribution to sorghum panicle architecture variability. Other architectural traits affecting grain yield such as, number of primary branches per panicle, number of secondary branches on primary branches at second node and rachis base diameter have also a role to play in the variability of panicle architecture variability. Transgressive segregation was identified for most of the panicle architecture traits. Across the three sowing dates, the parent Lata-3 panicles consistently displayed more nodes per panicle and more secondary branches per primary branch than Tiandougou. Panicle, peduncle and primary branches at the base and middle of the panicle were longer in Lata-3 while Tiandougou displayed more primary branches and more grains per primary branch at the top of the panicle. Nevertheless, panicle grain yield, number of grains per panicle, grain weight, rachis base diameter and number of primary branches per panicle were similar in the two parents. Additive, non-epistasis effects and less environmental effect may explain the stability of the genotypes across the different sowing dates. Branching and grain number decrease progressively from the base to the top of the panicle. Causal relationships were detected between architecture traits and grain yield per panicle. Therefore, indirect selection responses between number of primary branches per panicle or number of internodes per panicle and rachis base diameter on grain yield per panicle were promising and need to be further investigated.

# CHAPTER FOUR

## 4.0. QUANTITATIVE TRAIT LOCUS (QTL) ANALYSIS OF PANICLE ARCHITECTURE IN THE TIANDOUGOU/ LATA-3 BREEDING POPULATION

### 4.1. Introduction

Molecular markers are useful tools for dissecting the variation of complex traits, such as grain yield and its components into simply inherited factors. A quantitative trait locus (QTL) represents the statistical association observed in a segregating population between the quantitative variation of a given trait and the genotype (homozygous for one of the two parental alleles or heterozygous) of progenies at a given locus as revealed by molecular markers. In addition to the identification of the most probable location in the genome of such QTL, molecular markers allow estimation of their genetic effects and interactions among them. The incorporation through various ways of such information in a breeding program provides a strong basis for improving and optimizing plant breeding process.

The identification of QTLs affecting sorghum panicle architecture will permit an understanding of the genetic factors, their interactions, and elucidate their relationship to grain yield. It will provide useful information for the definition of target genotypes to breed for in the breeding population. Indeed, the Marker Assisted Recurrent Selection (MARS) approach constitutes a framework for the valorization of QTL results together with other traits such as grain quality and plant adaptation. QTL studies on the panicle architecture in sorghum were mainly done using Recombinant Inbreds Lines (RILs) population. The first reports of genomic regions associated with grain yield and grain yield components were reported by Tuinstra *et al*. (1996), while genomic regions controlling sorghum panicle architecture traits were reported by Pereira *et al*. (1995); Rami *et al*. (1998); Hart *et al*. (2001); Brown *et al*. (2006) and Srinivas *et al*. (2009).

The objectives of this research were (1) to identify QTL responsible for the phenotypic variation of sorghum panicle architecture traits in $F_3$ segregating population, (2) to determine the consistency of the detected QTL across different sowing dates, and (3) to determine genomic regions with pleiotropic effects on two (or more) different traits.

## 4.2. Material and methods

### 4.2.1. Plant material

A mapping population of 401 $F_4$ families obtained from a cross between Tiandougou and Lata-3 was used for QTL detection. The population is described in the chapter III.

### 4.2.2. Leaf sampling and DNA extraction

A sample of leaf tissue (400g) was collected for each of the 401 families during the $F_4$ to $F_5$ multiplication in 2010. For each family, a bulk of 10 $F_4$ plants were sampled to represent the parental $F_3$ line. Leaf samples of the parental lines, Tiandougou and Lata-3, were collected during the initial crossing made to produce the population. Leaf samples were collected in individual zipper storage plastic bags together with silica gel and allowed to dry during shipment to Montpellier, France. DNA extraction was performed at CIRAD, Montpellier following the MATAB isolation protocol as described by Risterucci *et al*. (2000).

### 4.2.3. Genotyping

DNA of the $F_3$ population and the 2 parents were sent to LGC genomics (formerly KBioscience) for SNP genotyping using KASP$^{TM}$ technology. Additional genotyping was performed at CIRAD Montpellier on another five (5) SNP markers using the same KASP$^{TM}$ technology on the same DNA samples (Appendix 4.1).

### 4.2.4. Phenotyping

A great number of panicle architecture traits related to geometry, topology and grain yield and its components were collected. However, focus was on selected traits based on the result of Pearson correlation and principal component analysis. These traits were grain yield per panicle, number of primary branches per panicle, primary branch length, number of grains per panicle, number of internodes per panicle, number of grains per primary branch, panicle length, panicle weigh and rachis base diameter (refer to Chapter III).

### 4.2.5. Construction of Genetic maps

Genetic markers were chosen for their position on the genome to get a good coverage of the genome and a good distance between markers for QTL detection. A total of 228 polymorphic markers (225 markers from LGC Genomics and 3 markers from the CIRAD lab) were used for the construction of genetic map. The genotypes of the individuals were coded as A for the allele of the parent Lata-3, B for the allele of the parent Tiandougou, and H for the heterozygous genotype. Linkage groups were determined by results of pair-wise comparisons at a minimum likelihood of odds (LOD) value of 3. The best order was determined by comparing the goodness of fit of the resulting map for each tested order using a threshold of 0.5 and 1.0 for the linkage groups and the loci, respectively. The functions order sequences, check inversion, ripple sequences and draw maps were used to elaborate the preliminary genetic map. Kosambi mapping function was used to translate recombination frequencies into map distances. The genetic map obtained with Mapdisto software was drawn using spiderMap software. The 10 linkage groups were assigned to sorghum chromosomes and named SBI-01 to SBI-10, according to the *a priori* known physical position of the SNP markers on the genome sequence. The constructed genetic map was compared to the sorghum consensus map published by Mace *et al*. (2009).

### 4.2.6. QTL Detection

QTL detection was performed on trait mean values in each individual sowing date and BLUP values that integrate all sowing dates. QTL analyses were performed using Simple interval mapping (SIM) with the regression method described (Halley and Knott, 1992) using the software R/qtl (Broman and Sen, 2009). A thousand-permutation test was applied to each trait variable to determine the LOD threshold ($P$ = 0.05) for considering significant levels of identified QTLs (Churchill and Doerge, 1994). SIM detection was followed by the "refine qtl" procedures that refine the position of the detected QTLs using an iterative scan of QTL positions using a multiple QTL model. Support interval of LOD was calculated for each QTL to obtain a 95% confidence interval. Adjacent QTLs on the same chromosome were considered different when the support intervals were not overlapping. Attention was also accorded to declare two peaks in the same chromosome for the same trait as two QTLs only if the distance between the QTLs was greater than 20 cM otherwise the higher peak was considered for better estimation of the QTL position (Parth *et al*., 2008; Ungerer *et al*., 2002). The percentage of phenotypic variance, the additive and dominance effects attributable to an individual QTL were estimated using an additive multi-QTL model involving all QTLs detected for a given trait, using the function "fitqtl" of the R/qtl package. An average level of dominance for QTL (d/a) was calculated as the ratio of dominance effect (d) over additive effect (a). The QTL action was determined according to guidelines presented by (Stuber *et al.* 1987): additive QTL action (A) = 0 to 0.20; partial dominance (PD) = 0.21 to 0.80; dominance (D) = 0.81 to 1.20; and over dominance (OD) = >1.20.

According to R/qtl documentation the "additive effect is derived from the coding scheme -1/0/+1 for genotypes AA/AB/BB, and so is half the difference between the phenotype averages for the two homozygotes". For a given QTL, if the additive effect is positive, the increasing allele came from the Tiandougou parent; if negative the increasing allele came from Lata-3 parent.

QTLs were designated with italicized symbols composed of a *Q* followed by trait name, a hyphen and the chromosome on which the QTL is detected. Serial numbers were used to design more than one QTL controlling the same trait detected on the same chromosome. For instance, *QPA_L-SBI-03-2* refers to the second panicle length QTL detected on SBI-03.

### 4.2.7. Digenic epistasis detection

The digenic epistasis interaction was analyzed within $F_4$ families using bi-parental populations (BIP) mode in QTLIciMapping software version 3.2. The ICIM-EPI (Inclusive Composite Interval Mapping for epistasis mapping method was used with a step size of 5 cM and a probability in stepwise regression of 0.0001. Deletion was selected as the means for dealing with missing phenotypic data and the LOD threshold was fixed for all traits at 5.

### 4.2.8. QTLs overlapping confidence interval across studies

A sorghum consensus map (Mace *et al.*, 2009), with multiple anchor points to the whole genome sequence was used to identify QTLs for the same trait overlapping confidence intervals across four (4) studies on sorghum panicle architecture traits previously reported.

## 4.3. Results

### 4.3.1. Summary of QTLs detected in Tiandougou/Lata-3 $F_4$ families population

Simple Interval Mapping followed by refine QTLs revealed significant QTLs for sorghum panicle architecture and grain yield (Table 4.1).

Table 4.1: Summary of significant QTLs detected for sorghum panicle architecture traits and grain yield in Tiandougou / Lata-3 $F_4$ families using Simple Interval Mapping followed by refine QTLs

| **Chrs** | **Nb_M** | **T_L (cM)** | **Av_DM (cM)** | **N_Q_ PVE>7** | **T_Q** | **Add** | **PD** | **Dom** | **Over Dom** | **Tiand** | **Lata** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 29 | 178.3 | 6.1 | 5 | 18 | 5 | 11 | 2 | 0 | 10 | 8 |
| 2 | 25 | 169.1 | 6.8 | 15 | 33 | 12 | 14 | 6 | 1 | 15 | 18 |
| 3 | 26 | 163.7 | 6.3 | 16 | 47 | 20 | 21 | 5 | 1 | 23 | 24 |
| 4 | 20 | 135.6 | 6.7 | 1 | 13 | 2 | 11 | 0 | 0 | 2 | 11 |
| 5 | 22 | 119.7 | 5.4 | 0 | 2 | 0 | 0 | 1 | 1 | 1 | 1 |
| 6 | 20 | 110.1 | 5.5 | 15 | 27 | 13 | 11 | 2 | 1 | 10 | 17 |
| 7 | 21 | 123.1 | 5.9 | 2 | 17 | 6 | 9 | 1 | 1 | 11 | 6 |
| 8 | 16 | 115.4 | 7.2 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| 9 | 23 | 118.5 | 5.1 | 1 | 4 | 1 | 2 | 0 | 1 | 1 | 3 |
| 10 | 26 | 133.8 | 5.1 | 0 | 8 | 3 | 5 | 0 | 0 | 5 | 3 |
| **Total** | **228** | **1362.3** | **6.01** | **53** | **164** | **62** | **80** | **17** | **5** | **75** | **89** |

*NB: Chrs = chromosomes; Nb_M = Number of Markers; T_L (cM) = Total length (centiMorgan); Av_DM (cM) = Average Distance between Markers (centiMorgan); N_Q_PVE>7 = Number of QTLs with Percentage of variation explained superior to 7%; T_Q = Total QTLs; Add = Additive; PD = Partial Dominance; Dom = Dominance; Over Dom = Over Dominance; Tiand = Tiandougou; Lata = Lata-3.*

The ten chromosomes were covered with 228 SNP markers (Appendix 4.2). The average distance between markers was 6.01 cM. The total length of the constructed genetic map was 1362.3 cM that was shorter compare to the sorghum consensus genetic maps (1603.5 cM). A total of 164 QTLs were detected with phenotypic variation explained in general low. Only 53 QTLs presented the phenotypic variation explained more than 7% (Appendix 4.3). Non-additive gene actions was predominant in the control of sorghum panicle architecture. The parent Lata-3 contributed favorable alleles that increased the trait value than the parent Tiandougou. The SBI-02, SBI-03 and SBI-06 bear a great number of QTLs for panicle architecture traits and grain yield.

#### 4.3.2. QTLs detected for the most important sorghum panicle architecture traits and grain yield in Tiandougou/Lata-3 $F_4$ families using Simple Interval Mapping followed by refine QTLs

Panicle grain yield, panicle length, rachis base diameter, number of primary branches per panicle, primary branch length, number of grains per primary branch, panicle weight and number of internodes per panicle were the most important traits related to sorghum panicle architecture and grain yield (refers ACP result in chapter III).

##### 4.3.2.1. Panicle grain yield

Two QTLs were detected for panicle grain yield on the SBI-03 and SBI-06 (Figure 4.1). The parent Lata-3 contributed the allele that increased the trait value in the QTL detected on SBI-06 with 11.86% of phenotypic variation explained. The parent Tiandougou contributed to the second QTL with 8.55% of phenotypic variation explained. Panicle grain yield was governed by non-additive gene action.

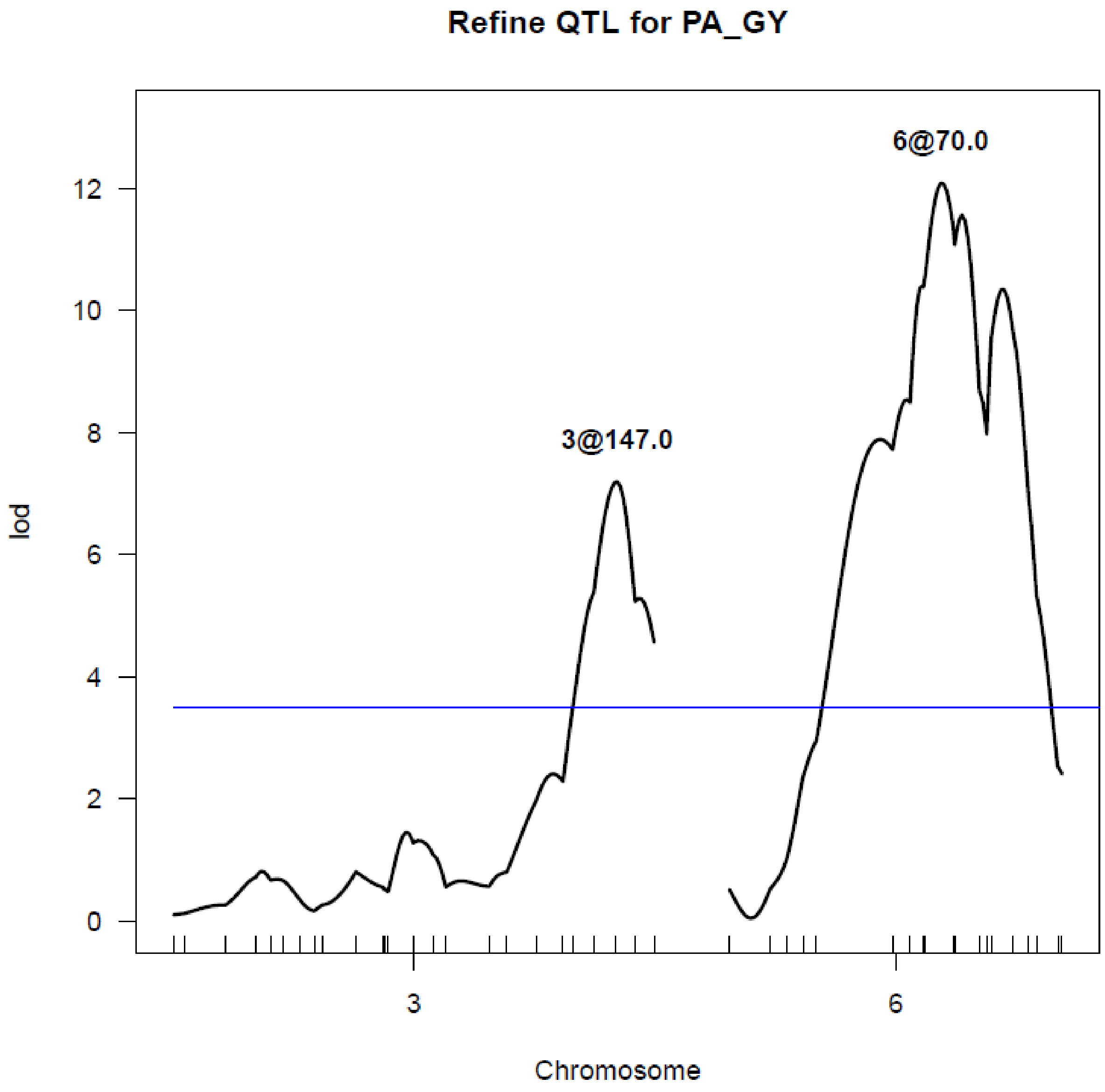


*Figure 4.1: QTLs detected for panicle grain yield in Tiandougou/Lata-3 $F_4$ families population*

### 4.3.2.2. Number of primary branches per panicle

Six QTLs were identified to control number of primary branches per panicle in the Tiandougou/Lata-3 population (Figure 4.2). The parent Tiandougou contributed the alleles increasing the trait value for four QTLs, while the parent Lata-3 provided increasing allele in two QTLs.

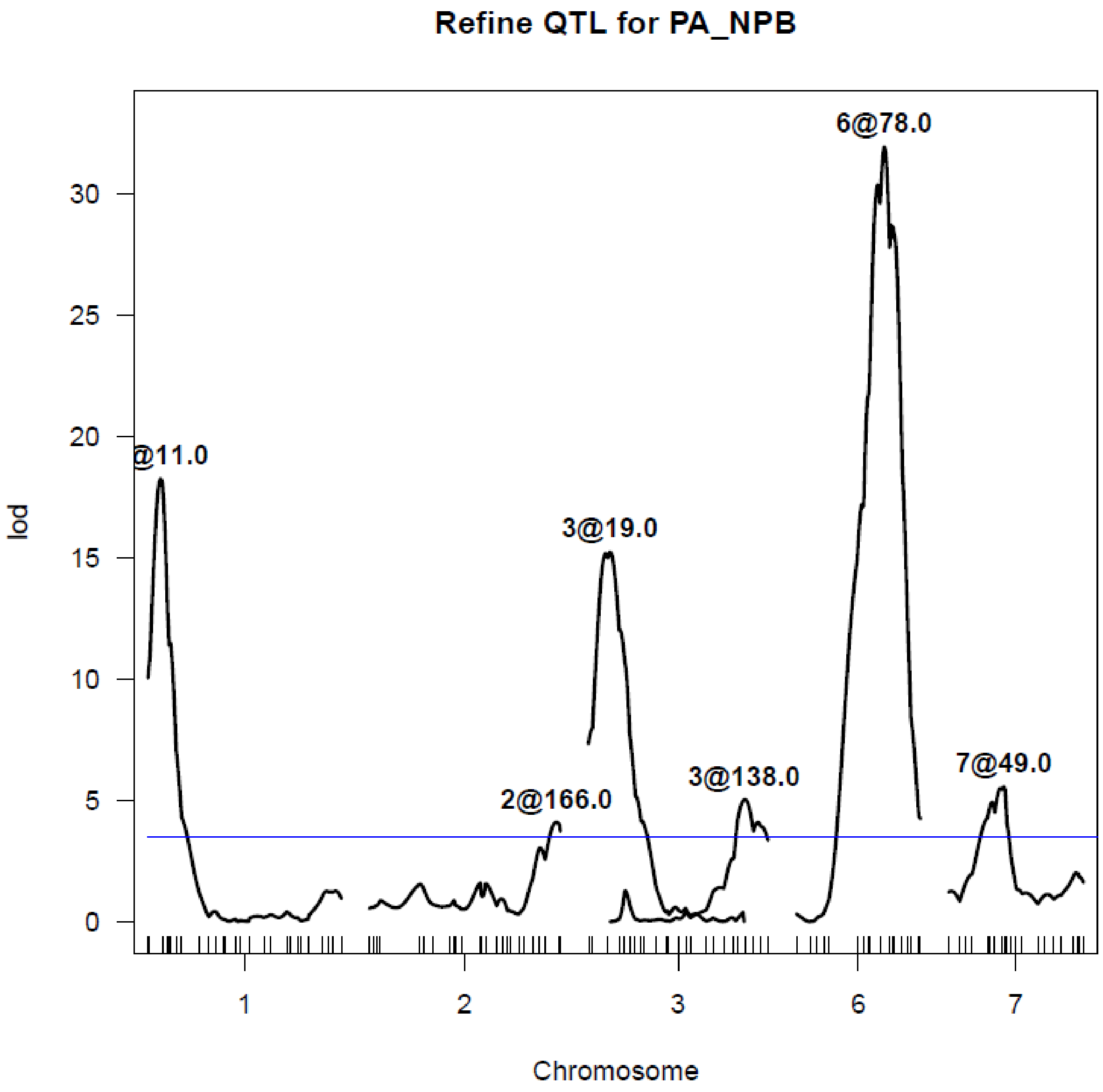


*Figure 4.2: QTLs detected for number of primary branches per panicle in Tiandougou/Lata-3 $F_4$ families population*

#### 4.3.2.3. Number of internodes per panicle

Three (3) QTLs for the number of internodes per panicle were identified (Figure 4.3). The percentage variation explained by the QTLs ranged from 2.95 (*QPA_IN_N-SBI-02-2*) to 7.92% (*QPA_IN_N-SBI-02-1*).

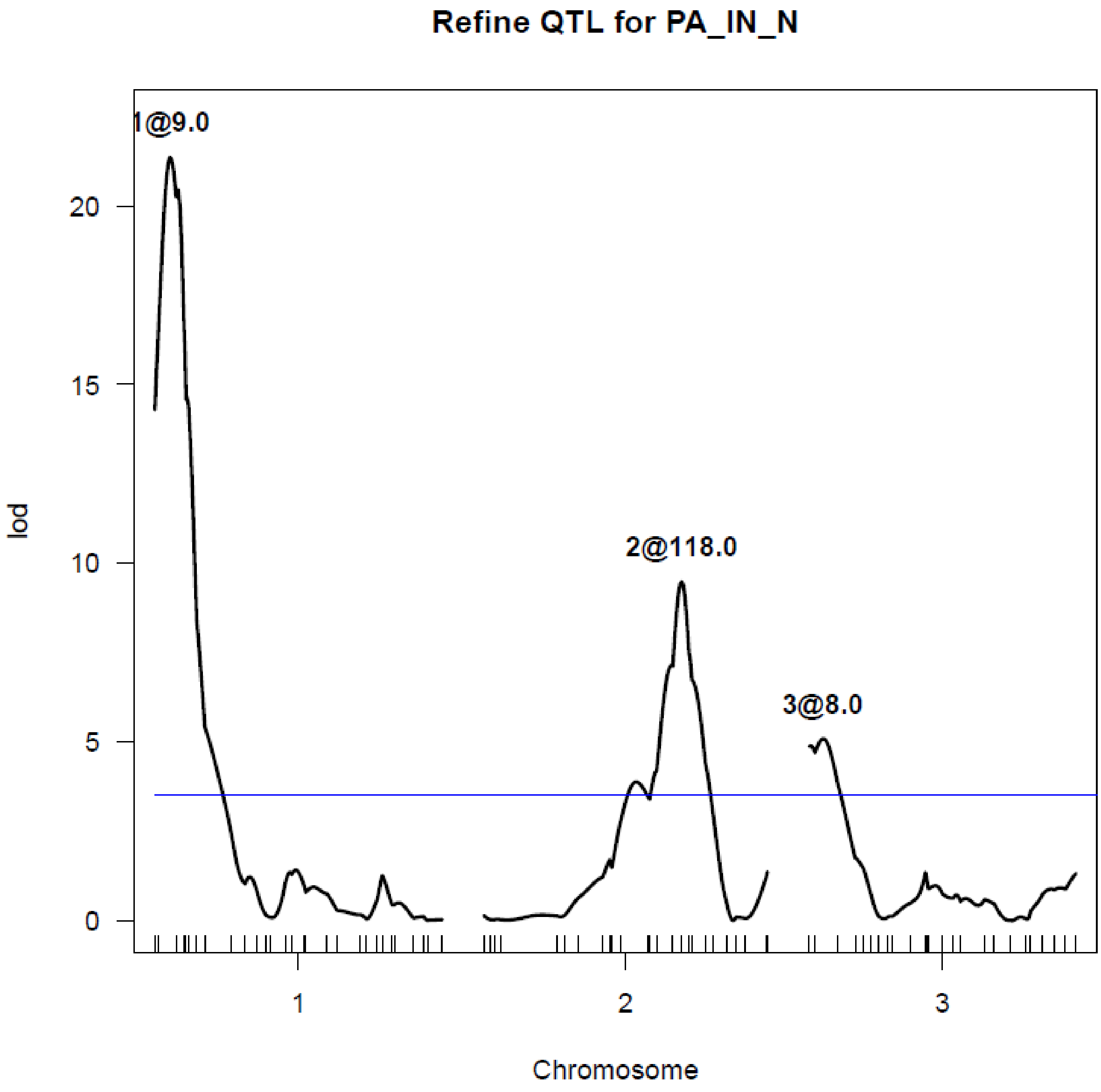


*Figure 4.3: QTLs detected for number of internodes per panicle in Tiandougou/Lata-3 $F_4$ families population*

The parent Lata-3 contributed to beneficial alleles increasing trait values for the QTL on the SBI-02 with 7.92 % of phenotypic variation explained. Tiandougou contributed to the two QTLs with partial dominance and additive gene action.

### 4.3.2.4. Rachis base diameter

A total of six QTLs were detected for rachis base diameter (Figure 4.4).

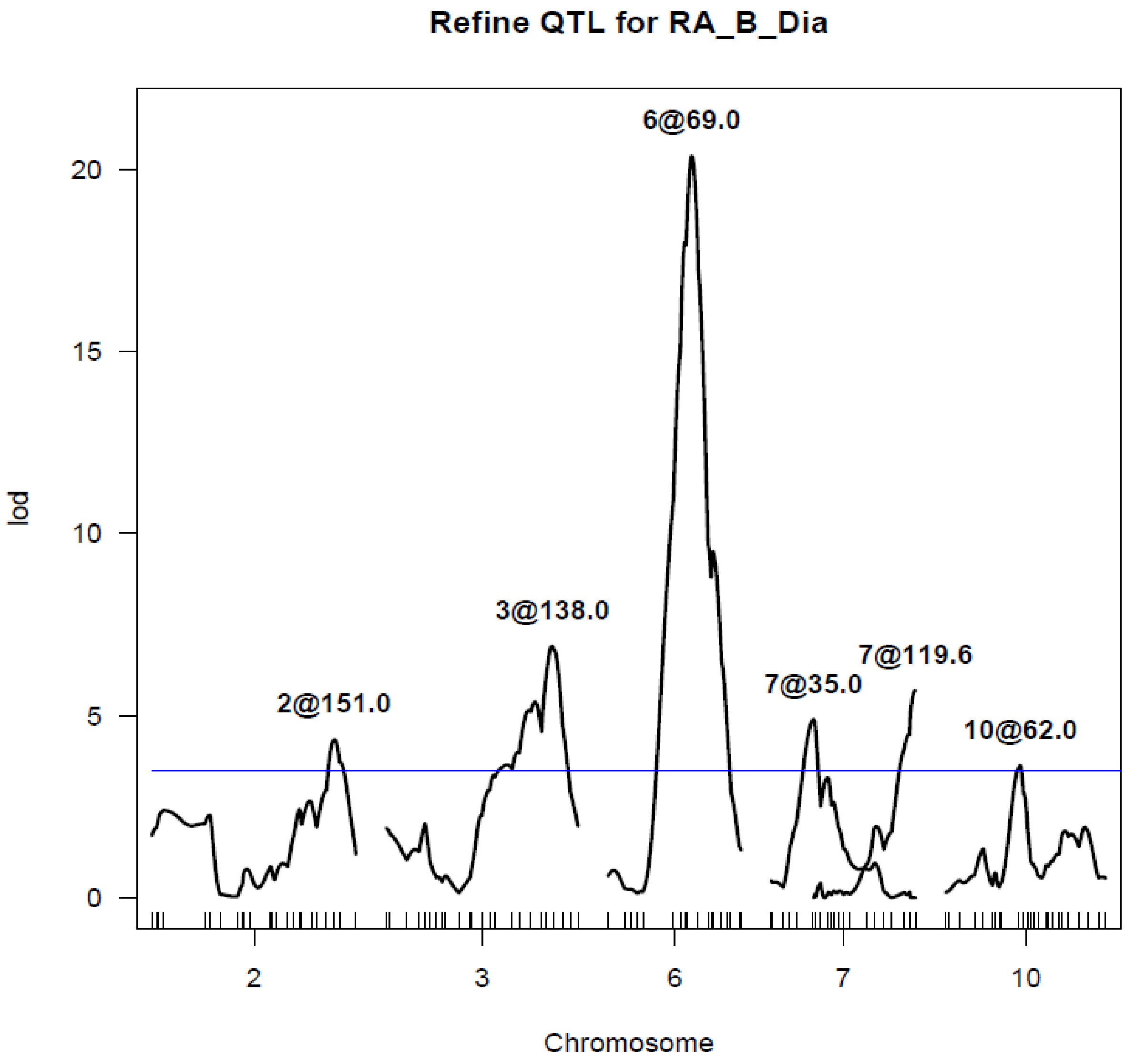


*Figure 4.4: QTLs detected for rachis base diameter in Tiandougou/Lata-3 $F_4$ families population*

All the QTLs have partial dominance gene action and the parent Tiandougou contributed for beneficial allele except the major QTL in which Lata-3 contributed for favorable allele. The percentage of variation explained by those QTLs ranged from 2.67 (QRA_B_Dia-SBI-10) to 16.60% (QRA_B_Dia-SBI-06).

#### 4.3.2.5. Panicle length

Four QTLs on SBI01, SBI02, SBI03, and SBI04 were detected for panicle length (Figure 4.5). The percentage of variation explained by those QTLs ranged from 6.74 (*QPA_L-SBI-01*) to 11.19% (*QPA_L-SBI-03*). The parent Lata-3 contributed mostly alleles that increase trait values of three QTLs. Among those three QTLs, two QTLs displayed partial dominance gene action while one QTL showed additive effect. The parent Tiandougou contributed a beneficial allele increasing trait values for only one partial dominance QTL of panicle length. One major QTL for panicle length was identified (*QPA_L_SBI-03)* on SBI-03 at the position 143 cM. The flanking markers were SB03137 on left and SB03164 at right. The percentage of variation explained by the QTL was 11.19%. The parent Lata-3 allele increased the trait value with partial dominance gene action.

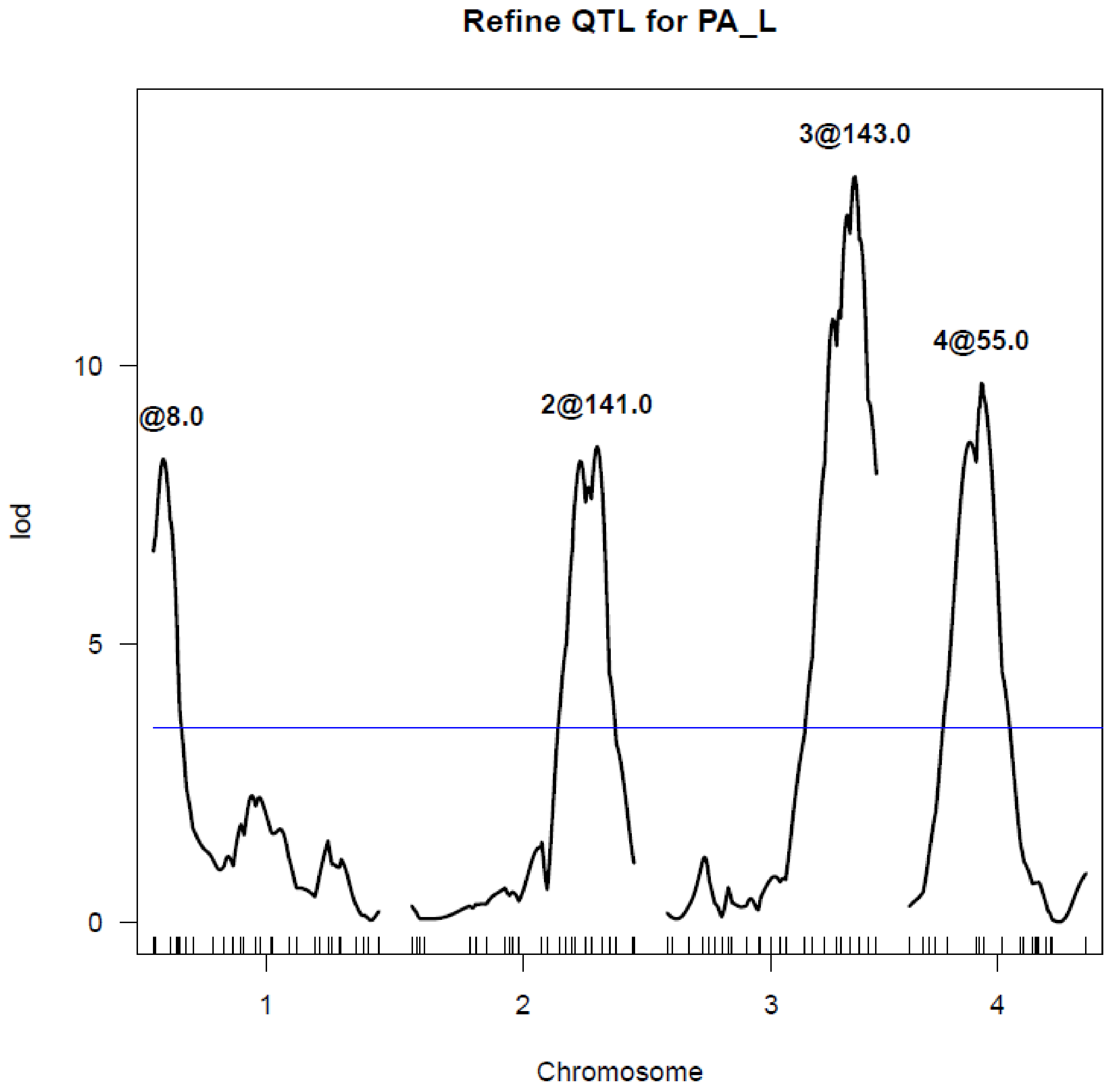


*Figure 4.5: QTLs detected for panicle length in Tiandougou/Lata-3 $F_4$ families population*

### 4.3.2.6. Average number of grains per primary branch

A total of four QTLs were detected for the average number of grains (Figure 4.6). The percentage of phenotypic variation explained by these QTLs ranged from 3.40 (*QAv_NG-SBI-01*) to 6.66%

(*QAv_NG-SBI-02*). Two QTLs have partial dominance gene effect and another two QTLs have additive gene action. Each parent contributed in alleles increasing trait value for two QTLs.

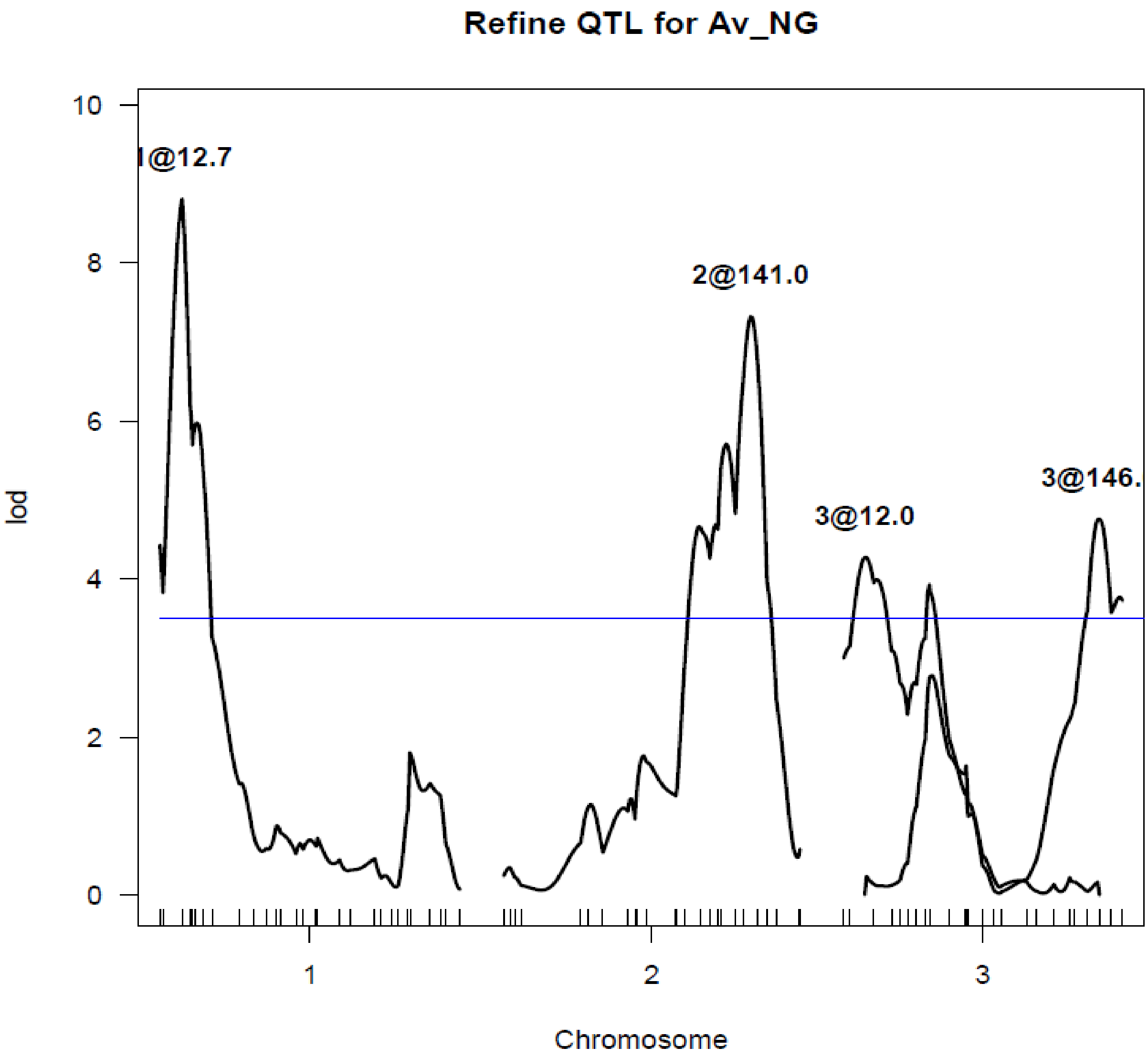


*Figure 4.6: QTLs detected for average number of grains per primary branch in Tiandougou/Lata-3 $F_4$ families population*

### 4.3.3. Consistent QTLs detected in Tiandougou/Lata-3 $F_4$ population across the three sowing dates in the combined data (BLUP)

Consistent QTLs across sowing dates and in BLUP data were indicated in Table 4.1 and Table 4.2.

Table 4.2: Topological consistent QTLs detected in Tiandougou/Lata-3 population

| **Traits** | **Sowing dates** | **Chr** | **Position cM** | **LOD** | **PVE** | **Add** | **Dom** | **Dom/Add** | **Gene action** | **Dir Parent** |
|---|---|---|---|---|---|---|---|---|---|---|
| PA_NPB | *BLUP* | 6 | 76 | 31.914 | 20.186 | -10.97 | 2.451 | -0.13 | PD | Lata |
| | SB1 | 6 | 79 | 14.9 | 13.11 | -11.58 | 1.97 | -0.17 | A | Lata |
| | SB2 | 6 | 72 | 18.69 | 17.9 | -14.73 | 4.1 | -0.28 | PD | Lata |
| | SB1’ | 6 | 79 | 16.49 | 15.02 | -13.25 | 3.76 | -0.28 | PD | Lata |
| NG_PA | *BLUP* | 6 | 62 | 12.833 | 12.46 | -141.5 | 6.899 | -0.05 | A | Lata |
| | SB1 | 6 | 79 | 8.52 | 6.54 | -256.3 | 187.22 | -0.73 | PD | Lata |
| | SB1’ | 6 | 69 | 8.24 | 8.61 | -385.9 | -10.15 | 0.03 | A | Lata |
| PA_IN_N | SB1 | 1 | 15 | 10.22 | 9.93 | 0.87 | -0.47 | -0.54 | PD | Tiand |
| | SB2 | 1 | 8 | 11.4 | 11.42 | 0.93 | -0.22 | -0.24 | PD | Tiand |
| | SB1’ | 1 | 9 | 9.57 | 10.23 | 0.93 | -0.04 | -0.04 | A | Tiand |
| PB_BZ_SN | SB2 | 2 | 116 | 8.03 | 7.23 | -5.13 | 2.75 | -0.54 | PD | Lata |
| | SB1’ | 2 | 141 | 9.53 | 10.24 | -7.05 | -1.38 | 0.20 | A | Lata |
| PB_Dens | *BLUP* | 6 | 72 | 28.82 | 18.35 | -0.036 | -0.009 | 0.03 | A | Lata |
| | SB1 | 6 | 79 | 14.05 | 11.74 | -0.04 | 0 | 0.00 | A | Lata |
| | SB2 | 6 | 76 | 14.17 | 13.6 | -0.04 | 0.008 | -0.20 | A | Lata |
| | SB1’ | 6 | 79 | 16.33 | 13.72 | -0.04 | 0.009 | -0.23 | PD | Lata |
| Nb_PB_max | *BLUP* | 6 | 67 | 24.551 | 19.352 | -1.25 | 0.07 | -0.06 | A | Lata |
| | SB1 | 6 | 69 | 17.19 | 14.87 | -1.67 | -0.23 | 0.14 | A | Lata |
| | SB2 | 6 | 70 | 16.31 | 14.39 | -1.92 | 0.86 | -0.45 | PD | Lata |
| | SB1’ | 6 | 87 | 9.4 | 9.92 | -1.89 | 0.77 | -0.41 | PD | Lata |

*NB: PA_NPB = Number of Primary Branches per panicle; NG_PA = Number of Grains per panicle; PA_IN_N = Number of Internodes per panicle; PB_BZ_SN = Primary branch Zone at second node; PB_Dens = Primary Branch Density; Nb_PB_max = Maximum number of primary branches per node; SBI = Chromosome; LOD = Logarithm of Odd; PVE = Percentage of Variance Explained; Add = Additive; Dom = Dominance; Additive gene action (A) if Dom/Add = 0 to 0.20; partial dominance (PD) if Dom/Add = 0.21 to 0.80; dominance (D) if Dom/Add = 0.81 to 1.20; and overdominance (OD) if Dom/Add > 1.20. Additive effects are associated with the allele from Tiandougou. Thus, a negative value means that the Tiandougou allele decreases the value of the trait. Dir_ Parent = Direction of response of the parent whose additive value of a marker allele increased the value of the trait. (Tiand = Tiandougou; Lata = Lata-3).*

One consistent QTL for Number of Primary Branches per panicle (*QPA_NPB_SBI-06)* was detected on SBI-06 in both combined analyses (BLUP) and individual analysis of the three sowing dates. The percentage of variation explained ranged from 13.11 to 20.18%. The parent Lata-3 allele increased the trait value with partial dominance gene action.

One consistent QTL for primary branch density on SBI-6. The percentage of variation explained ranged from 11.74 to 18.35%. The major QTL had additive gene action with the contribution of Lata-3 increasing trait value.

A consistent QTL for the maximum number of primary branches per node was identified on SBI-06. The phenotypic variance explained by the QTL ranged from 9.92 to 19.35%. The parent Lata-3 contributed favorable alleles that increased the trait value with additive gene action. However, one consistent QTL for number of internodes per panicle was detected in each the sowing dates on SBI-01. This QTL was not detected in the combined analysis. Also one consistent QTL was detected on SBI-06 in the combined and individual sowing date analyses excluding the second sowing date (SB2) in 2011.

One consistent QTLs for panicle grain yield was detected in each of the sowing dates and in the combined data on SBI-06 with the percentage of variation explained ranging from 4.7 (SB1) to 11.86% (BLUP). The parent Lata-3 contributed to beneficial allele that increased the trait value.
The date from sowing to flag leaf appearance that gave indication on flowering time displayed one major consistent QTL in the first and second sowing dates in 2011 on SBI-03. The QTL explained 45.64% and 53.88% of phenotypic variation respectively for SB1 and SB2. The QTL had partial dominance gene action and the parent Tiandougou allele increased the flowering time in the two sowing dates.

One consistent QTL was identified for rachis base diameter on SBI-06. The percentage of variation explained by the QTL ranged from 6.17% (SB1') to 16.60% (BLUP). The parent Lata-3 contributed alleles increasing the trait value with both additive and non-additive gene actions.

Table 4.3: Geometrical and grain yield consistent QTLs detected in Tiandougou/Lata-3 population

| Traits | Sowing dates | Chr | Position | LOD | PVE | Add | Dom | Dom/Add | Gene action | Dir Parent |
|---|---|---|---|---|---|---|---|---|---|---|
| PA_GY | *BLUP* | 6 | 66 | 12.08 | 11.863 | -3.1024 | 0.9234 | -0.30 | PD | Lata |
| | SB1 | 6 | 82.8 | 4.77 | 4.7 | -4.53 | 4.96 | -1.09 | OD | Lata |
| | SB2 | 6 | 71 | 6.7 | 6.86 | -5.84 | 0.46 | -0.08 | A | Lata |
| | SB1’ | 6 | 54 | 6.26 | 5.8 | -7.96 | 4.04 | -0.51 | PD | Lata |
| SFD | SB1 | 3 | 74 | 53.08 | 45.64 | 4.67 | -2.36 | -0.51 | PD | Tiand |
| | SB2 | 3 | 71 | 67.39 | 53.88 | 5.02 | -2.46 | -0.49 | PD | Tiand |
| PE_L | BLUP | 3 | 71,2 | 28.305 | 20.436 | -25.389 | -2.722 | 0.11 | A | Lata |
| | SB1 | 3 | 73 | 14.6 | 13.97 | -27.23 | -5.82 | 0.21 | PD | Lata |
| | SB2 | 3 | 73 | 19.67 | 17.59 | -35.93 | -3.23 | 0.09 | A | Lata |
| | SB1’ | 3 | 73 | 9.8 | 9.04 | -25.87 | -9.01 | 0.35 | PD | Lata |
| PH | BLUP | 7 | 94 | 60.265 | 41.128 | -238.01 | 67.857 | -0.29 | PD | Lata |
| | SB1 | 7 | 95 | 51.79 | 42.57 | -316.76 | 106.23 | -0.34 | PD | Lata |
| | SB2 | 7 | 96 | 30.97 | 32.17 | -219.56 | 33.92 | -0.15 | A | Lata |
| | SB1’ | 7 | 96 | 29.69 | 29.57 | -219.02 | 48.64 | -0.22 | PD | Lata |
| RA_B_Dia | BLUP | 6 | 67 | 20.363 | 16.602 | -0.2342 | 0.01118 | -0.05 | A | Lata |
| | SB1 | 6 | 71 | 8.02 | 8.5 | -0.34 | 0.21 | -0.62 | PD | Lata |
| | SB2 | 6 | 70 | 14.7 | 13.01 | -0.45 | 0.12 | -0.27 | PD | Lata |
| | SB1’ | 6 | 69 | 7.12 | 6.17 | -0.42 | 0.07 | -0.17 | A | Lata |
| RA_coni | BLUP | 2 | 125 | 12.861 | 8.735 | 9.34 | -1.90 | -0.20 | A | Tiand |
| | SB1 | 2 | 128 | 6.51 | 23.34 | 0.001 | 0 | 0.00 | A | Tiand |
| | SB1’ | 2 | 140 | 8.1 | 7.53 | 0.001 | 0 | 0.00 | A | Tiand |
| RA_slend | BLUP | 2 | 138 | 16.918 | 11.701 | -3.39 | -6.13 | 0.18 | A | Lata |
| | SB1 | 2 | 130 | 8.03 | 7.59 | -5.3 | -0.9 | 0.17 | A | Lata |
| | SB1’ | 2 | 142 | 10.45 | 9.65 | -6.56 | -0.89 | 0.14 | A | Lata |
| RA_slend | BLUP | 3 | 134 | 21.016 | 14.895 | -3.67 | -7.43 | 0.00 | A | Lata |
| | SB1 | 3 | 138 | 9.48 | 9.13 | -5.68 | 1 | -0.18 | A | Lata |
| | SB2 | 3 | 135 | 6.01 | 5.87 | -4.45 | -1.06 | 0.24 | PD | Lata |

*NB: PA_GY = Grain Yield per panicle; SFD = Flowering time; PE_L = Peduncle Length; PH = Plant Height; RA_B_Dia = Rachis base Diameter; RA_coni = Rachis conicity; RA_Slend = Rachis Slenderness; SBI = Chromosome; LOD = Logarithm of Odd; PVE = Percentage of Variance Explained; Add = Additive; Dom = Dominance; Additive gene action (A) if Dom/Add = 0 to 0.20; partial dominance (PD) if Dom/Add = 0.21 to 0.80; dominance (D) if Dom/Add = 0.81 to 1.20; and overdominance (OD) if Dom/Add > 1.20. Additive effects are associated with the allele from Tiandougou. Thus, a negative value means that the Tiandougou allele decreases the value of the trait. Dir_ Parent = Direction of response of the parent whose additive value of a marker allele increased the value of the trait. (Tiand = Tiandougou; Lata = Lata-3).*

The traits such as peduncle length, plant height, rachis conicity and rachis slenderness also displayed consistent QTLs across the sowing dates.

### 4.3.4. Consistent major QTLs cluster

One cluster of QTLs for peduncle length and QTLs for flowering time was detected on SBI-03 (Figure 4.7)

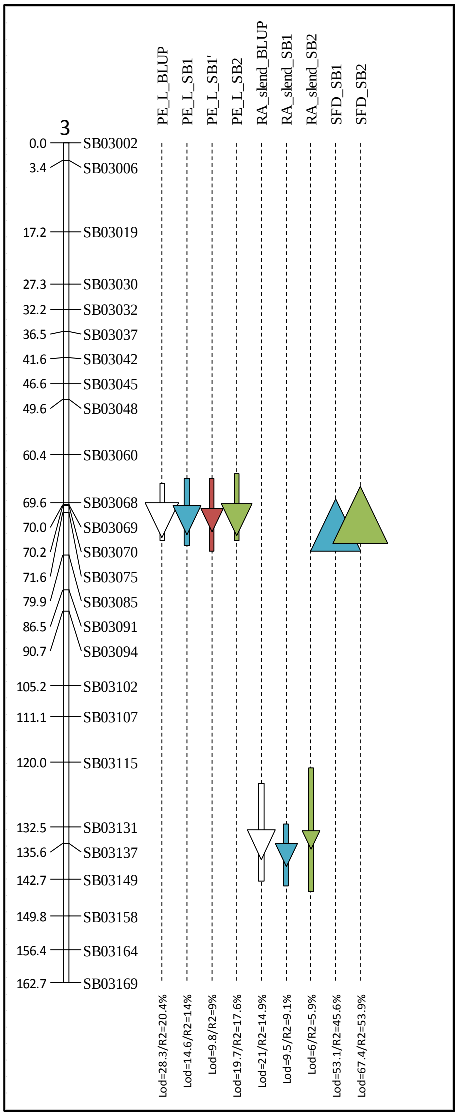


*Figure 4.7: Major consistent QTL cluster on SBI-03*

*NB: Blue color= SB1; Green color = SB2; Red color= SB1' and white color= BLUP*

One major cluster composed by the QTLs for panicle grain yield, number of primary branches per panicle, maximum primary branches per node, primary branches density, number of grains per panicle and rachis base diameter (Figure 4.8).

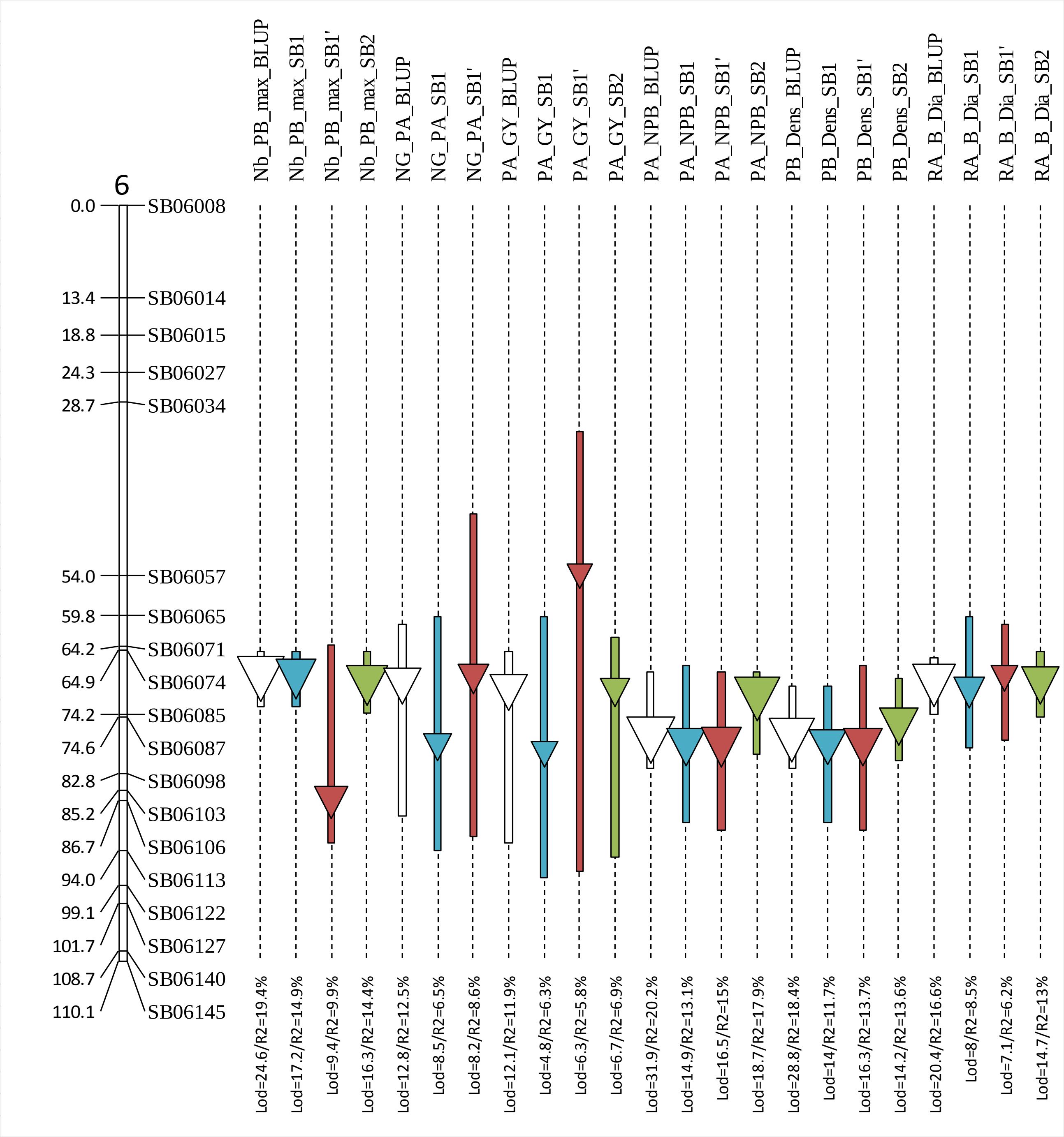


*Figure 4.8: Major consistent QTLs cluster on SBI-06*

*NB: Blue color= SB1; Green color = SB2; Red color= SB1' and white color= BLUP*

### 4.3.5. Pleiotropic effects

Many QTLs influencing multiple traits were observed at different positions on SBI-01, SBI-02, SBI-03, SBI-04 and SBI-06 (Table 4.4-6). Some of the most important traits affected by the pleiotropic QTLs were grain yield per panicle, rachis mean diameter, average primary branch length and panicle weight that were all affected by the same pleiotropic QTL on SBI-06 at the position 70 cM. Pleiotropic QTLs were detected which affect rachis base diameter, number of primary branches per panicle, number of grains per panicle and maximum number of primary branches per node. Rachis length, the longest secondary branch, average primary branch length and number of internodes per panicle were affected by pleiotropic QTLs.

Table 4.4: Pleiotropic QTLs detected on SBI-01 and SBI-02

| | | Traits influenced | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| QTLs | Position cM | *QPH-SBI-02* | *QRA_slend-SBI-02* | *QPB_L_PN-SBI-02* | *QAv_LPB-SBI-02* | *QSB_L_max-SBI-02* | *QPB_BZ_SN-SBI-02* | *QPA_IN_N-SBI-01* | *QPB_Dens-SBI-01* | *QPB_Dens-SBI-02* | *QPos_NPB_max-SBI-01* | *QAv_NG-SBI-02* | *QBending-SBI-02* |
| *QIN_L-SBI-1* | 7 | | | | | | | | | | x | | |
| *QRA_L-SBI-1* | 9 | | | | | | | x | | | | | |
| *QAv_NG-SBI-1* | 12.7 | | | | | | | | x | | | | |
| *QPA_IN_N-SBI-02* | 118 | | | x | | | | | | | | | |
| *QSB_L-SBI-02* | 127 | x | | | | | | | | | | | |
| *QRA_L-SBI-02* | 128 | | | | x | x | | | | | | | |
| *QPB_L_SN-SBI-02* | 140 | | | | | | x | | | | | | |
| *QPA_L-SBI-02* | 141 | | x | | | | | | | | | x | |
| *Qav_NPB-SBI-02* | 146 | | | | | | | | | | | | X |
| *QSC-SBI-02* | 147 | | | | | | | | | x | | | |

On SBI-01, pleiotropic effect was noted between the QTL for internode length and the position of the maximum number of primary branches per node. The traits rachis length and number of internodes per panicle were affected by the same QTL. Average number of grains per primary branch and primary branch density show pleiotropic QTL at the position 12.7 cM.

On SBI-02, seven pleiotropic QTLs were detected. Number of internodes and primary branch length at penultimate node; secondary branch length and plant height; rachis length, average primary branch length and the longest secondary branch were affected by the same QTL. Primary branch length at second node and primary branch zone at second node; average number of primary branch and panicle bending; panicle shape and compactness and primary branch density, displayed pleiotropic effects. A pleiotropic QTL affected panicle length, rachis slenderness and average number of grains per primary branch.

Table 4.5: Pleiotropic QTLs detected on SBI-03 and SBI-04

| QTLs | Position cM | Traits influenced | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *QRA_L-SBI-03* | *QRA_L-SBI-04* | *QRA_coni-SBI-3* | *QRA_coni-SBI-4* | *QPB_L_max-SBI-03* | *SB_L_max-SBI-04* | *PB_BZ_SN-SBI-03* | *PB_BZ_MN-SBI-03* | *Nb_PB_max-SBI-03* | *PA_NPB-SBI-03* | *Nb_SB_MN-SBI-03* | *Nb_SB_PN-SBI-03* | *SB_Dens_SN-SBI-03* | *NG_PA-SBI-03* | *NG_SN-SBI-03* |
| *QNG_MN-SBI-03* | 14 | | | | | | | | | | | x | x | | | |
| *QAv_LPB-SBI-03* | 135 | | | | | | | | x | | | | | | | |
| *QRA_slend-SBI-03* | 137 | | | | | | | | | x | | | | | | |
| *QRA_B_Dia-SBI-03* | 138 | | | | | | | | | | x | | | | | |
| *QPA_L-SBI-03* | 143 | x | | x | | | | | | | | | | | | |
| *QIN_L-SBI-03* | 144 | | | | | x | | x | | | | | | | | x |
| *QPA_W-SBI-03* | 146.3 | | | | | | | | | | | | | | x | |
| *QPB_L_SN-SBI-03* | 148 | | | | | | | | | | | | | x | | |
| *QPA_L-SBI-04* | 55 | | x | | x | | | | | | | | | | | |
| *QIN_L_max-SBI-04* | 56.5 | | | | | | x | | | | | | | | | |

On SBI-03, the QTL for the number of grains at median node influenced the QTLs for the number of secondary branches at median and penultimate nodes. The QTL for internode length showed pleiotropic effect with the QTLs for the longest primary branch, primary branch zone at second node and number of grains at second node. A pleiotropic QTL for panicle length affects rachis length and rachis conicity. The traits average primary branch length and primary branch zone at median node; Rachis slenderness and maximum number of primary branch per node; rachis base diameter and number of primary branch per panicle; panicle weight and number of grains per panicle were affected by the same QTLs.

On SBI-04, pleiotropic QTL for panicle length influenced rachis length and rachis conicity. The longest internode and the longest secondary branch were influenced by the same pleiotropic QTL.

Table 4.6: Pleiotropic QTLs detected on SBI-06 and SBI-07

| | | Traits influenced | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| QTLs | Position cM | *QPE_L-SBI-07* | *QRA__Dia-SBI-06* | *QAv_LPB-SBI-06* | *QNb_PB_max-SBI-06* | *QSB_Dens_SN-SBI-06* | *QPA_W-SBI-06* | *QNG_PA-SBI-06* | *QBending-SBI-07* |
| *QRA_B_Dia-SBI-06* | 69 | | | | x | x | | x | |
| *QPA_GY-SBI-06* | 70 | | x | x | | | x | | |
| *QSC-SBI-07* | 91 | | | | | | | | x |
| *QPH-SBI-07* | 95 | x | | | | | | | |

Two pleiotropic QTLs were detected on SBI-06 and another two on SBI-07.

On SBI-06, rachis base diameter, maximum number of primary branches per node, secondary branch density at second node and number of grains per panicle were affected by the same pleiotropic QTL at the position 69 cM. The QTL for grain yield per panicle displayed pleiotropic effect on rachis mean diameter, average primary branch length and panicle weight.

On SBI-07, panicle compactness and panicle bending were under the same pleiotropic QTL. Also plant height and peduncle length were influenced by a pleiotropic QTL at the position 95 cM.

### 4.3.6. Clustered heatmap analysis

The coded color heat map shows five main genomic regions corresponding to the SBI-01, SBI-02, SBI-03, SBI-04 and SBI-06 in which QTLs for several variable clusters. However, individual QTLs were spread along different chromosomes. Seven clusters or group of variables can be distinguished as follows:

The first cluster (Group-1) shows predominant contribution of Lata-3 alleles (Blue color) in the major QTLs on the SBI-02, SBI-03 and SBI-04. This group was composed of traits corresponding mainly to panicle, primary branches and internode elongation including the longest primary branch and the longest internode (Figure 4.9). These two variables represent a potential of elongation that will be expressed in function of the environment. If the conditions are favorable, the elongation will be at maximum. This cluster indicated relationship between panicle length, basal primary branch length and the longest primary branch and internode.

The second cluster (Group-2) indicated the contribution of Lata-3 in majors QTLs while Tiandougou contributed exclusively in minors QTLs. This group of traits was constituted by the QTLs related mainly to secondary branching and elongation, number of internodes per panicle and grain number on basal branches of the panicle.

In the third cluster, QTLs for peduncle length was observed with Lata-3 alleles in relation to primary branch zone at the middle of the panicle on SBI-03 and SBI-07.

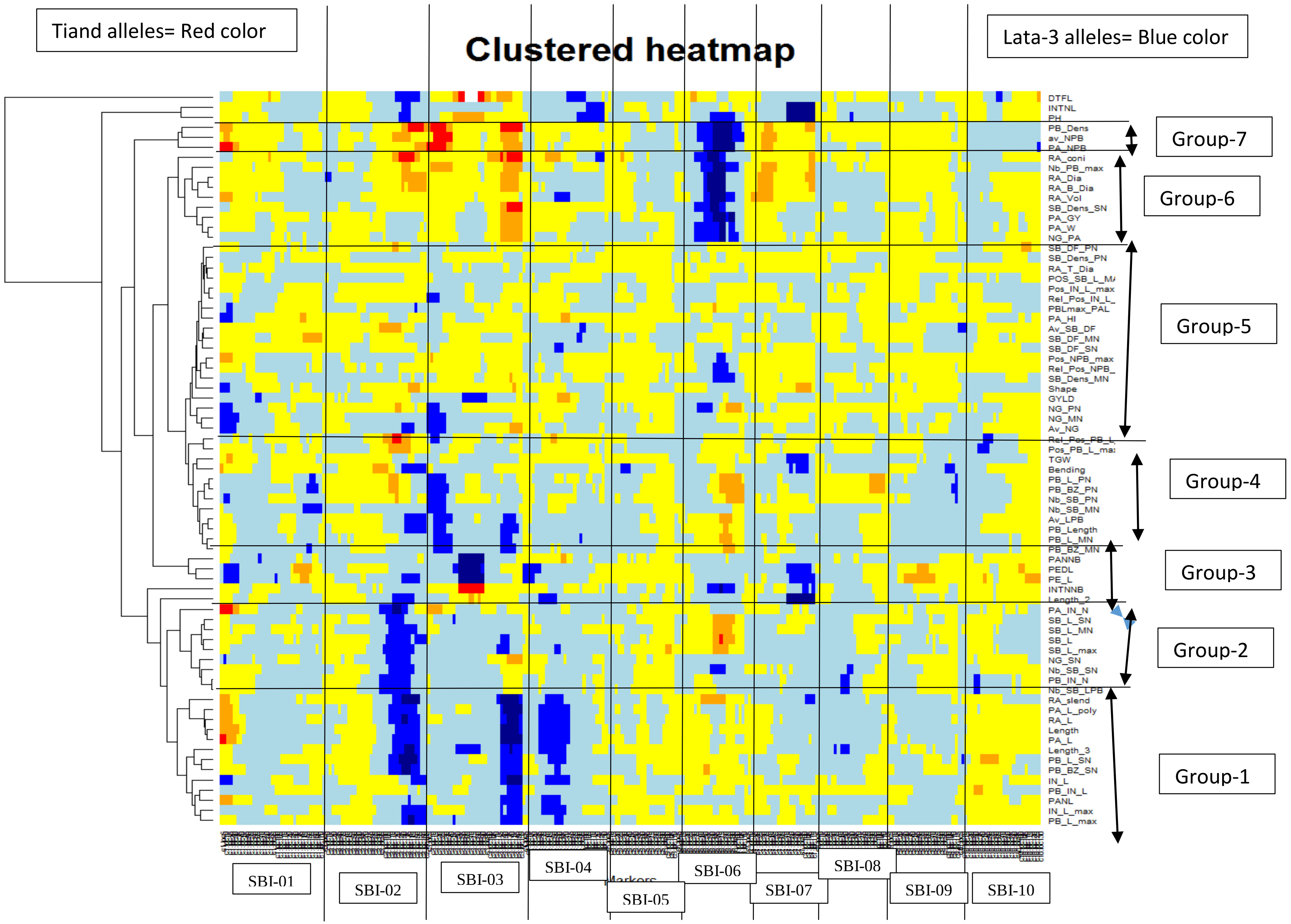


*Figure 4.9: QTLs Clustered heat map for sorghum panicle architecture traits in $F_4$ families derived from bi-parental population*

The fourth cluster presents major QTLs for both parents with more contribution of the parent Lata-3. This cluster was composed of QTLs related to grain weight, secondary branching and primary branch elongation.

In the fifth cluster (Group-5), few QTLs composed of variables at penultimate node and position of the longest primary branch were observed. However, some individual majors QTLs with contribution of Lata-3 and minors QTLs with contribution of Tiandougou were dispersed along all chromosomes except SBI-05 and SBI-08.

The sixth cluster (Group-6) shows grouping of many majors QTLs such as grain yield with the contribution of the two parents at three chromosomes (SBI-02, SBI-03 and SBI-06). This cluster was composed of rachis diameter, branching pattern and grain yield and its components.

The seventh cluster (Group-7) indicated the contribution of alleles from Tiandougou on SBI-03 and Lata-3 on SBI-06 to QTLs for number and density of primary branches.

### 4.3.6. Epistasis effect of QTLs detected in $F_4$ families derived from the cross between Tiandougou and Lata-3

Epistasis plays an important role in genetic determination of sorghum panicle architecture. Several epistasis interactions were observed for sorghum panicle traits, grain yield and its components on all the chromosomes at different positions in the genome (Figure 4.10)

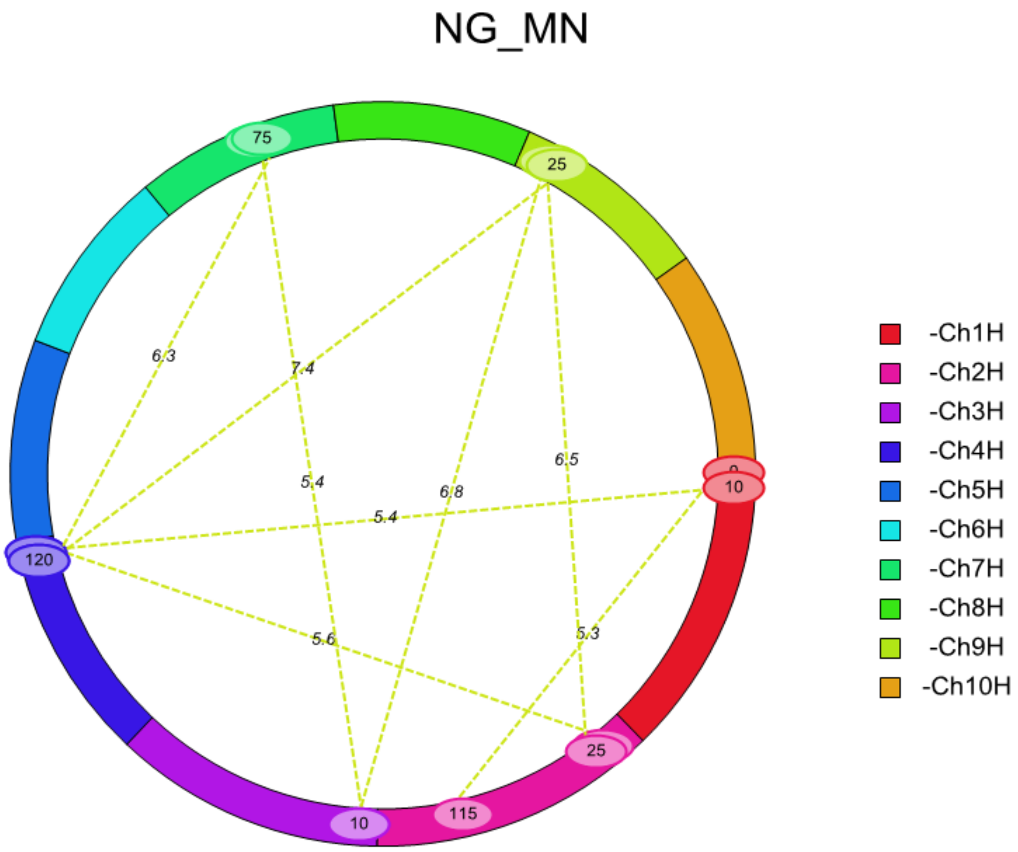


*Figure 4.10: Digenic epistasis detected for number of grains on primary branch at median node (NG_MN) using QTL IciMapping.*

The most important traits displaying major digenic epistasis effects with percentage of variance explained more than 7% are presented in Table 4.7.

Table 4.7: Significant digenic epistasis QTL detected in $F_4$ families derived from Tiandougou x Lata-3 using BLUP data

| TraitName | Chrs | Left_Marker1 | Chrs | Left_Marker2 | PVE | Add by Add |
|---|---|---|---|---|---|---|
| IN_L | 4 | SBI-04122 | 10 | SBI-10043 | 7.6523 | 0.082 |
| RA_Dia | 2 | SBI-02013 | 8 | SBI-08028 | 7.6068 | -0.0586 |
| RA_Vol | 2 | SBI-02086 | 3 | SBI-03164 | 7.228 | 122.8724 |
| PB_L_max | 1 | SBI-01103 | 9 | SBI-09091 | 7.3482 | 2.3068 |
| Av_LPB | 7 | SBI-07067 | 10 | SBI-10012 | 9.3613 | -0.7289 |
| SB_L | 2 | SBI-02013 | 3 | SBI-03102 | 13.0152 | 0.041 |
| SB_L_max | 2 | SBI-02013 | 3 | SBI-03102 | 16.8535 | 0.166 |
| SB_DF_SN | 2 | SBI-02040 | 9 | SBI-09003 | 7.6676 | -0.3041 |
| SB_DF_MN | 6 | SBI-06098 | 8 | SBI-08042 | 7.1256 | -0.4199 |
| Av_SB_DF | 2 | SBI-02013 | 3 | SBI-03002 | 22.1997 | 0.1669 |
| Av_SB_DF | 4 | SBI-04090 | 5 | SBI-05105 | 11.3372 | -0.0288 |
| Pos_NPB_max | 1 | SBI-01062 | 6 | SBI-06106 | 7.2599 | -0.1476 |
| Pos_NPB_max | 8 | SBI-08042 | 10 | SBI-10064 | 7.0829 | -0.3316 |
| NG_MN | 3 | SBI-03006 | 9 | SBI-09014 | 18.03 | -0.2119 |
| NG_MN | 4 | SBI-04136 | 7 | SBI-07067 | 15.3263 | -0.097 |
| NG_MN | 4 | SBI-04136 | 9 | SBI-09014 | 24.6544 | -1.0632 |
| Av_NG | 1 | SBI-01038 | 2 | SBI-02013 | 18.5469 | 0.2117 |
| Av_NG | 2 | SBI-02013 | 3 | SBI-03137 | 18.3073 | -0.3306 |
| Av_NG | 2 | SBI-02013 | 6 | SBI-06127 | 17.0226 | 0.0658 |

NB: PVE = Percentage of Variance Explained; addxadd = additive x additive; chrs = chromosomes

A total of nineteen significant digenic epistatic interactions (additive by additive) were detected for 13 traits using the BLUP extracted from all three sowing dates indicating that two loci interactions were widespread in the entire genome. A total of three digenic epistasis were found in number of grains per primary branch at median node and average number of grains per primary branch. Two were found in average distances to the first secondary branch on the primary branch and position of the maximum number of primary branches per node. One digenic epistasis QTLs was found in each of the following traits: internode length, rachis mean diameter, rachis volume, the longest primary branch, average primary branch length, secondary branch length, the longest secondary branch on the longest primary branch, distance to the first secondary branch at second and median nodes.

Internode length, the one significant additive x additive interactions, was detected between SBI-04 and SBI-10 with the effect of 0.082 explaining 7.65% of phenotypic variance. For rachis mean diameter, additive x additive interaction was observed between SBI-02 and SBI-08 with negative effects of -0.05 and 7.60% of phenotypic variance explained by the epistasis QTL. Positive Epistasis effect of 122. 87 was noted for rachis volume between SBI-02 and SBI-03 with the percentage of variation explained by the epistasis QTL of 7.22%. The one significant additive x additive interaction detected for the longest primary branch explained 7.34% of phenotypic variance with 2.30 interaction effects. Average primary branch length displayed negative additive x additive interaction between SBI-07 and SBI-10 with the percentage of phenotypic variance of 9.36% explained. Between SBI-02 and SBI-03 epistasis effect was noted for secondary branch length with 13.01% of phenotypic variance explained. For the longest secondary branch on the longest primary branch the one significant epistasis QTL was detected on the SBI-02 and SBI-03 with the percentage of variance explained of 13.85%. The additive x additive interaction observed for distance to the first secondary branch at second and median nodes were between SBI-02 and SBI-09 at second node and between SBI-06 and SBI-08 at median node with respectively 7.66 and 7.12% of phenotypic variance explained.

The total phenotypic variation explained by interactions for average distance to the first secondary branch on the primary branch and position of the maximum number of primary branch per node were 33.54% and 14.34% respectively from two digenic pairs; number of grains on primary branch at median node and average number of grains per primary branch were respectively 58.01% and 53.88% from three digenic pairs.

In general, for the traits with major digenic epistasis interaction, the total phenotypic variations explained by the interactions were greater than that of corresponding main effects for the different traits. The importance of additive x additive interaction effects in total genetic effects may be trait-dependent.

### 4.3.7. Identification of QTLs with overlapping confidence interval for sorghum panicle architecture traits across reported studies

QTLs with overlapping confidence interval for panicle architecture traits from four previous studies and the present study were identified for the following traits: primary branch length, secondary branch number at second node, distance to the first secondary branch on the primary branch, panicle length, panicle weight and thousand grain weight (Table 4.8). Most of the QTLs identified were composed of two individual QTLs for the concerned traits. Primary branch length presented the maximum QTL with overlapping confidence interval with four individual QTLs on SBI-03. The present study on panicle architecture using $F_4$ families confirmed results from Pereira *et al*. (1995) and Brown *et al*. (2006) on SBI-03 two QTLs with overlapping confidence interval for primary branch length.

Table 4.8: QTLs with overlapping confidence interval identified from five (5) studies on sorghum panicle architecture traits.

| Panicle traits | Number of QTLs | Chrs | Mean CI position | CI Start | CI End | LOD | PVE | Sources |
|---|---|---|---|---|---|---|---|---|
| Primary branch length | 2 | SBI-01 | 69.5 | 60.3 | 78.7 | 6.6 | 19.0 | Pereira *et al*. (1995) |
| | | | 65.8 | 61.5 | 70.1 | 7.0 | 13.8 | Brown *et al*. (2006) |
| Primary branch length | 4 | SBI-03 | 22.5 | 0.7 | 44.3 | 2.6 | 8.0 | Pereira *et al*. (1995) |
| | | | 13.3 | 8.7 | 17.8 | 6.5 | 13.0 | Brown *et al*. (2006) |
| | | | 13.0 | 6.0 | 16.0 | 4.4 | 4.1 | This study |
| | | | 14.0 | 8.0 | 26.0 | 4.5 | 4.5 | This study |
| Panicle length | 2 | SBI-02 | 141.0 | 137.7 | 144.3 | 4.9 | 14.7 | Srinivas *et al*. (2009) |
| | | | 141.0 | 125.0 | 147.0 | 8.6 | 6.9 | This study |
| Panicle length | 2 | SBI-03 | 155.0 | 135.6 | 174.4 | 3.0 | 9.0 | Pereira *et al*. (1995) |
| | | | 143.0 | 135.0 | 147.0 | 13.4 | 11.2 | This study |
| Panicle weight | 2 | SBI-06 | 84.0 | 80.7 | 87.3 | 4.9 | 14.7 | Srinivas *et al*. (2009) |
| | | | 70 | 64.9 | 78.0 | 12.8 | 12.3 | This study |

For panicle length, this study showed two QTLs with overlapping confidence interval with the study of Srinivas *et al*. (2009) on SBI-02 and Pereira *et al*. (1995) on SBI-03. QTLs with overlapping confidence interval were recorded on SBI-06 for panicle weight in the Srinivas *et al*. (2009) study and in this study. However this study showed no QTL with overlapping confidence interval with those already identified (result not shown) for primary branch length on SBI-02 (Pereira *et al*., 1995 and Brown *et al*., 2006) and thousand seed weight on SBI-07 (Rami *et al*., 1998). Distance to the first secondary branch or the sterile portion of the seed branch seems to be affected by the genetic background of the different parents used in the population. Indeed QTLs with overlapping confidence interval were identified for each specific study.

## 4.4. Discussion

A great number of QTLs were identified for sorghum panicle architecture traits in different studies. Some interesting QTLs detected across studies were used in the current discussion. Pereira *et al*. (1995) identified three QTLs for primary branch number per panicle on SBI-02, SBI-07 and SBI-08. Brown *et al*. (2006) reported two QTLs for primary branch number on SBI-03 and SBI-06 and Srinivas *et al*. (2009) identified four QTLs for primary branch number per panicle on SBI-01, SBI-05, SBI-07 and SBI-08 that did not relate to the QTLs detected by Brown *et al*. (2006). In this study, QTLs for primary branch number were detected on SBI-02, SBI-03, SBI-06 and SBI-07. One common QTL for primary branch number detected on SBI-03 seems to be similar to the one detected by Brown *et al*. (2006) based on the position of the QTLs in the genome. Three other common QTLs on the SBI-02, SBI-06 and SBI-07 across studies were different and situated very far away from each other in the genome. This result may suggest that depending on the genetic background. QTLs for the number of primary branches per panicle were less closely related. Therefore, there were different loci regulating the primary branching pattern in sorghum panicle resulting in their involvement in panicle architecture. Rachis diameter QTLs were detected on SBI-02, SBI-03, SBI-06, SBI-07 and SBI-10 in this study. Brown *et al*. (2006) reported one QTL for rachis diameter on SBI-06. However the one common QTLs on SBI-06 from this study and the study of Brown *et al*. (2006) were respectively detected at the position 69 and 55. These positions were close and it is possible to assume that they were the same QTLs. Brown *et al*. (2006) reported four QTLs for primary branch length, two on SBI-01 and one on each of SBI-03 and SBI-07 while this study detected five QTLs two on SBI-03 and one on each SBI-02, SBI-06 and SBI-07. The QTLs detected on SBI-03 and SBI-07 in the two studies were different.

Thousand seed weight QTLs were identified by Rami *et al*. (1998) on SBI-01, SBI-03 and SBI-07, Murray *et al*. (2008) also reported three QTLs for thousand seed weight on SBI-01, SBI-06 and SBI-08. In this study two different QTLs on SBI-03 and SBI-07 other than those detected by Rami *et al*. (1998) for thousand seed weight were found based on the position in the genome. Feltus *et al*. (2006) found one QTLs on SBI-10 for panicle weight while Srinivas *et al*. (2009) reported one QTL for panicle weight on SBI-06. This study identified two QTLs for panicle weight on SBI-01 and SBI-06. The QTLs on SBI-06 from Srinivas and this study were close in the genome respectively at position 84 and 70 cM. The proximity of location on the chromosome may suggest that they are the same QTL. Rami *et al*. (1998) reported four QTLs for Number of kernels per panicle. Two QTLs were detected on SBI-01, one on SBI-02 and another one on SBI-07 while this study identified two QTLs on SBI-02 and SBI-06. The QTLs detected on SBI-02 in these studies were at different positions on the chromosome. Rami *et al*. (1998) and Pereira *et al*. (1995) found two QTLs for panicle length, this study identified one QTL on SBI-01. These QTLs were spread along SBI-01. On SBI-02, this study detected one QTL for panicle length that was not similar to any of the QTLs detected and reported in the literature. Rami *et al*. (1998) found two QTLs for panicle length while Parh (2005) and Srinivas *et al*. (2009) reported one QTL for panicle length on

SBI-02. Pereira *et al*. (1995) and Rami *et al*. (1998) also reported one QTL for panicle length. This study identified one QTL on SBI-03 that is close to the QTL detected by Pereira *et al*. (1995). There were no QTLs detected in this study on SBI-06, SBI-07, SBI-08, SBI-09 and SBI-10. Parh (2005) and Srinivas *et al*. (2009), however, found two QTLs for panicle length on SBI-06. Rami *et al*. (1998) and Pereira *et al*. (1995) reported one QTL for panicle length on SBI-06. Hart *et al*. (2001), Srinivas *et al*. (2009), Rami *et al*. (1998) and Pereira *et al*. (1995) reported one QTL for panicle length on SBI-07. Hart *et al*. (2001) reported one QTL for panicle length on SBI-08 and SBI-09. Rami *et al*. (1998) and Pereira *et al*. (1995) reported one QTL for panicle length on SBI-10.

QTLs for grain yield per panicle were reported by Ritter *et al*. (2008) in R9188/R9403463-2-1 sweet sorghum population on SBI-03 at the position 133 cM in the genome and Srinivas *et al*. (2009) in 296B/IS18551 sorghum on SBI-06 at 83.5 cM. In this study two QTLs were detected for grain yield per panicle on SBI-03 at 146.3 cM and SBI-06 at 70 cM in Tiandougou/Lata-3 population. The QTL for grain yield per panicle detected on SBI-06 in this study is likely the same detected by Srinivas *et al*. (2009) based on their positions in sorghum genome. Nagaraja Reddy *et al*. (2013) detected one major QTL for grain yield per panicle on SBI-06 and one minor QTL on SBI-03. The position of these QTLs are not related to the QTLs for grain yield per panicle detected in this study. Traits correlated are known generally to be mapped together (Hittalmani *et al*., 2002), Lin *et al*. (1995) and Klein *et al*. (2008) detected pleiotropic QTLs for maturity and height. Zou *et al*. (2012) detected pleiotropic QTLs for heading date, number of nodes, plant height and stem diameter in long and short day photoperiod.

Pleiotropic QTLs in this study suggest that increasing grain yield may be obtained by positively influencing primary branch length, rachis diameter and the weight of the panicle. Nagaraja Reddy *et al*. (2013) also found pleiotropic QTL affecting grain yield per panicle and panicle weight. Rachis growth and primary branches pattern are under the same genetic control. The number of internodes per panicle determine rachis, primary and secondary branches elongation and flowering time might be affected by the mechanism regulating peduncle elongation. Srinivas *et al. (2009)* reported the QTL cluster for plant height, days to anthesis, green leaf area at maturity, panicle length, grain yield, panicle weight, and seed weight. In this study, QTLs clustered heatmap confirmed the link between panicle grain yield and some panicle architecture traits such as the number of primary branches per panicle and rachis base diameter. Indeed this cluster points out the traditional relationship between grain yield and grain yield components like panicle weight and number of grains per panicle. There was somehow a link between plant growths in term of plant height that may also affect the grain yield. Therefore, the improvement of sorghum grain yield should combine certain grain yield components as well as associating some of panicle architecture traits such as secondary branch density at the base of the panicle, rachis base diameter and the number of primary branches per panicle.

Epistasis refers to the phenotypic effects of interactions among alleles at multiple loci (Xing *et al*., 2002). The advent of molecular makers and their utilization has revealed that epistastatic interactions play an important role on the genetic basis of quantitative traits (Paterson *et al*., 1991; Li *et al*., 1997). Liao *et al*. (2001) suggests that there are three types of epistasis affecting complex traits: (1) interactions between QTL, (2) interactions between QTL and modifying loci, and (3) interaction between complementary loci. Shiringani (2009) reported epistasis in seven out of ten traits studied, Digenic epistasis interactions were observed to be very important in the pattern of the number of grains per primary branches and the length of the secondary branches in this study. Routman and Cheverud. (1997) reported that epistasis in both negative and positive directions is important for the breeding populations even if the net effects are not observed. Overlapping confidence interval of QTLs across studies on sorghum panicle architecture denote the consistency of these QTLs and may be used in QTL Meta- analysis to declare them as Meta-QTL by refining their position and by projecting onto the unique consensus genetic map.

## 4.5. Conclusion

Both additive and non-additive gene effects control most of sorghum panicle architecture traits. Additive gene action was determinant in the control of the number of internodes per panicle, primary branches zone and maximum primary branches per node, while the length and number of secondary branches were governed by non-additive gene effects. Grain yield and some of its components such as panicle weight and thousand grain weight were under non additive gene action. Number of grains per panicle was predominantly governed by additive gene effects; however, the number of grains per primary branches at different positions in the panicle was controlled by both additive and non-additive gene action. Most of the major QTLs were consistently detected across the three sowing dates. QTLs clustered for grain yield per panicle, rachis diameter, number of primary branches per panicle and secondary branch density at the base of the panicle were observed in this study. Therefore it is possible to improve grain yield through indirect selection for these traits.

Fourteen out of forty eight traits studied showed digenic epistasis interaction indicating the important role of epistasis in the regulation of the pattern of internode length, primary and secondary branches length and the number of grains per primary branch that showed the maximum percentage of variation explained (36%).

QTLs showing multiple effects or pleiotrophism were detected on six chromosomes out of ten. The major pleiotropic QTL affecting grain yield per panicle, rachis mean diameter, average primary branch length and panicle weight was found at 70 cM on SBI-06. Enhancement of grain yield-related traits could be faster if pleiotropic effects favor the positive alleles.

QTLs with overlapping confidence intervals were consistently identified across studies for primary branch length, panicle length and panicle weight. The flanking markers both left and right SB06074 and SB06098 of grain yield per panicle QTL on SBI-06 were consistently associated with QTLs for panicle weight, secondary branch length and primary branch density, whereas the flanking markers both left and right SB03149 and SB03164 of grain yield per panicle QTL on SBI-03 were consistently associated with QTLs for thousand grain weight, internode length and the longest secondary branch.

# CHAPTER FIVE

## 5.0. IDENTIFICATION OF CANDIDATE GENES INVOLVED IN SORGHUM PANICLE ARCHITECTURE IN MAIZE AND RICE

### 5.1. Introduction

Sorghum panicle architecture is determined by meristem activities during the reproductive phase. Axillary meristems specify the formation of branches and spikelets which are defined as short branch floret-bearing branches in the panicle. Axillary meristem initiation and development are regulated by developmental, environmental, and hormonal controls (Barazesh and McSteen, 2008). Understanding the regulation of axillary meristem formation is essential to improving grain crops as panicle structure directly affects grain yield. The genes required to maintain spikelet meristem identity belong to the SEPALLATA group. Sorghum MADS box genes *SbMADS1* and *SbMADS2* are the two MADS box genes whose cDNAs have been isolated from sorghum (Greco *et al*., 1997). PANICLE PHYTOMER2 (*PAP2* or rice *MADS34*) controls loss of function in a panicle composed of many primary, but few secondary, branches because many of the early spikelets are transformed into rachis branches (Kobayashi *et al*., 2010; Gao *et al*., 2010). Also the APETALA2 (*AP2*) family genes, such as *AP2-like* genes, have been implicated in spikelet determinacy and the transition to the floral meristem. These include BRANCHED SILKLESS1/FRIZZY PANICLE1/BRANCHED FLORETLESS 1 (*BD1/FZP1/BFL1*) (Komatsu *et al*., 2003b; Chuck *et al*., 2002), INDETERMINATE SPIKELET 1 (*ZmIDS1/OsIDS1*) (Chuck *et al*., 2008; Chuck *et al*., 1998) and SISTER OF INDETERMINATE SPIKELET1/SUPER NUMERARY BRACT1 (*ZmSID1/OsSNB1*) (Chuck *et al*., 2008; Lee *et al*., 2007). It has been reported that *OsSNB1* and

*OsIDS1* act synergistically to regulate the transition of spikelet meristem into floral meristem and to control inflorescence architecture in rice (Lee and An, 2012).

Several downstream genes have been identified as being involved in spikelet meristem identity in rice; these include ABERRANT PANICLE ORGANIZATION1 (*APO1*) (which encodes an F-box protein related to Arabidopsis UFO) (Sreenivasulu and Schnurbusch, 2012). The panicles of *APO1* loss-of-function mutants form fewer spikelets than the wild-type panicles. Elevated transcript levels of *APO1* in the rice stems or panicles are accompanied by a higher growth rate of the inflorescence meristem and by enlarged vascular bundles, thus producing a higher number of spikelets per panicle (Terao *et al*., 2010; Ikeda-Kawakatsu *et al*., 2009).

Spikelet, the primary panicle architecture unit in sorghum, development is dictated by several specific genes. Spikelets bear one to several florets and each floret develops a set of grain contributing to yield potential and final grain number. The ability to manipulate the spikelet formation process may allow for re-engineering of panicle architecture in a way that increases yield potential (Sreenivasulu and Schnurbusch, 2012).

Sorghum, together with maize and sugarcane, belongs to the group of grasses that develop spikelet pairs and the ramosa pathway is particularly important (Kellogg, 2007; McSteen *et al*., 2007; Vollbrecht *et al*., 2005). In maize, analyses of the highly branched mutants *Zmramosa1* (*ra1*), *Zmra2* and *Zmra3* have revealed a pathway that negatively regulates inflorescence branching (Bortiri *et al*., 2006; Vollbrecht *et al*., 2005). Only *ramosa2*, which is a putative LOB domain-containing a transcription factor, is well conserved across the Poaceae family (Kellogg, 2007).

In rice, MONOCULM 1, MOC1 (Li *et al*., 2003), LAX PANICLE 1, *LAX1* (Komatsu *et al*., 2003a), LAX PANICLE 2, *LAX2* (Tabuchi *et al*., 2011) and SHORT PANICLE 1, *SP1* (Li *et al*., 2009), all regulate the degree of panicle branching, as well as panicle size. It was established that *LAX1* and *LAX2* synergistically function in regulating the axillary meristem and thereby panicle branching

(Tabuchi *et al*., 2011). Higher numbers of branches and spikelets have been associated with increased grain number associated with the recessive *Gn1a* (=*OsCKX2*) allele in rice (Ashikari *et al*., 2005). Huang *et al*. (2009) reported that Dense and Erect panicle1 (*DEP1*) acts upstream of OsCKX2 to control cytokinin homeostasis in the panicle meristem (Huang *et al*., 2009). To date, only a few number of genes have been implicated in controlling panicle architecture in sorghum (Brown *et al*., 2006). The need to identify and characterize the function of novel panicle architecture genes cannot be ever emphasized. Therefore, the objectives of this study were 1). to identify candidate genes regulating sorghum panicle architecture. 2). to establish protein sequence similarity with sorghum, maize and rice genes and 3). to determine co-localization between sorghum candidate gene predictions with QTLs detected for panicle architecture traits in Tiandougou/ Lata-3 segregating population.

## 5.2. Methods

An inventory of genes known to be involved in panicle architecture in rice and maize was made based on the literature and their homologs were retrieved from the sorghum genome sequence based on protein sequence similarity. The hypothesis underlying this work was that genes involved in panicle architecture in species closely related to sorghum, such as rice (*Oryza sativa*) and maize (*Zea mays*), have conserved similar functions in Sorghum and could be good candidate genes for the control of panicle architecture in sorghum.

Rice and maize protein sequences corresponding to genes involved in meristem regulation during inflorescence development, specification of the spikelet meristem identity and plant growth and development were retrieved from the NCBI (National Center for Biotechnology Information) database (http://www.ncbi.nlm.nih.gov/). A total of 43 protein sequences were mined from the

NCBI database, including 12 accessions common to rice and maize; 21 accessions specific to rice and 10 accessions specific to maize.

Sorghum homologs for these genes were searched *in silico* within the sorghum whole-genome sequence v.1.4. (Paterson *et al*., 2009) using BLASTP (Basic Local Alignment Search Tool for Protein) versus sorghum gene predictions (amino acid) (http://www.phytozome.net/search.php?show=blast&method=Org_Sbicolor_v1.4).

Twenty five (25) gene predictions having the best BLAST (Basic Local Alignment Search Tool) expected values were selected for each rice and maize genes searched. The chromosome and their positions were noted in order to identify the best rice and maize proteins related to each sorghum gene prediction (E-value <1E-50). Alignments and phylogenetic tree analyses were carried out to determine the relationship between sorghum gene predictions and maize and rice genes. Predicted sorghum genes were named based on their similarity with rice and maize proteins. Deduced amino acid sequences were analyzed using the Phylogeny.fr platform (http://www.phylogeny.fr) using the one click mode that includes the pipeline chaining programs: MUSCLE for multiple alignment (Edgar, 2004), PhyML for tree building (Guindon and Gascuel, 2003; Anisimova and Gascuel, 2006), and TreeDyn for tree drawing (Chevenet *et al*., 2006). The tree building was based on an approximation of the maximum likelihood ratio test. A physical map was generated that included the positions in Megabases (Mb) of the predicted genes and of the SNP markers present on the genetic map. The positions of the QTLs detected on the Tiandougou/Lata-3 genetic map without candidate genes were then compared with the physical map.

## 5.3. Results

The availability of sorghum genome sequences allowed the search for candidate genes involved in meristem regulation during inflorescence development and specification of spikelet meristem identity in relation to plant growth and development. Therefore, co-localization of candidate genes and QTLs detected for panicle architecture traits that provide new information on putative genetic control of sorghum panicle architecture could be established. However, it is necessary to note that co-localization between candidate genes and QTLs clusters was not always evident for a large number of candidate genes involved in inflorescence architecture. A total of 42 candidate genes for inflorescence architecture, growth and development were selected on the basis of their known function in rice and maize. The list of selected candidate genes included 17 genes related to meristem regulation during inflorescence development (Table 5.1); 15 related to specification of spikelet meristem identity (Table 5.2) and 10 related to plant growth and development (Table 5.3). The candidate gene identification gave an indication of the position in the genome of the 43 genes involved in inflorescence, growth and development and enabled determination of predicted genes in sorghum named on the basis of similarity with rice and maize genes. Emphasis has been maintained on 25 candidate genes showing high sequence similarity and co-localization with the QTLs detected for sorghum panicle architecture traits in relation to the three traits concerned, which are meristem regulation during inflorescence development, specification of spikelet meristem identity and plant growth and development.

### 5.3.1. Candidate genes related to meristem regulation during inflorescence development

The focus on candidate genes for meristem regulation during inflorescence development (Table 5.1), was with regard to Aberrant spikelet and panicle1 (*ASP1*) in rice which encodes a transcriptional co-repressor important in branching pattern, transition from the branch meristem to the spikelet meristem and stem cell maintenance in both the branch meristem and the spikelet meristem. The function of this gene is also closely associated with auxin action. Therefore, in rice, ASP1 regulates various aspects of developmental processes and physiological responses as a transcriptional co-repressor (Yoshida *et al*., 2012). The corresponding genes to ASP1 in maize is RAMOSA ENHANCER LOCUS2 (*REL2*) which is a recessive mutation affecting both tassel and ear branching (Soderlund *et al*., 2009). These candidate genes co-localized with the QTLs for average primary branch length and density on SBI-07 at the position 5.31 cM. This suggest that the sorghum homologue of *ASP1* (*SbASP1*) may be responsible for branch pattering during panicle development and elongation.

Lax panicle1 (*LAX1*) encodes a basic helix-loop-helix (bHLH) domain transcription factor. In rice, *LAX1* regulates the number of branches as a mutation affecting *LAX1* presents fewer branches in the inflorescence (Komatsu *et al*., 2003a). *LAX1* protein sequence showed high sequence similarity to the sorghum Sb03g038820 predicted gene. Sorghum *LAX1* (*SbLAX1*) on SBI-03 co-localize with the QTLs for number of primary branches per panicle; maximum number of primary branches per node and rachis base diameter. This result suggests that *LAX1* in sorghum may regulate primary branch number and also play an important role in rachis size.

The rice *Gn1a/OsCKX2* (Grain number 1a/Cytokinin oxidase 2) gene, which encodes a cytokinin oxidase, has been identified as a major quantitative trait locus contributing to grain number improvement in rice. The candidate gene GRAIN NUMBER 1a (*GN1a*)/*osCKX2* in rice accession Q4ADV8 (Ashikari *et al*., 2005) had sequence similarity with sorghum gene prediction (Sb03g002810). *GN1* encodes cytokinin oxidase/dehydrogenase, an enzyme that catalyzes the degradation of cytokinin, implying the involvement of that phytohormone in panicle branching (Ashikari *et al*., 2005). Sorghum GRAIN NUMBER 1a (*SbGN1a*) on SBI-03 at the position 2.72 cM co-localized with the QTLs for average grain number per primary branch and the QTLs for grain number on the primary branch at median and penultimate nodes. This suggests that this gene in sorghum regulates grain number at different stages of primary branches on the main rachis and may act upstream of grain production at a different stratum of branching.

Table 5.1: List of candidate genes related to meristem regulation during inflorescence development

| Sorghum name | Sorghum gene prediction # | Chr | Position | Reference gene in *Oryza sativa* | | | Reference gene in *Zea mays* | | | QTL co-location | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Oryza name | Oryza accession # | E-value | Zea name | Zea accession # | E-value | | |
| ***Sbra2*** | **Sb03g004590** | **3** | **4,862** | | | | ***ramosa2* (*Zmra2*)** | **ABC54560** | **4,20E-102** | **Av_LPB, PB_L_MN** | **Bortiri *et al.* 2006** |
| *Sbra3* | Sb02g039820 | 2 | 73,973 | | | | *ramosa3* (*Zmra3*) | ABD92779 | 3,50E-149 | | Satoh-Nagasawa *et al.* 2006 |
| *Sbsra3* | Sb02g039810 | 2 | 73,961 | | | | *Sister of ramosa3* | DAA63682 | 2,00E-160 | | |
| ***Sbfl*** | **Sb06g027340** | **6** | **56,245** | | | | ***floricaula (zfl1, zfl2)*** | **AAO43174** | **8,70E-132** | **Nb_SB_SN** | **Bomblies *et al.* 2003** |
| *Sbfea2* | Sb04g025240 | 4 | 55,066 | | | | *fasciated ear2 (fea2)* | AAL17871 | 0,00E+00 | | Taguchi-Shiobara *et al.* 2001 |
| *SbTu1* | Sb04g033930 | 4 | 63,833 | | | | *Tunicate1 (Tu1)* | NP_001105148 | 5,10E-106 | PH | Mendez-Vigo *et al.* 2013 |
| ***SbASP1*** | **Sb07g004180** | **7** | **5,318** | ***ABERRANT SPIKELET AND PANICLE1 (ASP1)*** | **BAL44266** | **0,00E+00** | ***ramosa enhancer locus2 (rel2)*** | **NP_001167872** | **0,00E+00** | **Av_LPB, PB_Dens** | **Yoshida *et al.* 2012; Soderlund *et al.* 2009** |
| *Sbbif2* | Sb08g021520 | 8 | 53,232 | PINOID (OsPID) | Q2QM77 | 2,50E-135 | *barren inflorescence2 (bif2)* | ABR13340 | 2,50E-130 | | McSteen *et al.* 2007 |
| ***SbLAX1*** | **Sb03g038820** | **3** | **66,623** | ***LAX PANICLE 1 (LAX1)*** | **Q7XAQ6** | **6,00E-47** | | | | **PA_NPB, Nb_PB_max, RA_B_Dia** | **Komatsu *et al.* 2003a** |
| *SbLAX2* | Sb06g014520 | 6 | 40,193 | *LAX PANICLE 2 (LAX2)* | BAL03066 | 2,70E-19 | | | | | Tabuchi *et al.* 2011 |
| *Sbba1* | Sb02g026970 | 2 | 62,203 | | | | *BARREN STALK (ba1)* | AEL78914 | 7,30E-72 | PA_IN_N | Gallavotti *et al.* 2011 |
| *SbAPO1* | Sb10g026580 | 10 | 56,003 | *ABERRANT PANICLE ORGANIZATION1 (APO1)* | BAF75467 | 3,60E-129 | | | | PE_L, PH | Ikeda *et al.* 2007 |
| *SbMOC1* | Sb10g023950 | 10 | 52,722 | *MONOCULM 1 (MOC1)* | BAD35485 | 6,80E-136 | | | | | |
| ***SbCKX2*** | **Sb03g002810** | **3** | **2,722** | ***GRAIN NUMBER 1a (GN1a)/OsCKX2*** | **Q4ADV8** | **4,40E-150** | | | | **Av_NG, NG_MN, NG_PN** | **Ashikari *et al.* 2005** |
| *SbDEP1* | Sb02g025860 | 2 | 60,87 | *DENSE AND ERECT PANICLE 1 (DEP1)* | ACI25447 | 2,60E-33 | | | | Nb_SB_LPB | Huang *et al.* 2009 |
| *SbIPA1* | Sb07g027740 | 7 | 62,69 | *IDEAL PLANT ARCHITECTURE (IPA1)* | ADJ19220 | 2,50E-105 | | | | RA_Bdia, RA_Dia, RA_Slend | Jiao *et al.* 2010 |
| *SbGS3* | Sb01g032830 | 1 | 55,779 | *GRAIN LENGTH AND WEIGHT PROTEIN (GS3)* | ABC84855 | 1,20E-32 | | | | | Fan *et al.* 2006 |

### 5.3.2. Candidate genes related to specification of the spikelet meristem identity

For candidate genes related to specification of the spikelet meristem identity (Table 5.2), focus has been on rice FRIZZY PANICLE (*FZP*), an ortholog of the maize BRANCHED SILKLESS 1 (*BD1*), that regulates spikelet meristem identity (Chuck *et al*., 2002; Komatsu *et al*., 2003b) and has sequence similarity with sorghum prediction Sb02g042400 on 2 and co-localize with the QTLs for the number of primary branches per panicle in this study. This result may indicate that spikelet meristem development may act on the whole process of branch formation. Maize INDETERMINATE SPIKELET1 (*IDS1*) regulates spikelet-meristem determinacy (Chuck *et al*., 1998). Spikelet of the IDS1 mutant are indeterminate, producing extra florets. SISTER OF IDS1 (*SID1*), a paralog in maize, is important for floral meristem initiation and spikelet regulation (Chuck *et al*., 2008). *OsIDS1* positively regulates panicle branching by repressing spikelet meristem identity (Yeon Lee and An, 2012).

In this study, INDETERMINATE SPIKELET1 (*OsIDS1; ZmIDS1*) candidate genes in rice and maize presented sequence similarity with sorghum gene prediction (Sb01g003400) on SBI-01 at the position 2.73 which co-localized with the QTLs for average grain number per primary branch and thousand grain weight. The *OsFOR1* (*Oryza sativa floral organ regulator 1*) gene encodes a protein that contains a leucine-rich repeat (LRR) domain and suggests that *OsFOR1* plays a role in the formation and/or maintenance of floral organ primordia. In this study, the candidate gene FLORAL ORGAN REGULATOR1 (*OsFOR1*) in rice accession AA017320 showed sequence similarity with sorghum gene prediction (Sb02g036750) and co-localize with QTLs for panicle shape and compactness and average primary branches per node. This result suggests that *SbFOR1* may play a role in the organization of the panicle that determines sorghum panicle shape and compactness.

Table 5.2: List of candidate genes related to specification of spikelet meristem identity

| Sorghum name | Sorghum gene prediction # | Chr | Position | **Reference gene in *Oryza sativa*** | | | **Reference gene in *Zea mays*** | | | QTL co-location | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Oryza name | Oryza accession # | E-value | Zea name | Zea accession # | E-value | | |
| **Specification of the spikelet meristem identity** | | | | | | | | | | | |
| ***SbBD1*** | **Sb02g042400** | **2** | **76,137** | **FRIZZY PANICLE (*OsFZP*)** | **BAC79264** | **4,20E-53** | ***BRANCHED SILKLESS1 (ZmBD1)*** | **NP_001105200** | **1,00E-60** | **PA_NPB** | **Chuck *et al.* 2002; Komatsu *et al.* 2003b** |
| ***SbIDS1*** | **Sb01g003400** | **1** | **2,731** | **INDETERMINATE SPIKELET 1 (*OsIDS1*)** | **BAC21448** | **3,20E-123** | ***INDETERMINATE SPIKELET 1 (ZmIDS1)*** | **AAC05206** | **0,00E+00** | **Av_NG, NG_MN, NG_PN, PA_HI, TGW** | **Chuck *et al.* 1998** |
| *SbSID1* | Sb02g007000 | 2 | 8,952 | *SUPERNUMERARY BRACT 1 (OsSNB1)* | ABD24033 | 2,70E-144 | *SISTER OF INDETERMINATE SPIKELET 1 (ZmSID1)* | NP_001139539 | 9,70E-180 | | Lee *et al.* 2007 |
| *SbMFO1* | Sb04g031750 | 4 | 61,676 | *MOSAIC FLORAL ORGANS1 (MFO1)* | ACV89861 | 4,00E-118 | *bearded-ear1 (bde)* | NP_001105332 | 4,20E-139 | | Thompson *et al.* 2009 |
| *SbLHS1* | Sb01g042840 | 1 | 66,077 | *LEAFY HULL STERILE1 (LHS1)* | Q10PZ9 | 2,20E-101 | | | | | Jeon *et al.* 2000 |
| *SbMADS3* | Sb03g002525 | 3 | 2,406 | *OsMADS3* | Q40704 | 8,60E-111 | *zmm2, zmm23* | CAA57074 | 2,90E-109 | | Kang *et al.* 1995 |
| *SbMADS58* | Sb09g006360 | 9 | 9,847 | *OsMADS58* | BAE54300 | 4,40E-96 | *zag1* | NP_001105321 | 1,30E-139 | | Schmidt *et al.* 1993 |
| *SbMADS13* | Sb08g006460 | 8 | 10,063 | *OsMADS13* | Q2QW53 | 1,50E-91 | *zag2* | CAA56504 | 9,20E-140 | | Theissen *et al.* 1995 |
| *SbDL* | Sb01g042850 | 1 | 66,095 | *DROOPING LEAF (OsDL)* | AAR84663 | 4,60E-95 | *DROOPING LEAF (ZmDL)* | ACG32674 | 2,40E-106 | | |
| *SbFON1* | Sb03g037580 | 3 | 65,504 | *FLORAL ORGAN NUMBER1 (OsFON1)* | Q5Z9N5 | 1,50E-101 | | | | | Suzaki *et al.* 2004 |
| ***SbFOR1*** | **Sb02g036750** | **2** | **71,135** | ***FLORAL ORGAN REGULATOR 1 (OsFOR1)*** | **AAO17320** | **1,00E-142** | | | | **PB_Dens, Av_NPB, Shape and compactness** | **Jang *et al.* 2003** |
| *SbLP* | Sb04g009700 | 4 | 12,281 | *LARGER PANICLE (LP)* | ADQ13183 | 0,00E+00 | | | | | |
| *SbAP1* | Sb02g001090 | 2 | 0,956 | | | | *APETALA (AP1)* | NP_001105333 | 2,10E-102 | | Mena *et al.* 1995 |
| *SbCEN-like2* | Sb05g003200 | 5 | 3,587 | *TFL1/CEN-like* | AF159883 | 2,10E-92 | | | | | Zhang *et al.* 2005 |
| *SbCEN-like1* | Sb08g003210 | 8 | 3,572 | *TFL1/CEN-like* | AF159883 | 6,10E-94 | | | | | Zhang *et al.* 2005 |

### 5.3.3. Candidate genes related to plant growth and development

Candidate genes related to plant growth and development, HIGH-TILLERING DWARF1 (*OsCCD7*) in rice (Umehara *et al*., 2008), displayed sequence similarity with sorghum gene prediction (Sb06g024560) on chromosome 6. This sorghum gene prediction co-localized with the QTLs for number of primary branches per panicle and average primary branch per node. *SbCCD7* may play a role in the primary branch production. According to Zou *et al*. (2006), RNA gel blot analysis showed that the HIGH-TILLERING DWARF1 (*HTD1*) gene was expressed in all tissues examined. The levels of expression were generally higher in aerial tissues (leaf, stem and panicle) than in root tissue. Gibberellins are growth factors with a tetracyclic diterpenoid structure that are essential regulators of diverse growth and developmental processes of plants (Davies, 1995). The candidate gene Gibberellin 20-oxidase (*OsGA20ox*) in rice presented sequence similarity with sorghum gene prediction (Sb03g041900) on SBI-03 and co-localized with QTLs for internode length, primary branch length at second node, and primary and secondary branch density. This suggests the role played by Gibberellin 20 oxidase in sorghum panicle elongation and branch development. Toyomasu *et al*. (1997) demonstrated in rice that the *OsGA20ox* transcript level was controlled in a negative feedback manner by the level of bioactive Gibberellin.

Table 5.3: List of candidate genes related to plant growth and development

| Sorghum name | Sorghum gene prediction # | Chr | Position | Reference gene in *Oryza sativa* | | | Reference gene in *Zea mays* | | | QTL co-location | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Oryza name | Oryza accession # | E-value | Zea name | Zea accession # | E-value | | |
| **Plant growth and developement** | | | | | | | | | | | |
| ***SbCCD7*** | **Sb06g024560** | **6** | **53,677** | ***HIGH-TILLERING DWARF1 (OsCCD7)*** | **Q7XU29** | **0,00E+00** | | | | **Av_NPB, PA_NPB** | **Umehara *et al.* 2008** |
| *SbCCD8* | Sb03g034400 | 3 | 62,608 | *HIGH-TILLERING DWARF1 (OsCCD8b)* | Q8LIY8 | 0,00E+00 | | | | PB_BZ_MN | Umehara *et al.* 2008 |
| *SbTB1* | Sb01g010690 | 1 | 9,506 | *TEOSINTE BRANCHED 1 (Oryza latifolia)* | BAM13331 | 4,60E-54 | *TEOSINTE BRANCHED 1 (ZmTB1)* | AFI70995 | 5,20E-79 | | |
| *SbPLA1* | Sb01g022690 | 1 | 29,065 | *PLASTOCHRON1 (PLA1)* | Q7Y1V5 | 0,00E+00 | | | | | Miyoshi *et al.* 2004 |
| *SbPLA2* | Sb03g043230 | 3 | 70,604 | *PLASTOCHRON2 (PLA2)* | BAE79763 | 8,10E-171 | *TERMINAL EAR1* | AAK29419 | 0,00E+00 | | Kawakatsu *et al.* 2006 |
| *SbPLA3* | Sb01g005710 | 1 | 4,17 | *PLASTOCHRON3 (PLA3)* | BAH22722 | 0,00E+00 | | | | | Kawakatsu *et al.* 2009 |
| *SbDWARF8* | Sb01g010660 | 1 | 9,419 | | | | *DWARF8* | AAL10393 | 0,00E+00 | | |
| ***SbGA20ox*** | **Sb03g041900** | **3** | **69,376** | ***Gibberellin 20-oxidase (OsGA20ox)*** | **AAM56041** | **1,00E-166** | | | | **IN_L, PB_L_SN, PB_Dens, SB_DENS_SN** | **Spielmeyer *et al.* 2002** |
| *SbGA20ox* | Sb09g020760 | 9 | 50,182 | *Gibberellin 20-oxidase (OsGA20ox)* | AAM56041 | 9,70E-127 | | | | | Spielmeyer *et al.* 2002 |
| *SbGA20ox* | Sb01g000580 | 1 | 0,462 | *Gibberellin 20-oxidase (OsGA20ox)* | AAM56041 | 2,10E-95 | | | | | Spielmeyer *et al.* 2002 |

*NB: sorghum candidate genes that show meaningful co-localization with panicle architecture QTL are shown in bold*

### 5.3.4. Phylogenetic analysis

A phylogenetic analysis of the candidate genes indicated the presence of 13 distinct clades within the sorghum genome for meristem regulation during inflorescence development (Fig 5.1).

Twelve clades for specification of spikelet meristem identity and five separate clades for plant growth and development with one sorghum predicted gene per clade except the clade TFL1/CEN-like where two paralogous predicted gene copies were observed and the clade Gibberellin 20 oxidase presented three paralogous sorghum genes prediction. In many clades, sorghum predicted genes share more homology with maize than rice.

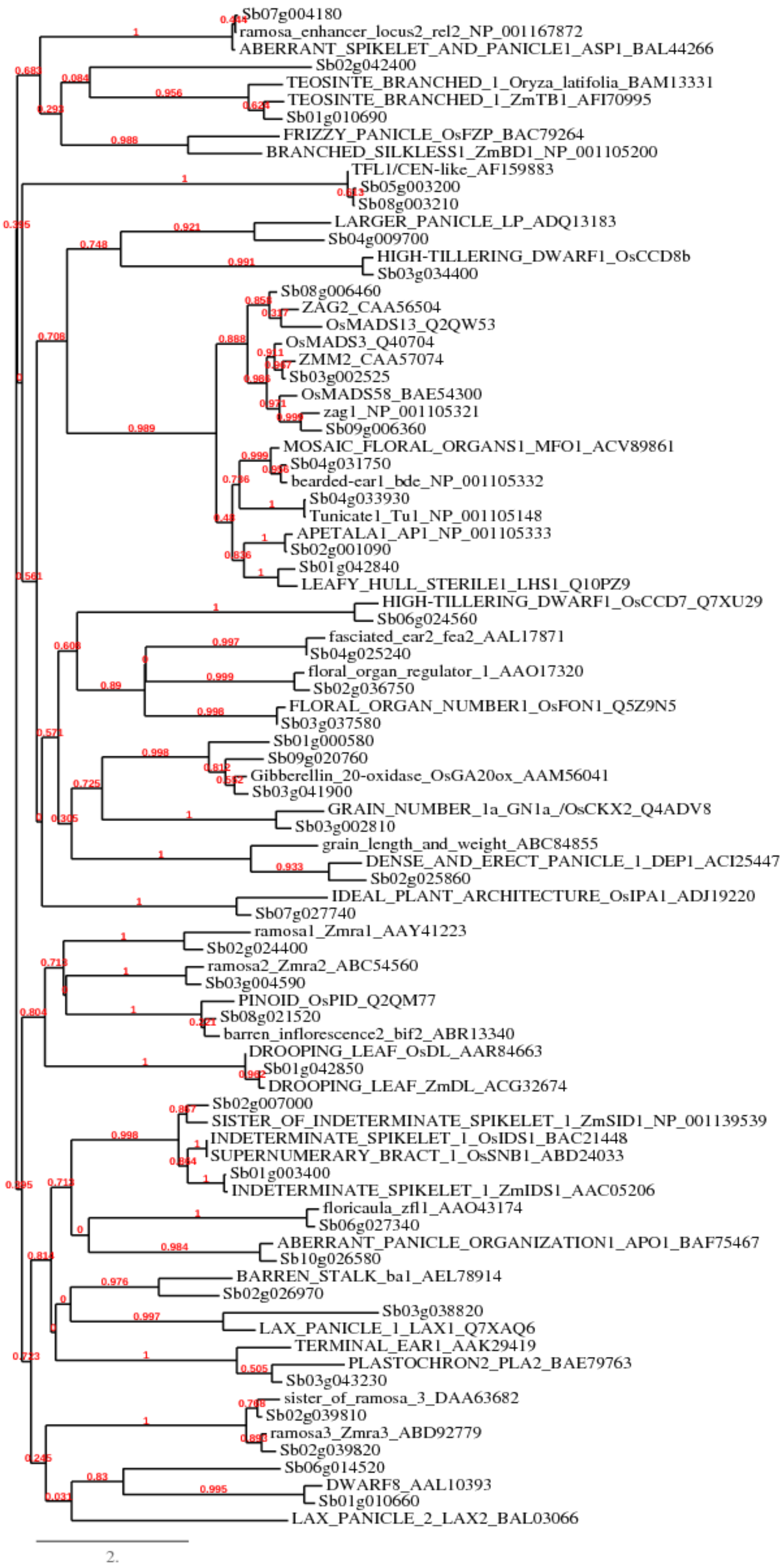


*Figure 5.1: Phyllogenetic tree for candidate genes involved in inflorescence architecture*

## 5.4. Conclusion

Protein sequence similarities with sorghum for a number of candidate genes in rice and maize involved in meristem regulation during inflorescence development, specification of spikelet meristem identity and plant growth were established. Several of these candidate genes co-localize closely with QTLs for relevant inflorescence architecture traits in a sorghum mapping population derived from the cross between Tiandougou (caudatum-guinea type) and Lata3 (guinea type). The results of this study suggest promising confirmation of the function of the candidate genes in rice and maize in relation to the QTLs detected for panicle architecture traits. BLAST may give indication of possible colinearity and homology of genes that can serve as a starting point for further investigation of the effectiveness of studying candidate genes in related species such as rice and maize to elucidate orthologous roles and function of these genes in sorghum and provide the basic understanding of sorghum grain yield improvement through panicle architecture. Candidate gene cloning studies in large genetic background of sorghum germplasm may contribute to a better understanding of the relationship between genes involved in sorghum panicle architecture and grain yield. The candidate genes identified in sorghum and localized with QTLs detected in this study for panicle architecture may help design breeding strategies based on genetic and physical architecture to improve sorghum productivity.

# CHAPTER SIX

## 6.0. GENERAL DISCUSSION

### 6.1. Phenotyping for sorghum panicle architecture and yield in Tiandougou/Lata-3 $F_4$ population

The relationship between panicle architecture and grain yield, the determination of the pattern of panicle branching and the number of grains at different positions such as the basal, medium and the top of the panicle were investigated in this study. The relationship between sorghum panicle architecture and grain yield was established through the identification of some highly heritable architectural and easy measurable traits. The number of internodes per panicle and the basal diameter of the panicle contribute to thirty two percent (32%) of grain yield per panicle. Branching and grain pattern at different positions on the panicle were characterized to capture panicle architecture variability.

Gradual decrease was seen in the length of the primary branch, the number of secondary branches and number of grains from the base of the panicle toward the top of the panicle.

Significant variation existed between the $F_4$ families for panicle architecture traits in the second sowing date compared to the first sowing date in 2011. This is common in sorghum because of photoperiod differences and late sowing reduction of sorghum grain yield per panicle.

Broad sense heritability estimated for panicle length, panicle weight, the number of primary branches per panicle, and the number of nodes per panicle corroborate most previous reports. Different degrees of heritability for number of secondary branches, number of grains per primary branches and rachis diameter were noted across studies. This difference may be explained by the dependence of some of these traits on others. For instance, number of secondary branches depends on the length of the primary branch. Broad sense heritability estimated in this study for grain yield per panicle corroborates some previous findings. Any differences may be explained by difference in the genetic background, the statistical method of estimation and interaction of genotype with environment.

The existence of decreasing gradient for the length and number of primary branches from the base of the panicle toward the top of the panicle may refine previous findings by giving meaningful information for phenotyping strategies.

Correlation between grain yield per panicle and a number of sorghum panicle architecture traits agrees with most previous findings. However, this study extends the correlation of grain yield per panicle to the pattern of branching and number of grains at different positions in the panicle base, median and top. Genetic variability in sorghum panicle architecture traits is important for the diversity in sorghum. Variability is expressed in terms of elongation, branching pattern and growth. The length of the panicle, rachis, primary and secondary branches at different positions in the panicle in relation to the number of primary and secondary branches per panicle at different positions plays an important role in capturing panicle architecture. Rachis diameter at different positions along the rachis influence the branching pattern, number of grains and consequently the grain yield per panicle. The contribution of panicle length, primary and secondary branches length and the number of primary branches per panicle to sorghum panicle variability agree with the previous findings. This study established other contributing traits such as the variation in length and number of primary branches at different positions in the panicle as well as the position of the longest primary branch. These traits characterized sorghum panicle architectural variability by identifying an evolutionary trend from the base toward the top of the panicle. Grain yield per panicle was associated with the number of primary branches per panicle and rachis base diameter explaining important sorghum panicle architecture variation. Transgressive segregation in sorghum panicle architecture traits and grain yield is common for quantitative traits in a bi parental population.

In sorghum, sowing date affects panicle architecture traits and grain yield. Indeed, sorghum is photoperiod sensitive for floral initiation. This physiological mechanism makes sorghum one of the most adapted crops to climate change. Variability in sorghum panicle architecture is expressed according to the sowing date that determines the vegetative cycle of the genotype. The longer the vegetative cycle, the more the dry matter which is accumulated and which is further mobilized for grain formation, thus affecting grain yield. Delay in sowing date influences both vegetative and reproductive growth. Sorghum panicle architecture traits were influenced by the late sowing date in term of elongation, branching pattern and growth. Consequently the potential yield is negatively affected. This implies an optimum sowing date is necessary for a genotype to be able to express its full yielding potential. In Mali, at Bamako area the optimum sowing date should be in June.

Branching pattern indicated decreasing heritability of branch length and number from the base to the top of the panicle. This implies that variability within the panicle and the pattern of this variation may be under different genetic control, therefore, the phenotyping of sorghum panicle architecture should focus on the basal zone of the panicle followed by the middle part of the panicle.

Sorghum panicle architecture traits such as rachis base diameter and number of internodes per panicle contributed more (31.8%) to the grain yield per panicle than other traits. They were followed in importance by the number of primary branches per panicle, number of secondary branches on primary branches at second node. This demonstrated the contribution of sorghum panicle architecture to grain yield, and suggests that grain yield per panicle can be estimated based on architecture traits. Sorghum panicle architecture may be used in breeding programs to improve the grain yield. The ideotype genotype to be targeted in breeding should have big basal rachis diameter with many primary branches.

The results of this study open up ground for further research on sorghum panicle architecture such as phenotyping to capture variability mainly at the basal part of the panicle. Designing breeding strategies using contrasting parents for rachis basal diameter and number of primary branches per panicle to elucidate the contribution of these architectural traits in grain yield through indirect selection should be successful.

### 6.2. QTLs analysis of panicle architecture in the Tiandougou/Lata-3 breeding population

The genetic mechanisms underlying branching patterns were also studied. Sorghum panicle architecture was dissected into individual QTL which clearly establish causal relationships between grain yield and panicle architecture traits by identifying the genomic region and deciphering the control of sorghum panicle branching patterns.

Both additive and non-additive gene effects control most sorghum panicle architecture traits. Additive gene action was determinant in the control of the number of internodes per panicle, primary branches zone and maximum primary branches per node. These traits will be easy to fix in a breeding program at early generations. Panicle traits such as the length and number of secondary branches were governed by non-additive gene effect. These findings extend the genetic mechanism controlling sorghum panicle traits. Grain yield per panicle, panicle weight, and thousand grain weight were under non additive gene action. This results agree with previous findings. Number of grains per panicle was predominantly governed by additive gene effect, however, the number of grains per primary branch at different positions in the panicle was controlled by both additive and non-additive gene action. It is possible to breed for traits controlled by both additive and non-additive gene effects, however, the selection will be delayed to later generations.

The position of the QTL for primary branch number detected on SBI-03; rachis base diameter on SBI-06; panicle weight on SBI-06; panicle length on SBI-03; and grain yield per panicle on SBI-06 corroborate previous findings (Brown *et al*., 2006; Srinivas *et al*., 2009). However, the position in sorghum genome of the QTLs for primary branch length, thousand seed weight, Number of grains per panicle, panicle length conflict with previous findings. This discrepancy may be explained by the different genetic backgrounds and the architectural variability in cultivated sorghum. This study extends the previous findings for the novel QTL for panicle length on SBI-04. The flanking markers both left and right SB06074 and SB06098 reveal that secondary branch length and primary branch density from the parent Lata-3 were linked to grain yield per panicle. While internode length, the longest secondary branch and number of secondary branches at penultimate node were associated to grain yield per panicle through the parent Tiandougou with the flanking markers SB03149 and SB03164. This marker information can be used to design breeding strategies targeting grain yield improvement through the recombination of these traits based on the relatedness of $F_4$ families to each parent to be selected for further crossing.

The QTL for grain yield per panicle on SBI-06 reveals pleiotropic effect on panicle weight, rachis diameter and average primary branch length. This result corroborates and extends the findings of Nagaraja Reddy *et al*. (2013). Therefore, sorghum improvement for grain yield should focus on these architectural traits such as secondary branch density at the base of the panicle, rachis base diameter and the number and length of primary branches per panicle. These architectural traits should be considered in the breeding program for further investigations to elucidate their impact on grain yield using molecular breeding approach. One parent to be used should have a big rachis base diameter with long primary branches and the other parent should have many primary branches. QTL region for grain yield on SBI-06 can be targeted with the QTL region for other favorable traits such as the number of primary branches per panicle to build an ideal genotype. In this study, digenic epistasis controlled the pattern of the grain number on primary branches at different positions in the panicle and the length of the primary and secondary branches as well as internode length. This finding extends the role of epistasis in sorghum particularly for panicle architecture traits. QTLs with overlapping confidence intervals for primary branch length, panicle length and panicle weight corroborate previous findings and may constitute potential Meta-QTLs. Therefore, Meta-analysis studies to determine the position of the Meta- QTLs and project them onto the consensus genetic map should be done.

### 6.3. Identification of candidate genes involved in sorghum panicle architecture in maize and rice

Candidate genes controlling sorghum panicle architecture traits were addressed in this study. Sequence similarity of candidate genes involved in inflorescence architecture in rice and maize with predicted genes in sorghum were evaluated. Co-localization between sorghum predicted genes and QTLs detected for panicle architecture traits was investigated to characterize the candidate genes and infer their role in sorghum based on known functions in rice or maize. Promising co-localization based on sequence similarity between QTLs for sorghum panicle architecture and candidate genes involved in inflorescence architecture in rice or/and maize suggested that: Sorghum homologue of ABERRANT SPIKELET AND PANICULE1 genes (*SbASP1*) on SBI-07 at the position 5.31 cM may regulate branching pattern during panicle development and elongation. LAX PANICLE1 (LAX1) homologue in sorghum (*SbLAX1)* on SBI-03 at the position 66.62 cM is suggested to regulate primary branch number and rachis size. Sorghum GRAIN NUMBER 1a (*SbGN1a*) on SBI-03 at the position 2.72 cM may regulates grain number on primary branches at different positions in the panicle (second, median and penultimate nodes). INDETERMINATE SPIKELET1 (*OsIDS1; ZmIDS1*) candidate genes in rice and maize homologues in sorghum

(SbIDS1) on SBI-01 at the position 2.73 cM may regulate grain size in relation to grain number. FLORAL ORGAN REGULATOR1 homologue in sorghum (*SbFOR1*) on SBI-02 at the position 71.13 cM may influence the general structure of the panicle such as the shape and compactness. GIBBERELLIN 20-OXIDASE (*SbGA20ox*) in sorghum on SBI-03 at the position 69.37 cM may play an important role in panicle elongation and branch development. Few studies were done on candidate genes controlling sorghum panicle architecture therefore, these findings extend the information that had been obtained earlier by other workers. There is a need to undertake further research on these candidate genes to elucidate and establish their functions in sorghum.

# GENERAL CONCLUSIONS AND RECOMMENDATIONS

Sorghum panicle architecture traits show tremendous variation in branching, elongation and growth. Most of sorghum panicle architecture traits were heritable in the $F_4$ families derived from Tiandougou/Lata-3. The basal parts of the panicle show high heritability compared to the top parts. Rachis base diameter and number of internodes per panicle were highly significant and positively correlated to grain yield per panicle. Indirect selection for these traits is feasible to improve grain yield. Panicle length, primary and secondary branches length at second, number of primary branches per panicle and position of the longest primary branch showed positive contributions to sorghum panicle architecture variability. Both additive and non-additive gene effects control most of sorghum panicle architecture traits. Grain yield per panicle was governed by non-additive gene action. Number of grains per panicle was under additive gene effect. However, at different positions, second, median and penultimate nodes, it was controlled by both additive and non-additive gene effects. The SBI-02, SBI-03 and SBI-06 were hot spots of major QTLs for panicle architecture traits and grain yield. The flanking markers (SB03149 - SB03164; SB06074 - SB06098) of grain yield per panicle QTL respectively on SBI-03 and SBI-06 were associated with architectural QTLs for secondary branch length, primary branch density, internode length and the longest secondary branch. Pleiotropic QTL affecting grain yield per panicle influences primary branches length and rachis diameter. This arrangement may be correlated to heterosis. Therefore, rachis base diameter and number and length of primary branches can be used in both conventional and molecular breeding programs to improve sorghum grain yield. Digenic epistasis determined the pattern of the grain number at different positions in the panicle and the length of the secondary branches. QTLs for panicle length, primary branch length and panicle weight were stable across different studies.

Sorghum homologues of *ABERRANT SPIKELET AND PANICULE1* genes *(SbASP1)* on SBI-07; *LAX PANICLE1 (LAX1)* gene *(SbLAX1)* on SBI-03; *GRAIN NUMBER 1a (SbGN1a)* gene on SBI-03; *INDETERMINATE SPIKELET1* gene *(SbIDS1)* on SBI-01; *FLORAL ORGAN REGULATOR1* gene *(SbFOR1)* on SBI-02; *GIBBERELLIN 20-OXIDASE (SbGA20ox)* on SBI-03 could play important role in the control of sorghum panicle architecture traits. However, further study should confirm and validate the function of these candidate genes in sorghum.

## RECOMMENDATIONS

Development of varieties in sorghum, based on panicle architecture traits should be addressed by other workers interested in sorghum cultivar development. Indirect selection for panicle architecture traits correlated to grain yield in conventional breeding should be initiated. At the molecular level, genomic regions related to grain yield and those contributing architectural traits should be combined in the ideal genotype by using the MBDT software to build ideal genotypes and OPTIMAS software should be used to determine different parents among the $F_4$ families to be cross to reach this ideal genotype. Information on QTLs for panicle architecture traits can be used in the first and second cycles of recombination in MARS breeding approach for sorghum grain yield improvement. It is valuable to address further studies to determine the relevance of panicle architecture traits in biotic and abiotic stresses such as diseases affecting sorghum panicle (smut, ergot) and drought for better food security in Mali.  Also, it is recommended to undertake further studies such as loss of function, *in situ* hybridization and Northern blot analysis for expression patterns to decipher the role of candidate genes involved in panicle architecture identified in sorghum and use such information to improve sorghum breeding.

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# APPENDIXES

## Appendix 4.1: Genotyping Material and method at CIRAD lab

### Material

- ✓ LightCycler® 480 Multiwell Plate 384, white (04 729 749 001)
- ✓ Plate PCR96 Biorad (réf : HSS9601)
- ✓ LightCycler® 480 Sealing Foil, (04 729 757 001)
- ✓ Robot Hamilton Microlab Star
- ✓ Thermocycler Veriti 384 Well (Applied Biosystems)
- ✓ LightCycler® 480 II (LC480), Roche

### Chemical and biological reagents

- ✓ KASPar 2X Reagent Mix (Kbioscience, PN KBS-1004-001), conserved at -20°C
- ✓ $MgCl_2$ 50mM, Kbiosciences, conserved at -20°C
- ✓ $H_2O$ milliQ

### Method

- ✓ *Dispense DNA in plate 384*

2µL of DNA at 10ng/µl was dispensed into PCR white plate 384 in the following order: A1, B1, A2, and B2.

- ✓ *Preparing and dispensing PCR mix*

Preparing « assays mix » (mix of primers); for 100µl assay mix:

- 12 µL primer specific for Allele 1 (100µM)_ 12 µM final
- 12 µL primer specific for Allele 2 (100µM)_ 12 µM final
- 30 µL common primer specific (100µM)_ 30 µM final

- 46 µl $H_2O$ mQ

The mix KASPAR has 1.8mM in $MgCl_2$ of concentration, when the percentage GC of the assay is very low. We need to add 2.2 mM $MgCl_2$ if the percentage of GC is between 33% and 55% and 2.5 mM $MgCl_2$ for GC less than 33%. The plates were sealed with LightCycler® 480 Sealing Foil

✓ *PCR*

Because of the great size of the sample, PCR were directly done in LightCycler® 480 II (LC480), Roche and in Thermocycler Veriti 384 Well (Applied Biosystems) using this program « outside KASPar Project»

- 94 °C for 15 minutes

  Hot-start enzyme activation

- 94 °C for 20 seconds

  Touchdown over 65-57°C for 60 seconds

  10 cycles (dropping 0.8°C per cycle)

- 94°C for 20 seconds; 57°C for 60 seconds; 26 cycles

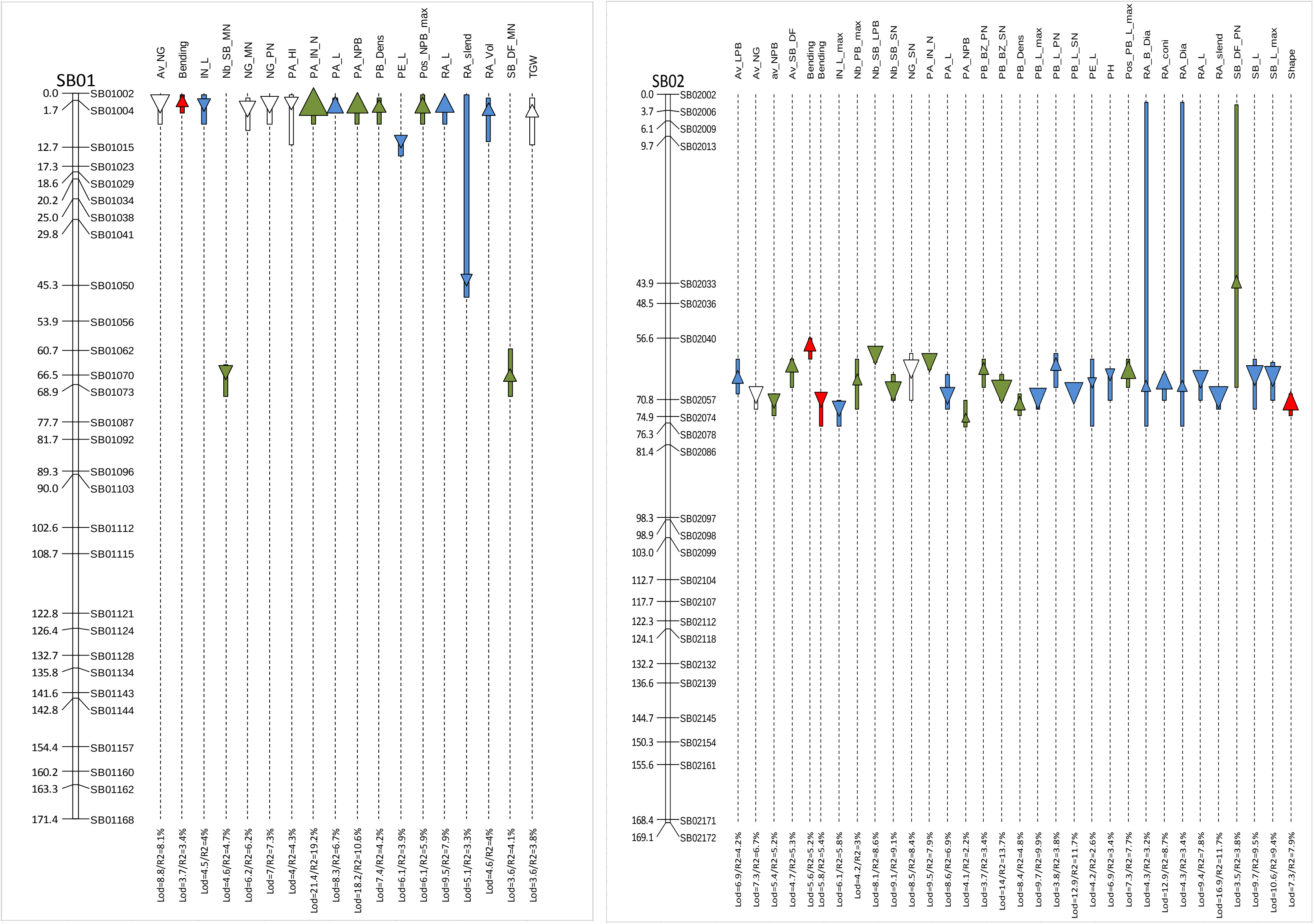
✓ *Reading on* LightCycler® 480 II (LC480), Roche

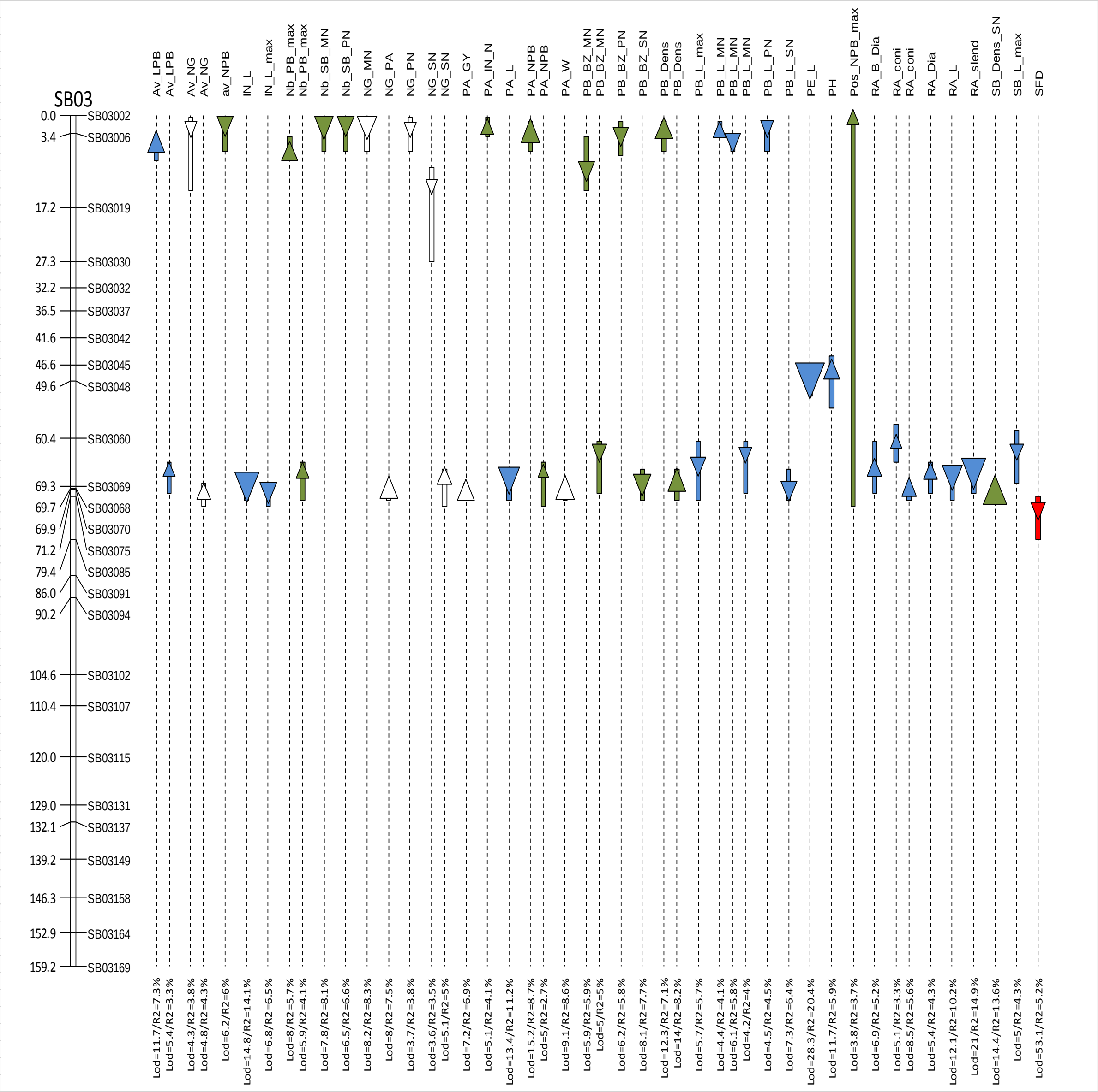
- Open the software « LightCycler® 480 SW 1.5 »
- Click on « New experiment from template »
- Highlight Run template : « read Kaspar Plateform »
- Click start Run
- Give a name to the plate and folder in which it will be saved.

✓ *Coding of the alleles*

The allale “B” is coded for the parent Tiandougou, “A” allele is noted for the parent Lata-3 and the heterozygous alleles were coded “H”. Then the genotype of the different $F_4$ families for each markers was established.

## Appendix 4.2: QTLs map for sorghum panicle architecture traits in F₄ families from Tiandougou/Lata-3

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3.4 SB03006
17.2 SB03019
27.3 SB03030
32.2 SB03032
36.5 SB03037
41.6 SB03042
46.6 SB03045
49.6 SB03048
60.4 SB03060
69.3 SB03069
69.7 SB03068
69.9 SB03070
71.2 SB03075
79.4 SB03085
86.0 SB03091
90.2 SB03094
104.6 SB03102
110.4 SB03107
120.0 SB03115
129.0 SB03131
132.1 SB03137
139.2 SB03149
146.3 SB03158
152.9 SB03164
159.2 SB03169
Av_LPB
Av_LPB
Av_NG
Av_NG
av_NPB
IN_L
IN_L_max
Nb_PB_max
Nb_PB_max
Nb_SB_MN
Nb_SB_PN
NG_MN
NG_PA
NG_PN
NG_SN
NG_SN
PA_GY
PA_IN_N
PA_L
PA_NPB
PA_NPB
PA_W
PB_BZ_MN
PB_BZ_MN
PB_BZ_PN
PB_BZ_SN
PB_Dens
PB_Dens
PB_L_max
PB_L_MN
PB_L_MN
PB_L_MN
PB_L_PN
PB_L_SN
PE_L
PH
Pos_NPB_max
RA_B_Dia
RA_coni
RA_coni
RA_Dia
RA_L
RA_slend
SB_Dens_SN
SB_L_max
SFD
Lod=11.7/R2=7.3%
Lod=5.4/R2=3.3%
Lod=4.3/R2=3.8%
Lod=4.8/R2=4.3%
Lod=6.2/R2=6%
Lod=14.8/R2=14.1%
Lod=6.8/R2=6.5%
Lod=8/R2=5.7%
Lod=5.9/R2=4.1%
Lod=7.8/R2=8.1%
Lod=6.5/R2=6.6%
Lod=8.2/R2=8.3%
Lod=8/R2=7.5%
Lod=3.7/R2=3.8%
Lod=3.6/R2=3.5%
Lod=5.1/R2=5%
Lod=7.2/R2=6.9%
Lod=5.1/R2=4.1%
Lod=13.4/R2=11.2%
Lod=15.2/R2=8.7%
Lod=5/R2=2.7%
Lod=9.1/R2=8.6%
Lod=5.9/R2=5.9%
Lod=5/R2=5%
Lod=6.2/R2=5.8%
Lod=8.1/R2=7.7%
Lod=12.3/R2=7.1%
Lod=14/R2=8.2%
Lod=5.7/R2=5.7%
Lod=4.4/R2=4.1%
Lod=6.1/R2=5.8%
Lod=4.2/R2=4%
Lod=4.5/R2=4.5%
Lod=7.3/R2=6.4%
Lod=28.3/R2=20.4%
Lod=11.7/R2=5.9%
Lod=3.8/R2=3.7%
Lod=6.9/R2=5.2%
Lod=5.1/R2=3.3%
Lod=8.5/R2=5.6%
Lod=5.4/R2=4.3%
Lod=12.1/R2=10.2%
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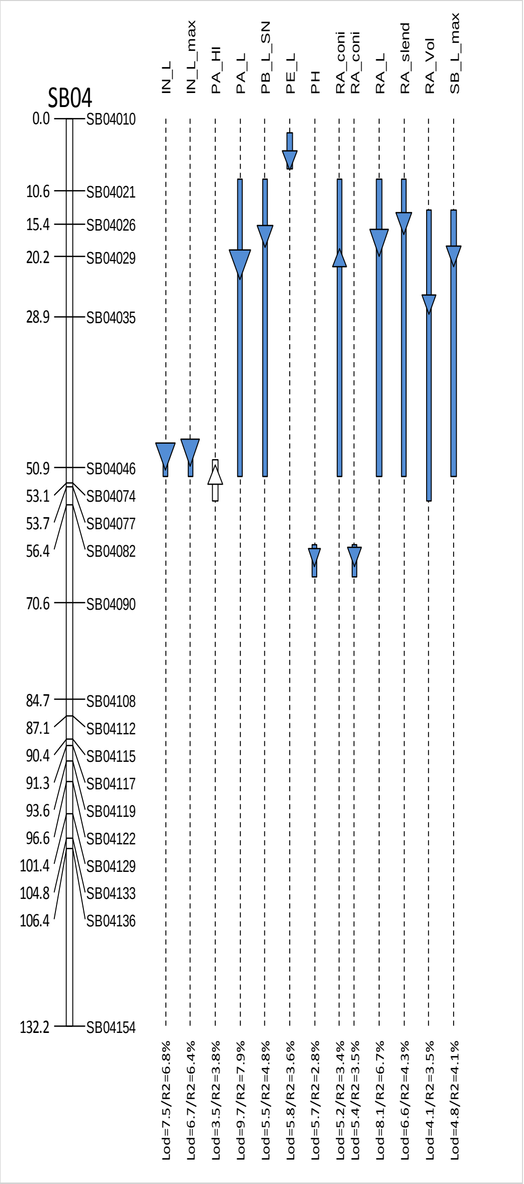
Lod=53.1/R2=5.2%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10.6 SB04021
15.4 SB04026
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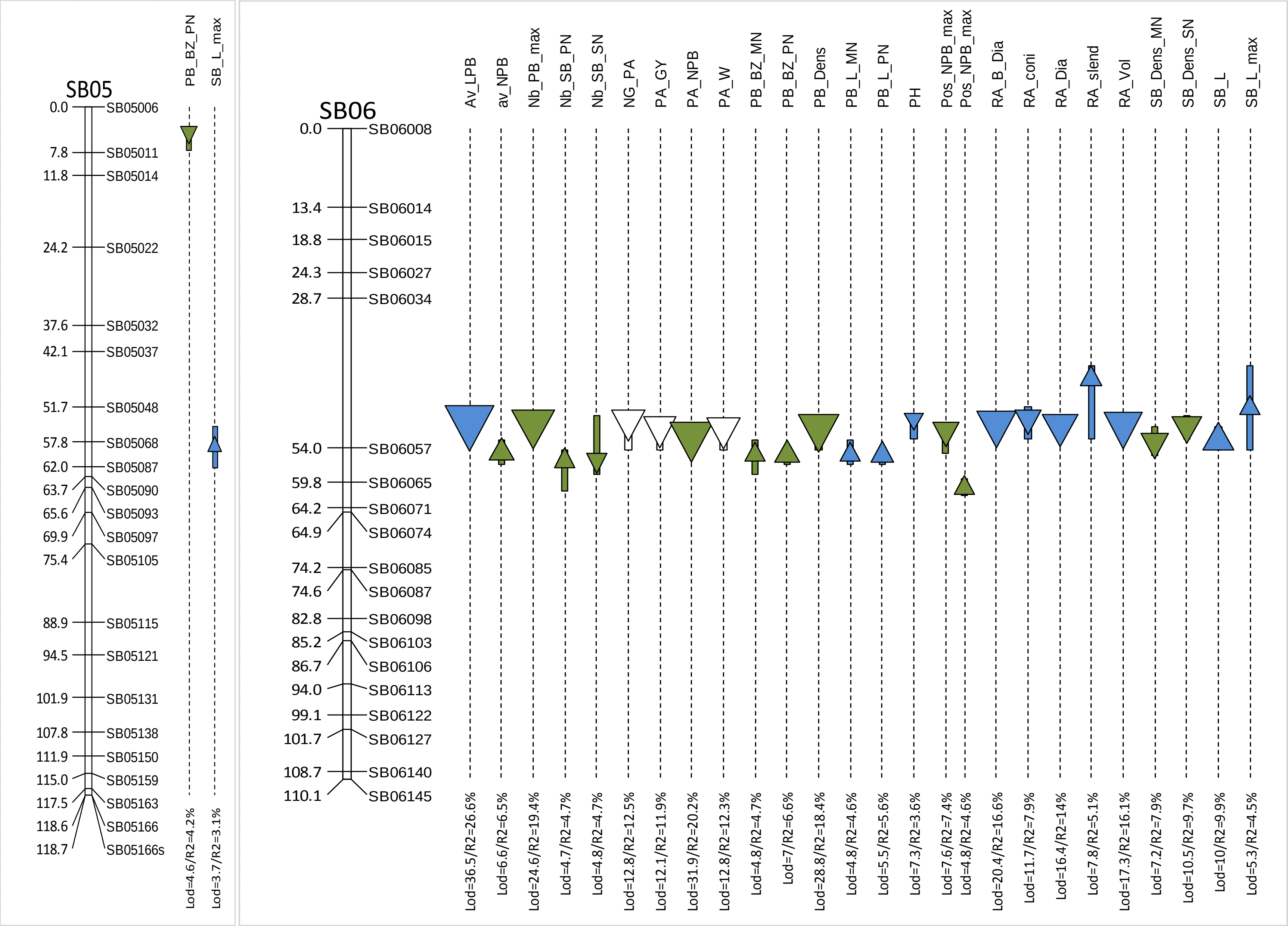
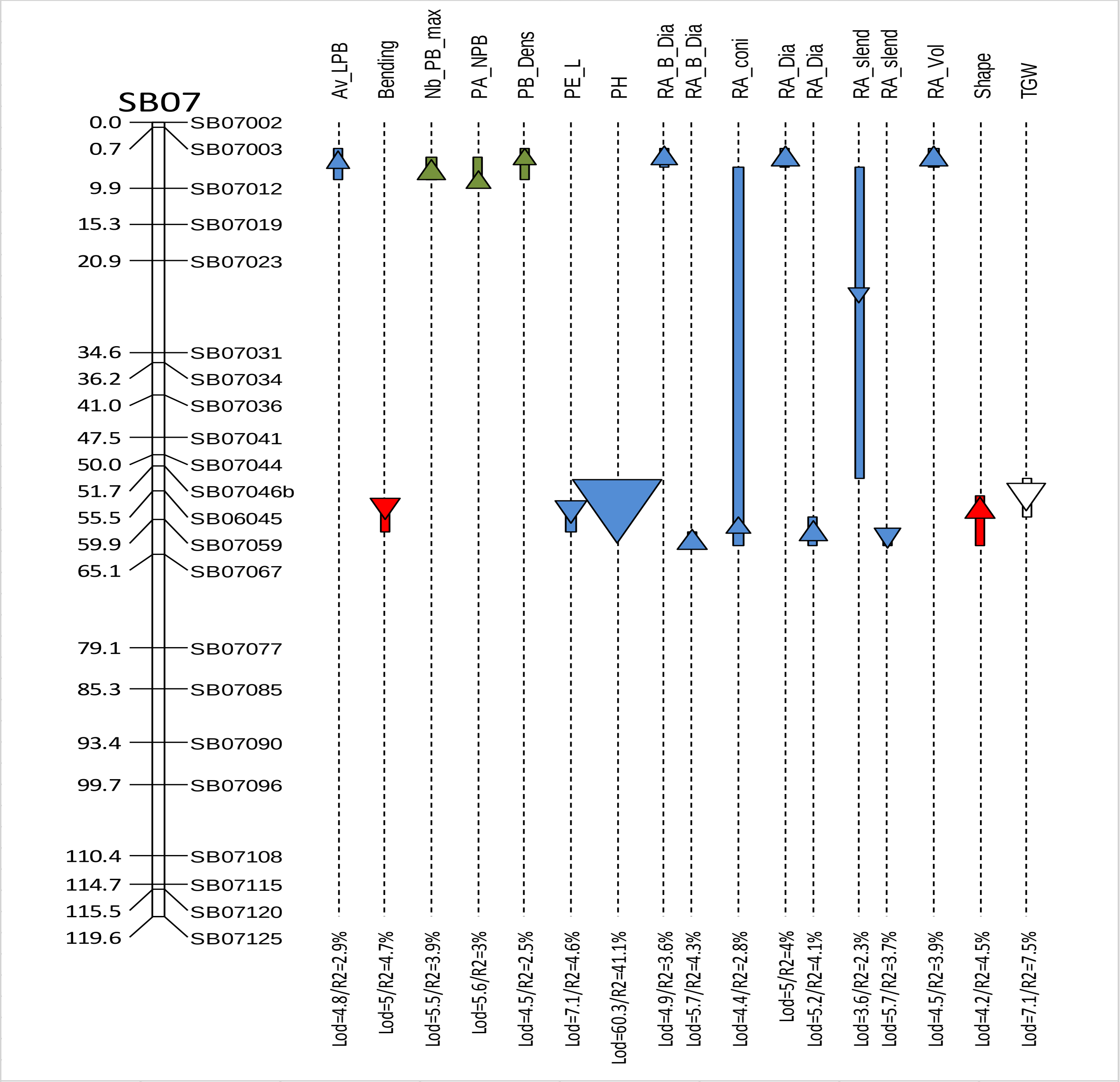
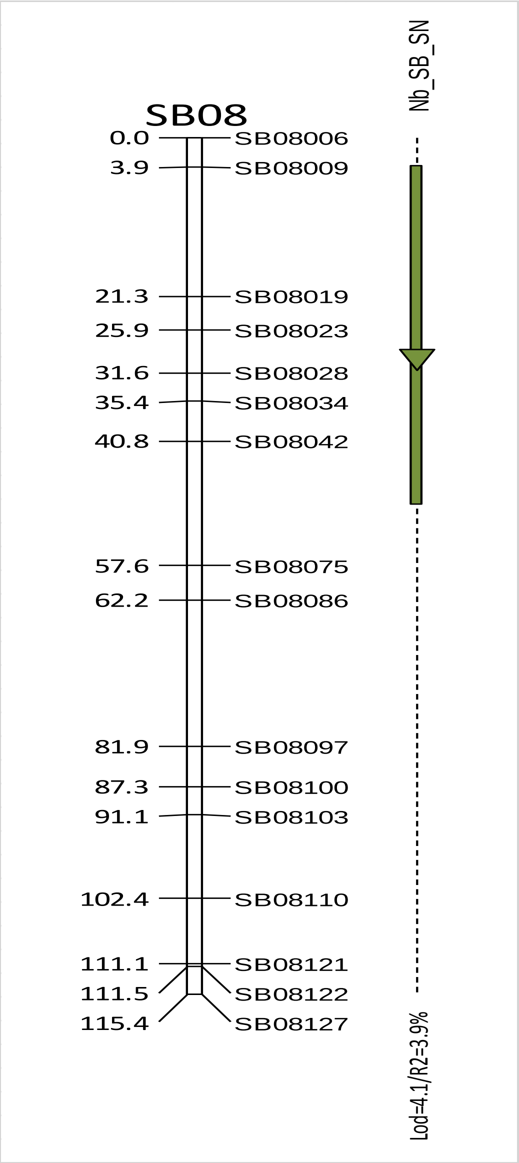
28.9 SB04035
50.9 SB04046
53.1 SB04074
53.7 SB04077
56.4 SB04082
70.6 SB04090
84.7 SB04108
87.1 SB04112
90.4 SB04115
91.3 SB04117
93.6 SB04119
96.6 SB04122
101.4 SB04129
104.8 SB04133
106.4 SB04136
132.2 SB04154
IN_L
IN_L_max
PA_HI
PA_L
PB_L_SN
PE_L
PH
RA_coni
RA_coni
RA_L
RA_slend
RA_Vol
SB_L_max
Lod=7.5/R2=6.8%
Lod=6.7/R2=6.4%
Lod=3.5/R2=3.8%
Lod=9.7/R2=7.9%
Lod=5.5/R2=4.8%
Lod=5.8/R2=3.6%
Lod=5.7/R2=2.8%
Lod=5.2/R2=3.4%
Lod=5.4/R2=3.5%
Lod=8.1/R2=6.7%
Lod=6.6/R2=4.3%
Lod=4.1/R2=3.5%
Lod=4.8/R2=4.1%


SB05
0.0 SB05006
7.8 SB05011
11.8 SB05014
24.2 SB05022
37.6 SB05032
42.1 SB05037
51.7 SB05048
57.8 SB05068
62.0 SB05087
63.7 SB05090
65.6 SB05093
69.9 SB05097
75.4 SB05105
88.9 SB05115
94.5 SB05121
101.9 SB05131
107.8 SB05138
111.9 SB05150
115.0 SB05159
117.5 SB05163
118.6 SB05166
118.7 SB05166s
PB_BZ_PN
SB_L_max
Lod=4.6/R2=4.2%
Lod=3.7/R2=3.1%
SB06
0.0 SB06008
13.4 SB06014
18.8 SB06015
24.3 SB06027
28.7 SB06034
54.0 SB06057
59.8 SB06065
64.2 SB06071
64.9 SB06074
74.2 SB06085
74.6 SB06087
82.8 SB06098
85.2 SB06103
86.7 SB06106
94.0 SB06113
99.1 SB06122
101.7 SB06127
108.7 SB06140
110.1 SB06145
Av_LPB
av_NPB
Nb_PB_max
Nb_SB_PN
Nb_SB_SN
NG_PA
PA_GY
PA_NPB
PA_W
PB_BZ_MN
PB_BZ_PN
PB_Dens
PB_L_MN
PB_L_PN
PH
Pos_NPB_max
Pos_NPB_max
RA_B_Dia
RA_coni
RA_Dia
RA_slend
RA_Vol
SB_Dens_MN
SB_Dens_SN
SB_L
SB_L_max
Lod=36.5/R2=26.6%
Lod=6.6/R2=6.5%
Lod=24.6/R2=19.4%
Lod=4.7/R2=4.7%
Lod=4.8/R2=4.7%
Lod=12.8/R2=12.5%
Lod=12.1/R2=11.9%
Lod=31.9/R2=20.2%
Lod=12.8/R2=12.3%
Lod=4.8/R2=4.7%
Lod=7/R2=6.6%
Lod=28.8/R2=18.4%
Lod=4.8/R2=4.6%
Lod=5.5/R2=5.6%
Lod=7.3/R2=3.6%
Lod=7.6/R2=7.4%
Lod=4.8/R2=4.6%
Lod=20.4/R2=16.6%
Lod=11.7/R2=7.9%
Lod=16.4/R2=14%
Lod=7.8/R2=5.1%
Lod=17.3/R2=16.1%
Lod=7.2/R2=7.9%
Lod=10.5/R2=9.7%
Lod=10/R2=9.9%
Lod=5.3/R2=4.5%


SB07
0.0 SB07002
0.7 SB07003
9.9 SB07012
15.3 SB07019
20.9 SB07023
34.6 SB07031
36.2 SB07034
41.0 SB07036
47.5 SB07041
50.0 SB07044
51.7 SB07046b
55.5 SB06045
59.9 SB07059
65.1 SB07067
79.1 SB07077
85.3 SB07085
93.4 SB07090
99.7 SB07096
110.4 SB07108
114.7 SB07115
115.5 SB07120
119.6 SB07125
Av_LPB
Bending
Nb_PB_max
PA_NPB
PB_Dens
PE_L
PH
RA_B_Dia
RA_B_Dia
RA_coni
RA_Dia
RA_Dia
RA_slend
RA_slend
RA_Vol
Shape
TGW
Lod=4.8/R2=2.9%
Lod=5/R2=4.7%
Lod=5.5/R2=3.9%
Lod=5.6/R2=3%
Lod=4.5/R2=2.5%
Lod=7.1/R2=4.6%
Lod=60.3/R2=41.1%
Lod=4.9/R2=3.6%
Lod=5.7/R2=4.3%
Lod=4.4/R2=2.8%
Lod=5/R2=4%
Lod=5.2/R2=4.1%
Lod=3.6/R2=2.3%
Lod=5.7/R2=3.7%
Lod=4.5/R2=3.9%
Lod=4.2/R2=4.5%
Lod=7.1/R2=7.5%


SB08
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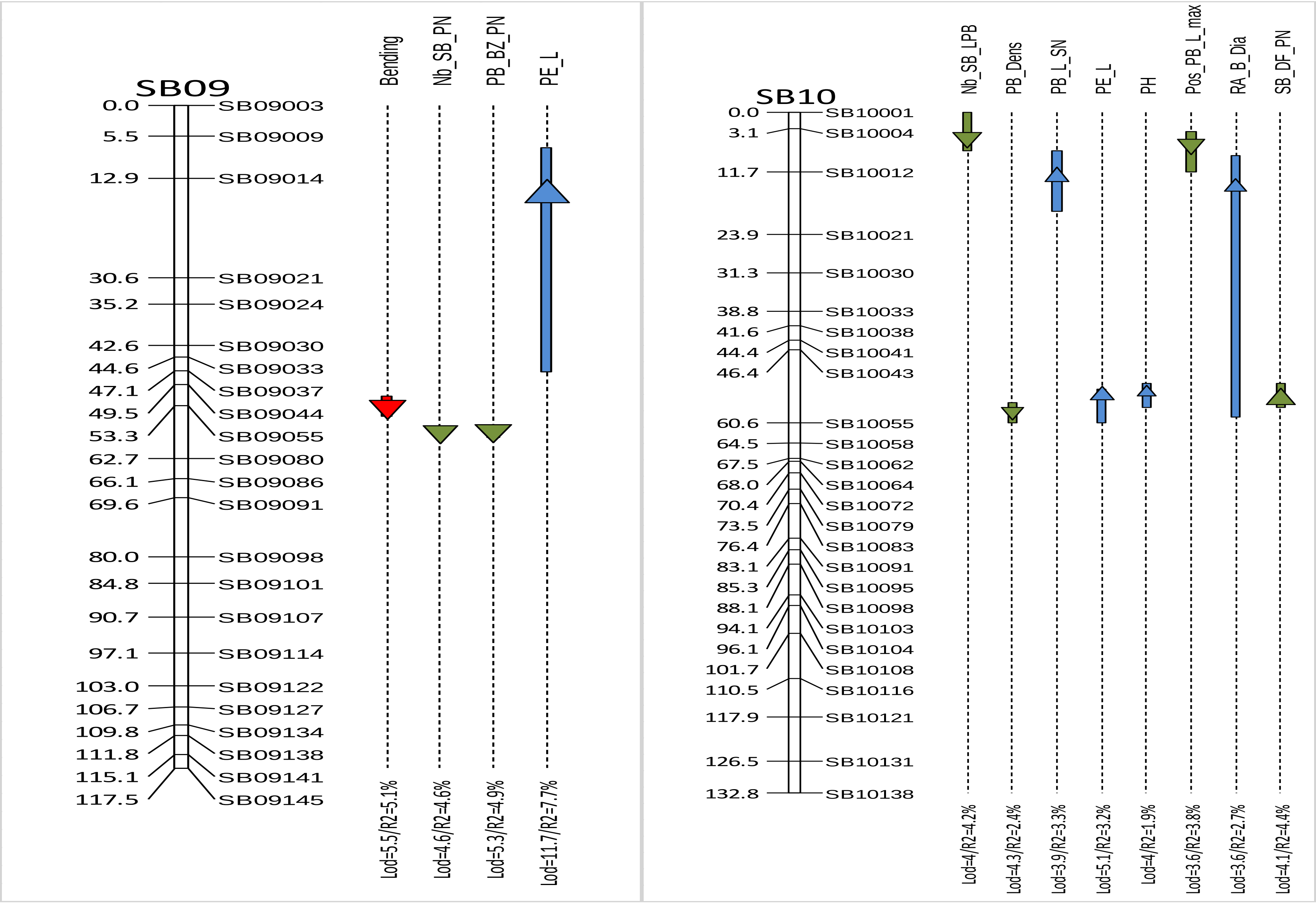
3.9 SB08009
21.3 SB08019
25.9 SB08023
31.6 SB08028
35.4 SB08034
40.8 SB08042
57.6 SB08075
62.2 SB08086
81.9 SB08097
87.3 SB08100
91.1 SB08103
102.4 SB08110
111.1 SB08121
111.5 SB08122
115.4 SB08127
Nb_SB_SN
Lod=4.1/R2=3.9%


SB09
0.0 SB09003
5.5 SB09009
12.9 SB09014
30.6 SB09021
35.2 SB09024
42.6 SB09030
44.6 SB09033
47.1 SB09037
49.5 SB09044
53.3 SB09055
62.7 SB09080
66.1 SB09086
69.6 SB09091
80.0 SB09098
84.8 SB09101
90.7 SB09107
97.1 SB09114
103.0 SB09122
106.7 SB09127
109.8 SB09134
111.8 SB09138
115.1 SB09141
117.5 SB09145
Bending
Nb_SB_PN
PB_BZ_PN
PE_L
Lod=5.5/R2=5.1%
Lod=4.6/R2=4.6%
Lod=5.3/R2=4.9%
Lod=11.7/R2=7.7%
SB10
0.0 SB10001
3.1 SB10004
11.7 SB10012
23.9 SB10021
31.3 SB10030
38.8 SB10033
41.6 SB10038
44.4 SB10041
46.4 SB10043
60.6 SB10055
64.5 SB10058
67.5 SB10062
68.0 SB10064
70.4 SB10072
73.5 SB10079
76.4 SB10083
83.1 SB10091
85.3 SB10095
88.1 SB10098
94.1 SB10103
96.1 SB10104
101.7 SB10108
110.5 SB10116
117.9 SB10121
126.5 SB10131
132.8 SB10138
Nb_SB_LPB
PB_Dens
PB_L_SN
PE_L
PH
Pos_PB_L_max
RA_B_Dia
SB_DF_PN
Lod=4/R2=4.2%
Lod=4.3/R2=2.4%
Lod=3.9/R2=3.3%
Lod=5.1/R2=3.2%
Lod=4/R2=1.9%
Lod=3.6/R2=3.8%
Lod=3.6/R2=2.7%
Lod=4.1/R2=4.4%

**Appendix 4.3: Quantitative Trait Loci (QTLs) detected in Tiandougou/Lata-3 $F_4$ families population**

| Trait | Chrs | Position (cM) | CI_low (0.95) | CI_high (0.95) | Left marker | Right marker | LOD | PVE % | Add | Dom | Dom/Add | Gene action | Dir_ Parents |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *QShcomp- SBI-02* | SBI-02 | 147 | 141 | 154 | SB02139 | SB02161 | 7.3 | 7.9 | 0.5 | 0.4 | 0.80 | D | Tiand |
| *QShcomp- SBI-07* | SBI-07 | 91 | 81 | 117 | SB07077 | SB07125 | 4.2 | 4.5 | 0.4 | 0.0 | -0.08 | A | Tiand |
| *QPH- SBI-02* | SBI-02 | 127 | 123 | 137 | SB02112 | SB02145 | 6.9 | 3.4 | -63.0 | -51 | 0.82 | D | Lata |
| *QPH- SBI-03* | SBI-03 | 74 | 69.9 | 85 | SB03070 | SB03091 | 11.7 | 5.9 | 91.8 | -49 | -0.54 | PD | Tiand |
| *QPH- SBI-04* | SBI-04 | 125 | 115 | 134 | SB04136 | SB04154 | 5.7 | 2.8 | -85.8 | -6.5 | 0.08 | A | Lata |
| *QPH- SBI-06* | SBI-06 | 67 | 63 | 72 | SB06065 | SB06085 | 7.3 | 3.6 | -71.9 | 11.1 | -0.15 | A | Lata |
| *QPH- SBI-07* | SBI-07 | 95 | 94 | 96 | SB07090 | SB07096 | 60.3 | 41.1 | -238 | 67.9 | -0.29 | PD | Lata |
| *QPH- SBI-10* | SBI-10 | 99 | 90 | 113 | SB10098 | SB10121 | 4.0 | 1.9 | 51.8 | 19.9 | 0.38 | PD | Tiand |
| *QSFD*- SBI-03 | SBI-03 | 71.2 | 74 | 79.4 | SB03075 | SB03085 | 53.1 | 45.6 | 5.2 | -2.6 | -0.49 | PD | Tiand |
| *QPA_L- SBI-01* | SBI-01 | 8 | 3 | 12.7 | SB01004 | SB01015 | 8.3 | 6.7 | 7.8 | -2.0 | -0.26 | PD | Tiand |
| *QPA_L- SBI-02* | SBI-02 | 141 | 125 | 147 | SB02118 | SB02154 | 8.6 | 6.9 | -8.1 | 1.0 | -0.13 | A | Lata |
| *QPA_L- SBI-03* | SBI-03 | 143 | 135 | 147 | SB03137 | SB03164 | 13.4 | 11.2 | -9.7 | -2.7 | 0.27 | PD | Lata |
| *QPA_L- SBI-04* | SBI-04 | 55 | 43 | 61 | SB04035 | SB04090 | 9.7 | 7.9 | -8.3 | -1.8 | 0.22 | PD | Lata |
| *QPE_L- SBI-01* | SBI-01 | 28 | 21 | 37 | SB01034 | SB01050 | 6.1 | 3.9 | -10.6 | 4.4 | -0.41 | PD | Lata |
| *QPE_L- SBI-02* | SBI-02 | 132.2 | 112.7 | 157 | SB02104 | SB02171 | 4.2 | 2.6 | -8.9 | 4.8 | -0.53 | PD | Lata |
| *QPE_L- SBI-03* | SBI-03 | 73 | 71.2 | 75 | SB03075 | SB03085 | 28.3 | 20.4 | -25.4 | -2.7 | 0.11 | A | Lata |
| *QPE_L- SBI-04* | SBI-04 | 15.4 | 0 | 20 | SB04010 | SB04029 | 5.8 | 3.6 | -10.1 | 1.6 | -0.16 | A | Lata |
| *QPE_L- SBI-07* | SBI-07 | 95 | 88 | 108 | SB07085 | SB07108 | 7.1 | 4.6 | -11.5 | 6.0 | -0.52 | PD | Lata |
| *QPE_L- SBI-09* | SBI-09 | 51 | 48 | 61 | SB09037 | SB09080 | 11.7 | 7.7 | 14.9 | -4.6 | -0.31 | PD | Tiand |
| *QPE_L- SBI-10* | SBI-10 | 103 | 99 | 130 | SB10104 | SB10138 | 5.1 | 3.2 | 10.2 | -0.7 | -0.07 | A | Tiand |
| *QRA_L- SBI-01* | SBI-01 | 9 | 6 | 14 | SB01004 | SB01023 | 9.5 | 7.9 | 8.6 | -2.5 | -0.29 | PD | Tiand |
| *QRA_L- SBI-02* | SBI-02 | 128 | 124.1 | 140 | SB02118 | SB02145 | 9.4 | 7.8 | -8.6 | 2.0 | -0.23 | PD | Lata |
| *QRA_L- SBI-03* | SBI-03 | 143 | 137 | 151 | SB03137 | SB03164 | 12.1 | 10.2 | -9.5 | -2.4 | 0.25 | PD | Lata |
| *QRA_L- SBI-04* | SBI-04 | 55 | 46 | 64 | SB04035 | SB04090 | 8.1 | 6.7 | -7.8 | -1.8 | 0.23 | PD | Lata |
| *QIN_L- SBI-01* | SBI-01 | 7 | 1 | 17.3 | SB01002 | SB01023 | 4.5 | 4.0 | -0.4 | -0.1 | 0.30 | PD | Lata |
| *QIN_L- SBI-03* | SBI-03 | 144 | 141 | 150 | SB03149 | SB03164 | 14.8 | 14.1 | -0.7 | -0.1 | 0.17 | A | Lata |
| *QIN_L-SBI-04* | SBI-04 | 60 | 55 | 66 | SB04077 | SB04090 | 7.5 | 6.8 | -0.5 | 0.2 | -0.33 | PD | Lata |

Appendix 4.3 continued

| **Trait** | **Chrs** | **Position (cM)** | **CI_low (0.95)** | **CI_high (0.95)** | **Left marker** | **Right marker** | **LOD** | **PVE %** | **Add** | **Dom** | **Dom/Add** | **Gene action** | **Dir_ Parents** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *QIN_L_max- SBI-02* | SBI-02 | 153 | 144.7 | 162 | SB02145 | SB02171 | 6.1 | 5.8 | -0.9 | 0.3 | -0.39 | PD | Lata |
| *QIN_L_max- SBI-03* | SBI-03 | 151 | 143 | 158 | SB03149 | SB03169 | 6.8 | 6.5 | -0.9 | -0.2 | 0.20 | A | Lata |
| *QIN_L_max- SBI-04* | SBI-04 | 56.5 | 54 | 64 | SB04077 | SB04090 | 6.7 | 6.4 | -0.9 | -0.4 | 0.49 | PD | Lata |
| *QRA_B_Dia- SBI-02* | SBI-02 | 151 | 9 | 164 | SB02009 | SB02171 | 4.3 | 3.2 | 0.1 | 0.1 | 0.65 | PD | Tiand |
| *QRA_B_Dia- SBI-03* | SBI-03 | 138 | 123 | 144 | SB03115 | SB03158 | 6.9 | 5.2 | 0.1 | 0.0 | -0.28 | PD | Tiand |
| *QRA_B_Dia- SBI-06* | SBI-06 | 69 | 67 | 72 | SB06074 | SB06085 | 20.4 | 16.6 | -0.2 | 0.0 | -0.05 | A | Lata |
| *QRA_B_Dia- SBI-07-1* | SBI-07 | 35 | 27 | 38 | SB07023 | SB07036 | 4.9 | 3.6 | 0.1 | 0.0 | 0.31 | PD | Tiand |
| *QRA_B_Dia- SBI-07-2* | SBI-07 | 119.6 | 113 | 119.6 | SB07108 | SB07125 | 5.7 | 4.3 | 0.1 | 0.0 | 0.41 | PD | Tiand |
| *QRA_B_Dia- SBI-10* | SBI-10 | 62 | 53 | 118 | SB10043 | SB10131 | 3.6 | 2.7 | 0.0 | 0.2 | 5.73 | PD | Tiand |
| *QRA_Dia- SBI-02* | SBI-02 | 151 | 9 | 163 | SB02009 | SB02171 | 4.3 | 3.4 | 0.0 | 0.0 | 0.42 | PD | Tiand |
| *QRA_Dia- SBI-03* | SBI-03 | 136 | 131 | 143 | SB03131 | SB03158 | 5.4 | 4.3 | 0.1 | 0.0 | -0.21 | A | Tiand |
| *QRA_Dia- SBI-06* | SBI-06 | 70 | 67 | 73 | SB06074 | SB06085 | 16.4 | 14.0 | -0.1 | 0.0 | -0.08 | A | Lata |
| *QRA_Dia- SBI-07-1* | SBI-07 | 34.6 | 27 | 38 | SB07023 | SB07036 | 5.0 | 4.0 | 0.1 | 0.0 | 0.10 | A | Tiand |
| *QRA_Dia- SBI-07-2* | SBI-07 | 119 | 108 | 119.6 | SB07096 | SB07125 | 5.2 | 4.1 | 0.1 | 0.0 | 0.22 | PD | Tiand |
| *QRA_coni- SBI-02* | SBI-02 | 129 | 125 | 144 | SB02118 | SB02145 | 12.9 | 8.7 | 0.0 | 0.0 | -0.20 | A | Tiand |
| *QRA_coni-SBI-03-1* | SBI-03 | 117 | 105 | 122 | SB03102 | SB03131 | 5.1 | 3.3 | 0.0 | 0.0 | 0.08 | A | Tiand |
| *QRA_coni-SBI-03-2* | SBI-03 | 143 | 140 | 151 | SB03149 | SB03164 | 8.5 | 5.6 | 0.0 | 0.0 | -0.09 | A | Tiand |
| *QRA_coni- SBI-04-1* | SBI-04 | 55 | 44 | 62 | SB04035 | SB04090 | 5.2 | 3.4 | 0.0 | 0.0 | 0.59 | PD | Tiand |
| *QRA_coni- SBI-04-2* | SBI-04 | 134.6 | 125 | 134.6 | SB04136 | SB04154 | 5.4 | 3.5 | 0.0 | 0.0 | -0.74 | PD | Lata |
| *QRA_coni- SBI-06* | SBI-06 | 68 | 58 | 72 | SB06057 | SB06085 | 11.7 | 7.9 | 0.0 | 0.0 | -0.03 | A | Lata |
| *QRA_coni- SBI-07* | SBI-07 | 119.6 | 42 | 119.6 | SB07036 | SB07125 | 4.4 | 2.8 | 0.0 | 0.0 | 0.76 | PD | Tiand |
| *QRA_Vol- SBI-01* | SBI-01 | 15 | 9 | 21 | SB01004 | SB01038 | 4.6 | 4.0 | 199.7 | -69 | -0.35 | PD | Tiand |
| *QRA_Vol- SBI-04* | SBI-04 | 64 | 53 | 77 | SB04046 | SB04108 | 4.1 | 3.5 | -200 | 78.3 | -0.39 | PD | Lata |
| *QRA_Vol- SBI-06* | SBI-06 | 71 | 68 | 74 | SB06074 | SB06085 | 17.3 | 16.1 | -417 | 55.0 | -0.13 | A | Lata |
| *QRA_Vol- SBI-07* | SBI-07 | 33 | 25 | 38 | SB07023 | SB07036 | 4.5 | 3.9 | 206.8 | 3.2 | 0.02 | A | Tiand |

Appendix 4.3 continued

| **Trait** | **Chrs** | **Position (cM)** | **CI_low (0.95)** | **CI_high (0.95)** | **Left marker** | **Right marker** | **LOD** | **PVE %** | **Add** | **Dom** | **Dom/Add** | **Gene action** | **Dir_ Parents** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *QRA_slend- SBI-01* | SBI-01 | 81.7 | 0 | 86 | SB01002 | SB01096 | 5.1 | 3.3 | -1.4 | 1.9 | -1.41 | PD | Lata |
| *QRA_slend- SBI-02* | SBI-02 | 141 | 138 | 145 | SB02139 | SB02154 | 16.9 | 11.7 | -3.4 | -0.6 | 0.18 | A | Lata |
| *QRA_slend- SBI-03* | SBI-03 | 137 | 134 | 141 | SB03137 | SB03158 | 21.0 | 14.9 | -3.7 | 0.0 | 0.00 | A | Lata |
| *QRA_slend- SBI-04* | SBI-04 | 53.2 | 47 | 60 | SB04035 | SB04090 | 6.6 | 4.3 | -1.9 | -0.9 | 0.48 | PD | Lata |
| *QRA_slend- SBI-06* | SBI-06 | 58 | 52 | 71 | SB06034 | SB06085 | 7.8 | 5.1 | 2.1 | 0.6 | 0.30 | PD | Tiand |
| *QRA_slend- SBI-07-1* | SBI-07 | 56 | 46 | 64 | SB07036 | SB07067 | 3.6 | 2.3 | -1.2 | -1.5 | 1.29 | OD | Lata |
| *QRA_slend- SBI-07-2* | SBI-07 | 119.6 | 114 | 119.6 | SB07108 | SB07125 | 5.7 | 3.7 | -1.7 | -0.9 | 0.52 | PD | Lata |
| *QPB_L_SN- SBI-02* | SBI-02 | 140 | 134 | 143 | SB02132 | SB02145 | 12.9 | 11.7 | -3.1 | 0.4 | -0.14 | A | Lata |
| *QPB_L_SN- SBI-03* | SBI-03 | 148 | 133 | 152 | SB03137 | SB03164 | 7.3 | 6.4 | -2.1 | -1.0 | 0.49 | PD | Lata |
| *QPB_L_SN- SBI-04* | SBI-04 | 57 | 49 | 66 | SB04035 | SB04090 | 5.5 | 4.8 | -1.8 | 0.5 | -0.26 | PD | Lata |
| *QPB_L_SN- SBI-10* | SBI-10 | 53 | 43 | 66 | SB10038 | SB10062 | 3.9 | 3.3 | 1.6 | 0.6 | 0.40 | PD | Tiand |
| *QPB_L_MN- SBI-03-1* | SBI-03 | 13 | 6 | 16 | SB03006 | SB03019 | 4.4 | 4.1 | 3.5 | 4.2 | 1.21 | D | Tiand |
| *QPB_L_MN- SBI-03-2* | SBI-03 | 18 | 14 | 19 | SB03019 | SB03030 | 6.1 | 5.8 | -4.4 | -3.7 | 0.83 | D | Lata |
| *QPB_L_MN- SBI-03-3* | SBI-03 | 132 | 124 | 140 | SB03115 | SB03158 | 4.2 | 4.0 | -1.0 | 0.1 | -0.11 | A | Lata |
| *QPB_L_MN- SBI-06* | SBI-06 | 85.2 | 76 | 89 | SB06087 | SB06113 | 4.8 | 4.6 | 1.2 | -0.1 | -0.12 | A | Tiand |
| *QPB_L_MN- SBI-02* | SBI-02 | 118 | 109 | 129 | SB02099 | SB02132 | 3.8 | 3.8 | 0.8 | 0.8 | 1.03 | D | Tiand |
| *QPB_L_PN- SBI-03* | SBI-03 | 14 | 8 | 26 | SB03006 | SB03030 | 4.5 | 4.5 | -1.0 | 0.6 | -0.57 | PD | Lata |
| *QPB_L_PN- SBI-06* | SBI-06 | 85.2 | 84 | 91 | SB06098 | SB06113 | 5.5 | 5.6 | 1.1 | 0.2 | 0.22 | PD | Tiand |
| *QPB_L_PN- SBI-02* | SBI-02 | 142 | 138 | 148 | SB02139 | SB02154 | 9.7 | 9.9 | -3.0 | 0.5 | -0.15 | A | Lata |
| *QPB_L_max- SBI-03* | SBI-03 | 144 | 128 | 152 | SB03115 | SB03164 | 5.7 | 5.7 | -2.1 | -0.1 | 0.06 | A | Lata |
| *QPB_L_max- SBI-02* | SBI-02 | 128 | 116 | 133 | SB02104 | SB02139 | 6.9 | 4.2 | 0.3 | -0.1 | -0.34 | PD | Tiand |
| *QAv_LPB- SBI-03-1* | SBI-03 | 23 | 19 | 33 | SB03019 | SB03037 | 11.7 | 7.3 | 0.3 | 0.1 | 0.23 | PD | Tiand |
| *QAv_LPB- SBI-03-2* | SBI-03 | 135 | 131 | 141 | SB03131 | SB03158 | 5.4 | 3.3 | 0.2 | 0.2 | 0.89 | D | Tiand |
| *QAv_LPB-* | SBI-06 | 70 | 69 | 72 | SB06074 | SB06085 | 36.5 | 26.6 | -0.6 | 0.0 | -0.07 | A | Lata |
| *QAv_LPB- SBI-07* | SBI-07 | 39 | 29 | 50 | SB07023 | SB07044 | 4.8 | 2.9 | 0.2 | 0.0 | 0.05 | A | Tiand |
| *QAv_LPB -SBI-02* | SBI-02 | 127 | 115 | 146 | SB02104 | SB02154 | 9.7 | 9.5 | -0.6 | 0.1 | -0.18 | A | Lata |

Appendix 4.3 continued

| **Trait** | **Chrs** | **Position (cM)** | **CI_low (0.95)** | **CI_high (0.95)** | **Left marker** | **Right marker** | **LOD** | **PVE %** | **Add** | **Dom** | **Dom/Add** | **Gene action** | **Dir_ Parents** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *QSB_L- SBI-06* | SBI-06 | 75 | 68 | 78 | SB06074 | SB06098 | 10.0 | 9.9 | 0.5 | 0.1 | 0.24 | PD | Tiand |
| *QSB_L- SBI-02* | SBI-02 | 128 | 118 | 143 | SB02107 | SB02145 | 10.6 | 9.4 | -1.1 | 0.3 | -0.24 | PD | Lata |
| *QSB_L_max- SBI-03* | SBI-03 | 126 | 113 | 133 | SB03107 | SB03149 | 5.0 | 4.3 | -0.7 | -0.5 | 0.72 | PD | Lata |
| *QSB_L_max- SBI-04* | SBI-04 | 56.5 | 53 | 65 | SB04046 | SB04090 | 4.8 | 4.1 | -0.6 | 0.4 | -0.71 | PD | Lata |
| *QSB_L_max- SBI-05* | SBI-05 | 101.9 | 90 | 118.7 | SB05115 | SB05166s | 3.7 | 3.1 | 0.5 | -0.5 | -1.04 | D | Tiand |
| *QSB_L_max- SBI-06* | SBI-06 | 72 | 47 | 79 | SB06034 | SB06098 | 5.3 | 4.5 | 0.7 | 0.3 | 0.46 | PD | Tiand |
| *QSB_L_max- SBI-01* | SBI-01 | 157 | 138 | 171.4 | SB01134 | SB01168 | 3.6 | 4.1 | 0.3 | 0.0 | 0.09 | A | Tiand |
| *QSB_DF_MN- SBI-02* | SBI-02 | 117.7 | 41 | 132.2 | SB02013 | SB02132 | 3.5 | 3.8 | 0.0 | 0.0 | 0.55 | PD | Tiand |
| *QSB_DF_PN- SBI-10* | SBI-10 | 108 | 92 | 117 | SB10098 | SB10121 | 4.1 | 4.4 | 0.0 | 0.0 | -0.79 | A | Tiand |
| *QSB_DF_PN- SBI-02-1* | SBI-02 | 117.7 | 114 | 126 | SB02104 | SB02132 | 4.7 | 5.3 | 0.2 | 0.2 | 0.99 | D | Tiand |
| *QAv_SB_DF- SBI-02-2* | SBI-02 | 140 | 126 | 143 | SB02118 | SB02145 | 14.0 | 13.7 | -3.4 | 0.7 | -0.19 | A | Lata |
| *QPB_BZ_SN- SBI-03-1* | SBI-03 | 144 | 133 | 152 | SB03137 | SB03164 | 8.1 | 7.7 | -2.4 | -0.8 | 0.32 | PD | Lata |
| *QPB_BZ_SN- SBI-03-2* | SBI-03 | 45 | 17.2 | 57 | SB03019 | SB03060 | 5.9 | 5.9 | -1.1 | 0.0 | 0.02 | A | Lata |
| *QPB_BZ_MN- SBI-03* | SBI-03 | 135 | 128 | 143 | SB03115 | SB03158 | 5.0 | 5.0 | -1.0 | 0.0 | 0.00 | A | Lata |
| *QPB_BZ_MN- SBI-06* | SBI-06 | 88 | 80 | 96 | SB06087 | SB06122 | 4.8 | 4.7 | 0.9 | -0.8 | -0.84 | D | Tiand |
| *QPB_BZ_MN- SBI-02* | SBI-02 | 121 | 115 | 127 | SB02104 | SB02132 | 3.7 | 3.4 | 0.6 | 1.2 | 2.08 | OD | Tiand |
| *QPB_BZ_PN- SBI-03* | SBI-03 | 22 | 9 | 28 | SB03006 | SB03032 | 6.2 | 5.8 | -1.0 | 1.0 | -1.01 | D | Lata |
| *QPB_BZ_PN- SBI-05* | SBI-05 | 28 | 18 | 32 | SB05014 | SB05032 | 4.6 | 4.2 | -0.2 | -2.1 | 10.98 | OD | Lata |
| *QPB_BZ_PN- SBI-06* | SBI-06 | 85.2 | 84 | 92 | SB06098 | SB06113 | 7.0 | 6.6 | 1.2 | 0.1 | 0.07 | A | Tiand |
| *QPB_BZ_PN- SBI-09* | SBI-09 | 115.1 | 113 | 117.5 | SB09138 | SB09145 | 5.3 | 4.9 | -0.9 | 0.7 | -0.82 | A | Lata |
| *QPB_BZ_PN- SBI-01* | SBI-01 | 9 | 7 | 14 | SB01004 | SB01023 | 21.4 | 19.2 | 0.6 | -0.1 | -0.16 | A | Tiand |
| *QPA_IN_N- SBI-02-1* | SBI-02 | 118 | 115 | 120 | SB02104 | SB02112 | 9.5 | 7.9 | -0.4 | -0.1 | 0.15 | A | Lata |
| *QPA_IN_N- SBI-03* | SBI-03 | 8 | 0 | 14 | SB03002 | SB03019 | 5.1 | 4.1 | 0.3 | 0.0 | -0.05 | A | Tiand |
| *QPA_IN_N- SBI-02-2* | SBI-02 | 130 | 115 | 147 | SB02104 | SB02154 | 4.2 | 3.0 | 0.5 | -0.2 | -0.51 | PD | Tiand |

Appendix 4.3 continued

| **Trait** | **Chrs** | **Position (cM)** | **CI_low (0.95)** | **CI_high (0.95)** | **Left marker** | **Right marker** | **LOD** | **PVE %** | **Add** | **Dom** | **Dom/Add** | **Gene action** | **Dir_ Parents** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *QNb_PB_max- SBI-03-1* | SBI-03 | 30 | 19 | 33 | SB03019 | SB03037 | 8.0 | 5.7 | 0.6 | -0.2 | -0.36 | PD | Tiand |
| *QNb_PB_max- SBI-03-2* | SBI-03 | 137 | 132 | 151 | SB03131 | SB03164 | 5.9 | 4.1 | 0.5 | 0.6 | 1.08 | D | Tiand |
| *QNb_PB_max- SBI-06* | SBI-06 | 69 | 67 | 71 | SB06074 | SB06085 | 24.6 | 19.4 | -1.3 | 0.1 | -0.06 | A | Lata |
| *QNb_PB_max- SBI-07* | SBI-07 | 44 | 36 | 50 | SB07031 | SB07044 | 5.5 | 3.9 | 0.6 | -0.1 | -0.20 | A | Tiand |
| *QNb_PB_max- SBI-01* | SBI-01 | 11 | 8 | 13 | SB01004 | SB01023 | 18.2 | 10.6 | 7.9 | -1.5 | -0.19 | A | Tiand |
| *QPA_NPB- SBI-02* | SBI-02 | 166 | 150 | 169.1 | SB02145 | SB02172 | 4.1 | 2.2 | 3.7 | 0.6 | 0.16 | A | Tiand |
| *QPA_NPB- SBI-03-1* | SBI-03 | 19 | 12 | 22 | SB03006 | SB03030 | 15.2 | 8.7 | 7.1 | -0.4 | -0.06 | A | Tiand |
| *QPA_NPB- SBI-03-2* | SBI-03 | 138 | 132 | 154 | SB03131 | SB03169 | 5.0 | 2.7 | 3.9 | 1.4 | 0.36 | PD | Tiand |
| *QPA_NPB- SBI-06* | SBI-06 | 78 | 76 | 80 | SB06087 | SB06098 | 31.9 | 20.2 | -11.0 | 2.5 | -0.22 | PD | Lata |
| *QPA_NPB- SBI-07* | SBI-07 | 49 | 36 | 51 | SB07031 | SB07046b | 5.6 | 3.0 | 4.1 | -0.8 | -0.21 | A | Tiand |
| *QPA_NPB- SBI-02* | SBI-02 | 146 | 140 | 151 | SB02139 | SB02161 | 5.4 | 5.2 | -1.1 | 0.4 | -0.38 | PD | Lata |
| *Qav_NPB- SBI-03* | SBI-03 | 19 | 15 | 26 | SB03006 | SB03030 | 6.2 | 6.0 | -1.2 | -0.1 | 0.10 | A | Lata |
| *Qav_NPB- SBI-06* | SBI-06 | 86 | 79 | 88 | SB06087 | SB06113 | 6.6 | 6.5 | 1.2 | 0.4 | 0.36 | PD | Tiand |
| *Qav_NPB- SBI-01* | SBI-01 | 12.7 | 8 | 15 | SB01004 | SB01023 | 7.4 | 4.2 | 0.0 | 0.0 | -0.10 | A | Tiand |
| *QPB_Dens- SBI-02* | SBI-02 | 147 | 139 | 153 | SB02139 | SB02161 | 8.4 | 4.8 | 0.0 | 0.0 | -0.03 | A | Tiand |
| *QPB_Dens- SBI-03-1* | SBI-03 | 20 | 15 | 24 | SB03006 | SB03030 | 12.3 | 7.1 | 0.0 | 0.0 | 0.25 | PD | Tiand |
| *QPB_Dens- SBI-03-2* | SBI-03 | 142 | 135 | 150 | SB03137 | SB03164 | 14.0 | 8.2 | 0.0 | 0.0 | 0.15 | A | Tiand |
| *QPB_Dens- SBI-06* | SBI-06 | 77 | 72 | 80 | SB06074 | SB06098 | 28.8 | 18.4 | 0.0 | 0.0 | 0.03 | A | Lata |
| *QPB_Dens- SBI-07* | SBI-07 | 38 | 31 | 50 | SB07023 | SB07044 | 4.5 | 2.5 | 0.0 | 0.0 | -0.50 | PD | Tiand |
| *QPB_Dens- SBI-10* | SBI-10 | 126.5 | 114 | 132.8 | SB10116 | SB10138 | 4.3 | 2.4 | 0.0 | 0.0 | -0.60 | PD | Lata |
| *QPB_Dens- SBI-02* | SBI-02 | 110 | 106 | 117 | SB02099 | SB02107 | 8.1 | 8.6 | -0.2 | 0.2 | -0.80 | PD | Lata |
| *QNb_SB_LPB- SBI-10* | SBI-10 | 32 | 2 | 41 | SB10001 | SB10038 | 4.0 | 4.2 | -0.1 | 0.0 | -0.09 | A | Lata |
| *QNb_SB_LPB- SBI-02* | SBI-02 | 139 | 125 | 143 | SB02118 | SB02145 | 9.1 | 9.1 | 0.0 | 0.0 | -0.87 | D | Lata |
| *QNb_SB_SN- SBI-06* | SBI-06 | 93 | 61 | 97 | SB06065 | SB06122 | 4.8 | 4.7 | 0.0 | 0.0 | 0.09 | A | Lata |
| *QNb_SB_SN- SBI-08* | SBI-08 | 62.2 | 30 | 71 | SB08023 | SB08097 | 4.1 | 3.9 | 0.0 | 0.0 | 1.07 | D | Lata |
| *QNb_SB_SN- SBI-01* | SBI-01 | 152 | 145 | 167 | SB01144 | SB01168 | 4.6 | 4.7 | 0.0 | 0.0 | -0.98 | D | Lata |

Appendix 4.3 continued

| **Trait** | **Chrs** | **Position (cM)** | **CI_low (0.95)** | **CI_high (0.95)** | **Left marker** | **Right marker** | **LOD** | **PVE %** | **Add** | **Dom** | **Dom/Add** | **Gene action** | **Dir_ Parents** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *QNb_SB_MN- SBI-03-1* | SBI-03 | 14 | 9 | 19 | SB03006 | SB03030 | 7.8 | 8.1 | 0.0 | 0.0 | -0.76 | PD | Lata |
| *QNb_SB_MN- SBI-03-2* | SBI-03 | 14 | 10 | 21 | SB03006 | SB03030 | 6.5 | 6.6 | 0.0 | 0.0 | -1.36 | OD | Lata |
| *QNb_SB_PN- SBI-06* | SBI-06 | 90 | 85 | 107 | SB06098 | SB06140 | 4.7 | 4.7 | 0.0 | 0.0 | -0.07 | A | Tiand |
| *QNb_SB_PN- SBI-09* | SBI-09 | 116 | 113 | 117.5 | SB09138 | SB09145 | 4.6 | 4.6 | 0.0 | 0.0 | -0.58 | PD | Lata |
| *QNb_SB_PN- SBI-03* | SBI-03 | 148 | 143 | 150 | SB03149 | SB03164 | 14.4 | 13.6 | 0.0 | 0.0 | 0.12 | A | Tiand |
| *QSB_Dens_SN- SBI-06-1* | SBI-06 | 69 | 59.8 | 73 | SB06065 | SB06085 | 10.5 | 9.7 | 0.0 | 0.0 | 0.48 | PD | Lata |
| *QSB_Dens_SN- SBI-06-2* | SBI-06 | 80 | 66 | 86 | SB06074 | SB06106 | 7.2 | 7.9 | 0.0 | 0.0 | 0.26 | PD | Lata |
| *QSB_Dens_MN- SBI-01* | SBI-01 | 7 | 1 | 13 | SB01002 | SB01023 | 6.1 | 5.9 | 0.4 | 0.2 | 0.56 | PD | Tiand |
| *QRel_Pos_IN_L_max- SBI-06* | SBI-06 | 73 | 67 | 78 | SB06074 | SB06098 | 4.9 | 5.5 | 0.0 | 0.0 | -0.70 | D | Lata |
| *QRel_Pos_NPB_max- SBI-02* | SBI-02 | 116 | 113 | 124.1 | SB02104 | SB02118 | 11.3 | 12.2 | 0.0 | 0.0 | -0.54 | PD | Tiand |
| *QRel_Pos_PB_L_max- SBI-03* | SBI-03 | 147 | 142 | 151 | SB03149 | SB03164 | 7.2 | 6.9 | 2.2 | 1.0 | 0.45 | PD | Tiand |
| *QBending- SBI-02-1* | SBI-02 | 102 | 94 | 106 | SB02086 | SB02104 | 5.6 | 5.2 | 0.2 | -0.2 | -0.73 | PD | Tiand |
| *QBending- SBI-02-2* | SBI-02 | 146 | 140 | 166 | SB02139 | SB02171 | 5.8 | 5.4 | -0.3 | 0.0 | -0.06 | A | Lata |
| *QBending- SBI-07* | SBI-07 | 91 | 87 | 102 | SB07085 | SB07108 | 5.0 | 4.7 | -0.2 | 0.2 | -1.17 | D | Lata |
| *QBending- SBI-09* | SBI-09 | 98 | 86 | 102 | SB09101 | SB09122 | 5.5 | 5.1 | -0.2 | -0.3 | 1.60 | OD | Lata |

Appendix 4.3 continued

| **Trait** | **Chrs** | **Position (cM)** | **CI_low (0.95)** | **CI_high (0.95)** | **Left marker** | **Right marker** | **LOD** | **PVE %** | **Add** | **Dom** | **Dom/Add** | **Gene action** | **Dir_ Parents** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *QPA_GY- SBI-06* | SBI-06 | 70 | 66 | 79 | SB06074 | SB06098 | 12.1 | 11.9 | -3.1 | 0.9 | -0.30 | PD | Lata |
| *QPA_GY- SBI-03* | SBI-03 | 146.3 | 143 | 150 | SB03149 | SB03164 | 9.1 | 8.6 | 3.1 | 1.3 | 0.42 | PD | Tiand |
| *QPA_W- SBI-06* | SBI-06 | 70 | 64.9 | 78 | SB06074 | SB06098 | 12.8 | 12.3 | -4.0 | 2.4 | -0.59 | PD | Lata |
| *QPA_W- SBI-01* | SBI-01 | 5 | 0 | 28 | SB01002 | SB01041 | 4.0 | 4.3 | 0.0 | 0.0 | -0.22 | PD | Lata |
| *QPA_HI- SBI-04* | SBI-04 | 72 | 62 | 83 | SB04082 | SB04108 | 3.5 | 3.8 | 0.0 | 0.0 | 0.64 | PD | Tiand |
| *QPA_HI- SBI-01* | SBI-01 | 18.6 | 11 | 28 | SB01004 | SB01041 | 3.6 | 3.8 | 0.2 | 0.1 | 0.35 | PD | Tiand |
| *QTGW- SBI-07* | SBI-07 | 90 | 73 | 99.7 | SB07067 | SB07096 | 7.1 | 7.5 | -0.3 | 0.1 | -0.26 | PD | Lata |
| *QTGW- SBI-03* | SBI-03 | 146.3 | 143 | 150 | SB03149 | SB03164 | 8.0 | 7.5 | 102.3 | 17.5 | 0.17 | A | Tiand |
| *QNG_PA- SBI-06* | SBI-06 | 69 | 62 | 77 | SB06065 | SB06098 | 12.8 | 12.5 | -141 | 6.9 | -0.05 | A | Lata |
| *QNG_PA- SBI-02* | SBI-02 | 126 | 112 | 138 | SB02099 | SB02145 | 8.5 | 8.4 | -3.3 | -0.2 | 0.06 | A | Lata |
| *QNG_SN- SBI-03-1* | SBI-03 | 49 | 43 | 69.7 | SB03042 | SB03068 | 3.6 | 3.5 | -1.6 | 2.6 | -1.66 | A | Lata |
| *QNG_SN- SBI-03-2* | SBI-03 | 144 | 139 | 159.2 | SB03137 | SB03169 | 5.1 | 5.0 | 2.5 | -0.7 | -0.28 | PD | Tiand |
| *QNG_SN- SBI-01* | SBI-01 | 14 | 8 | 18.6 | SB01004 | SB01029 | 6.2 | 6.2 | -1.3 | 0.7 | -0.49 | PD | Lata |
| *QNG_MN- SBI-03* | SBI-03 | 14 | 9 | 19 | SB03006 | SB03030 | 8.2 | 8.3 | -1.6 | 0.5 | -0.31 | PD | Lata |
| *QNG_MN- SBI-01* | SBI-01 | 13 | 9 | 16 | SB01004 | SB01023 | 7.0 | 7.3 | -0.6 | 0.5 | -0.82 | D | Lata |
| *QNG_PN- SBI-03* | SBI-03 | 11 | 1 | 21 | SB03002 | SB03030 | 3.7 | 3.8 | -0.5 | 0.3 | -0.67 | PD | Lata |
| *QNG_PN- SBI-01* | SBI-01 | 12.7 | 9 | 15 | SB01004 | SB01023 | 8.8 | 8.1 | -1.3 | 0.1 | -0.04 | A | Lata |
| *QAv_NG- SBI-02* | SBI-02 | 141 | 135 | 146 | SB02132 | SB02154 | 7.3 | 6.7 | -1.3 | 0.3 | -0.23 | PD | Lata |
| *QAv_NG- SBI-03-1* | SBI-03 | 12 | 2 | 54 | SB03002 | SB03060 | 4.3 | 3.8 | -1.0 | 0.1 | -0.07 | A | Lata |
| *QAv_NG- SBI-03-2* | SBI-03 | 146 | 139.2 | 159.2 | SB03149 | SB03169 | 4.8 | 4.3 | 1.0 | -0.1 | -0.09 | A | Tiand |
| *QAv_NG- SBI-01* | SBI-01 | 4 | 0 | 12.7 | SB01002 | SB01015 | 3.7 | 3.4 | 0.2 | -0.1 | -0.63 | PD | Tiand |