GENETIC ANALYSIS OF GRAIN YIELD AND RELATED TRAITS IN SORGHUM *(Sorghum bicolor* (L.) Moench*)* UNDER PHOSPHORUS DEFICIENT FIELD CONDITIONS IN SOUTHERN MALI

By

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DECLARARATION

I here 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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ABSTRACT

Sorghum (*Sorghum bicolor* (L) Moench), the fifth most important cereal in the world is a multipurpose crop that plays a crucial role in human diets. Sorghum grain yield is heavily constrained by low phosphorus levels that limit its availability for plant use. Genetic improvement approaches that combine high grain yields with tolerance to low phosphorus levels would greatly improve adoption of such sorghum varieties. High grain yield reduction as a results of low soil phosphorus levels have been reported in Mali and they form the primary focus of this study. Determination of farmer preferences for grain and panicle traits in sorghum was undertaken to understand farmer preferred traits in addition to high yield. Genomic segments responsible for grain quality, panicle traits and other yield related traits under high and low phosphorus levels were identified. Alternative selection strategies targeting grain yield improvement under both high and low phosphorus levels (P-deficient) field conditions were also explored. The influence of environment on sorghum 1083 BC1F5 BC-NAM progenies for grain yield were elucidated.

Results from focus group discussions with farmers at Sibi and Dioila clearly indicated that in addition to high grain yield, there were high preferences for grain-hardened open panicles with good threshability. Early maturing sorghum varieties were also much preferred especially under semi-arid conditions.

A high genetic correlation ($r_G = 0.81$) for grain yield was detected under HP and LP condition. The direct selection for grain yield was predicted to be 13% more efficient than the indirect selection. Using a nested mapping approach, a total of 84 QTLs detected by both bi-parental and Association mapping approaches were found to define the genetic architecture of tolerance to low and high phosphorus levels in sorghum. Field evaluations of a set of 13 backcross nested association mapping (BC-NAM) populations was done under contrasting P levels. A multi-environment evaluation of 298 sorghum lines for grain yield indicated that non-additive genetic variance was more important across years under contrasting phosphorus levels. The findings from this study will contribute to the improvement of knowledge about farmers' sorghum preferred traits and genetic control of those traits LP field conditions culminating in genetic improvement of the crop and better adoption of new varieties by farmers.

DEDICATION

I dedicate this thesis to my late father, my mother, my wife and daughter, for their prayers and supports throughout this study.

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LIST OF ABBREVIATIONS

BC	Backcross
BC-NAM	Backcross Nested Association Mapping
ci	Interval of confidence
CIRAD	Centre de Coopération internationale en recherche agronomique pour le développement
COPROSEM	Coopérative des producteurs de semences maraichers
DNA	Deoxyribonucleic acid
GBS	Genotyping By Sequencing
GWAS	Genome-wide association study
GxP	Genotype by Phosphorus
GxY	Genotype by Year
HP	High Phosphorus
ICRISAT	International Crop Research Institute for the Semi-Arid Tropics
IER	institut d'Economie Rurale
IPR/IFRA	Institut Polytechnique Rural de Formation et Recherche Appliquée
LG	Linkage Group
LP	Low Phosphorus
NAM	Nested Association Mapping
P	Phosphorus
Pop	population
PPB	Participatory plant breeding
PRA	Participtory Rural Appraisal
PVE	Phenotypic variation explain
QDA	Qualitative data analysis software
QTL	Quantitative Trait Loci
SNP	Single-nucleotide polymorphisms
ULPC	union locale des producteurs de céréale
WA	West Africa
WCA	West and Central Africa

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CHAPTER ONE

1.0. GENERAL INTRODUCTION

Sorghum (*Sorghum bicolor* (L) Moench) is a cereal of the family *Poaceae*. It is a short day C4 plant, and its capacity to adapt to arid and semi-arid and tropic zones in the world makes it a climate change-compliant crop (Madhusudhana *et al*., 2015). It is grown in 112 countries across the world and it is the fifth leading cereal crop, with production estimated at 94 million tons (FAO, 2016). Sorghum is listed among the 8 cereal grains that provide 56% of food energy and 50% of protein consumed in the world (FAO, 2014). It is one of the most adapted cereal crops to harsh environmental field conditions (D'amato & Lebel, 1998; Sivakumar, 1988; Dahlberg *et al*., 2011). In Mali, sorghum is produced on 1.4 million hectares, with average yield of 0.89 t/ha (FAO, 2016). Sorghum is a staple food crop for millions of poor peoples around the world, especially West Africa. It plays an important role in human nutrition as a source of energy, proteins, vitamins, minerals, and nutraceuticals such as antioxidant phenolic and cholesterol-lowering waxes (Taylor *et al*., 2006)**.** Sorghum is the major food crop in Mali mainly for smallholder farmers, therefore it plays an important role in achieving food security. Sorghum is also used as fodder for livestock and raw material for biofuel industries.

However sorghum production is limited by many biotic and abiotic constraints such as *Striga*, insects, diseases, drought, and poor soil fertility. Grain yield is highly influenced by environmental constraints in sub Saharan zones of Africa where farmers struggle for their livelihoods. The bulk of sorghum in West and Central Africa (WCA) is produced under low input farming systems (vom Brocke *et al*., 2010) mainly in low phosphorus field conditions (Buerkert *et al*., 2001; Leiser *et al*., 2012). Generally in Mali as well as in many parts of Africa, sorghum is cultivated on poor soils with low phosphorus, below the critical threshold level of 7 ppm (Bray-1) (Manu *et al*, 1991; Doumbia *et al*., 1998; Doumbia *et al*., 2003; Leiser *et al*., 2012). This P deficiency leads to reduced plant development, plant height and delayed maturity, consequently yield loss results (Wissuwa & Ae, 2001; Rossiter, 2003; Chen *et al*.,2008; Cichy *et al*., 2009; Parentoni *et al*., 2010; Leiser *et al*., 2012, 2015)**.** There is no doubt that one of the most limiting factors to crop production in soils of Africa is low soil P availability (Bationo *et al*., 1989; Payne *et al*., 1992; Hafner *et al*., 1993). Previous studies have demonstrated the important role played by phosphorus in the sorghum plant growth and development. Small scale farmers are known to generally have low income and as a consequence the application of chemical fertilizer is very low. The rate of application of fertilizer was reported to be lower than 5 kg P ha^{-1} (MacDonald *et al*., 2011)**.** Additionally the implementation of good agronomic practices like crop rotations, intercropping and the use of organic manures and compost provide options for small scale farmers. However, the applications these practices are difficult for small scale farmers due to the lack of high quality of manure recommended and available land is too small for crop rotations.

The recent efforts to improve sorghum production and grain quality have created breeding materials with a range of panicle types with increased grain number of panicle. In spite of these efforts the percentage of improved varieties adopted by farmers remains very low. Therefore the understanding of farmers' preferences for varietal traits have to be included in the breeding objectives. Breeding for grain yield under low input soil fertility system in particular under low P (LP) has been reported (Atlin & Frey , 1989; Mahamane, 2008; Leiser *et al*., 2012). Therefore breeding under phosphorous deficient field conditions could be one solution to the problem. Indeed sorghum grain yield is assessed by yield grain component traits like seed weight, seed number and number of harvested plants. The yield-related traits can affect indirectly or directly grain yield by affecting yield component traits or other mechanisms that are unknown (Shi *et al*., 2009)**.** Local varieties are generally adapted to many biotic and abiotic constraints and have good grain quality but have generally low grain yield potential. In recent decades, there has been remarkable progress in the development of molecular tools for plant breeding, which complement or support conventional breeding effort (Madhusudhana *et al*., 2015). QTL mapping is a tool to unveil/explain the factors underlying genetic control of complex traits. This could be done by either using bi-parental population QTL mapping or genome-wide association mapping (GWAS). The traditional bi-parental analysis has strong statistical power for QTL detection and is more convenient for understanding the allelic effect between two different genotypes to identify region of interest. However it provides low genetic resolution depending of the population size (Buckler & Thornsberry, 2002). Association mapping on the other hand has high resolution mapping QTL based on linkage disequilibrium (LD) (Buckler & Thornsberry, 2002; Holland, 2007). Both methods have been successfully applied to understand the genetics underlying abiotic stress in cereal crops. These two methods have advantages and limitations for the breeder, but they are still complementary depending on the breeding purposes. In order to overcome these limitations, the multi-parental population design multi-parental advanced generation inter-crosses (MAGIC) and nested association mapping (NAM) have been developed. The nested association mapping (NAM) design was created by Yu *et al*., (2008) to combine the advantages of the two methods for identifying quantitative trait loci: linkage analysis and association mapping. The NAM strategies combines the advantages of high recombination resolution, through a large number of genotypes of lines having a common parent, and high allelic diversity, due to the use of a broad range of diverse parents. One of the reasons of using both methods is to distinguish the recombination events that occur in the families or in population. Numerous genetic analysis studies have been conducted in sorghum with the main goal to improve sorghum grain yield and related traits (Lin *et al*., 1995; Pereira *et al*., 1995; Rami *et al*., 1998; Tuinstra *et al*., 1998; Crasta *et al*., 1999; Subudhi *et al*., 2000; Tao *et al*., 2000; Kebede et al., 2001; Haussmann *et al.*, 2002; Murray *et al*., 2009; Phuong *et al*., 2013b; Sako, 2013; Boyles *et al*., 2016) but most of these studies have been undertaken under favorable field conditions. A few studies have been conducted to map QTL under abiotic stress in particular under LP condition. Although NAM is a powerful multi-cross design for complex traits to identify QTLs, it is yet to be used in West African sorghum for identifying QTLs associated with yield and components. A variation of NAM, Back-cross nested association mapping (BC-NAM) uses a recurrent parent that is a locally-adapted elite line (Mace & Jordan, 2011). Local sorghum varieties in WA are generally adapted to low input soils condition specifically under LP conditions (Leiser *et al*., 2012; 2014). Additional to the NAM creation, all F1s are backcrossed once to this elite recurrent parent in order to obtain progenies with a genome of ~25% exotic and ~75% locally adapted origin. The single backcross is important for the context of a generally well-adapted background, and for quickly pyramiding valuable donor alleles into acceptable new cultivars.

The objectives of this study were to:

- determine smallholder farmers’ preferences for grain quality and panicle traits,
- identify QTL associated with grain quality and panicle traits and grain yield related traits,
- develop alternative selection strategies for improving grain yield and related traits under P deficient field conditions, and
- determine the importance of genotype by environment interaction of selected BC-NAM progenies for grain yield.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1 Origin and botanic classification

Sorghum is a cereal crop that belong to the family of Poaceae with chromosome number 2n=2x=20. Many studies have reported the origin of sorghum (Wet & Huckabay, 1967)**.** The African continent is the center of origin and domestication of sorghum (Dillon *et al*., 2007; Doggett, 1965; Wet & Huckabay, 1967). According to Vavilov, Ethiopia is one of the centers of origin for several species and hosts a wide genetic variability for sorghum. Several authors have discussed the systematics of sorghum (Harlan & de Wet, 1971; Wet & Huckabay, 1967). Linnaeus (1753) was the first to describe it under the name *Holcus*, Meonch later separated the genus *Sorghum* from the genus *Holcus* (Clayton, 1961). Sorghum is classified under family *Poaceae*, tribe *Andropogoneaes*, subtribe *Sorghinae*, genus *Sorghum Moench* (Clayton & Renvoize, 1986)**.** The genus has been divided into five subgenera (Celarier, 1959; Garber, 1950). Celarier (1959) described the variation within the five subgenera, except subgenera *Sorghum*. *Sorghum bicolor* sub spp. contains all of the cultivated sorghums. Harlan and de Wet, (1972) developed a simplified informal classification useful to plant breeders for the cultivated sorghum and their closest wild relatives. They classified *Sorghum bicolor* (L) Moench, subspp.*bicolor* into five basic and ten intermediates races (Table 2.1).

Table 2.1. Five basic races and ten intermediate races of sorghum

Basic races	Intermediate/hybrid races
1. Race bicolor (B)	6. Race guinea-bicolor (GB)
2. Race guinea (G)	7. Race caudatum-bicolor (CB)
3. Race caudatum (C)	8. Race kafir-bicolor (KB)
4. Race kafir (K)	9. Race durra-bicolor (DB)
5. Race durra(D)	10. Race guinea-caudatum (GC)
	11. Race guinea-kafir (GK)
	12. Race guinea-durra(GD)
	13. Race kafir-caudatum (KC)
	14. Race durra-caudatum (DC)
	15. Race kafir-durra (KD)

2.2 Global Importance and production constraints.

Sorghum is a major crop for over 30 countries in arid and semi-arid zones of the world (FAO, 2017). Africa is the largest producer worldwide with 43% of production. Sorghum is generally grown in drier zones with diverse rainfall under low soil fertility in WA and other semi-arid zones of Africa. Sorghum grain yield varies depending on the country and the type of producer, but it is particularly low in Africa, where it is used generally as food. Sorghum is an important source of energy including proteins, vitamins, minerals such as Fe and Zn (Kumar *et al*., 2011), and nutraceuticals (Taylor *et al*., 2006), which play an important role to enhance nutrition in the semi-arid zone of the world, in general, and in particular, in West Africa. Sorghum is known for its adaptation to the more hostile environments. Therefore it is an important crop for achieving food security in the semi-arid zones of Africa. Sorghum is also important as animal feed and raw material for biofuel and fiber.

Several agro-ecological factors constrain the production of major food crops in Sub-Sahara Africa (SSA) including sorghum (Reynolds *et al*., 2015). Low temperature, low soil P, Fe toxicity and soil acidity hinder yield, while downy mildew, insect pests, and weeds such as *Striga* cause severe losses (Michels *et al*., 1993; Singh *et al*., 2009). Sorghum cultivation in particular is vulnerable to various biotic and abiotic constraints (Ratnadass *et al*., 2003) that reduce yield and productivity. Among the biotic constraints, weeds and insect pest, are the most important in West Africa.

2.3 Participatory research technology development and transfer

The identification of adoption constraints and opportunities for improving farm performance with famers' involvement in the assessments of new technologies should result in more sustainable impact (Rusike *et al*., 2006). The participatory rural appraisal (PRA) is an excellent concept that socio-economic scientists use to interact with local people through participatory learning and action (Chambers, 2008). It aims to develop more consistent baseline data about constraints through involvement of local communities in the definition and documentation of those problems (Chambers, 2008). In participatory breeding programmes, knowledge about the most important selection criteria of male and female farmers for the cultivars preferred in the rural cropping systems environments is acquired. Danial *et al*. (2007) reported the difficulty of easily replicating on station the various environment of soil type and other abiotic stresses that farmers confront and the distribution of labor in farming system. For the success of breeding activities, farmers' varietal preferences should be clearly identified through participatory collaboration (Sibiya *et al*.,2013). The objectives and criteria of farmers and those from scientists should be complementary for the development of the new technology (Ashby, 1991; Soleri *et al*., 2000)**.** Odendo *et al*. (2002) used PRA to solicit farmer's views on the selection of varieties they planted and reported that earliness and high yield were the most desired traits to farmers. One of the reasons that limits wide adoption of technologies include inappropriateness of technologies, inaccessibility to required input and socio-economic condition (Adesina & Zinnah, 1993; Nkongolo *et al*., 2008; Nkonya *et al*., 1997; Upton, 1987). Nkongolo *et al* (2008) have used farmers participatory tools to assess farmer's knowledge of the major attributes of sorghum cultivars and reported that farmer characterization of sorghum varieties had enabled for the identification of landraces that had outperformed already commercialized varieties. Previous studies have revealed farmer conceptualization of and valuation of their farming systems is holistic, multi-faceted and often distinct from the scientific community's comprehension (Christinck *et al*., 2005b). Chamber (2008), reported, in 1987 the successive involvement of the farmer in seed selection of later generations in breeding process, but Witcombe *et al*. (1996) found that farmer involvement in the whole process, substantially improved outcomes.

2.4 Soils phosphorus availability

2.4.1 **Plant uptake of phosphorus**

Plants take up phosphorus (P) as orthophosphate ion (Pi) from the soil substance through the root and hyphae of associated mycorrhizal fungi (Schnug & De Kok, 2016). The quantity of annual P up take in agriculture systems by plants range from 10 kg to 50 kg ha-1 (Sinaj *et al*., 2009). Plants can use different mechanisms for low phosphorus adaptation, either by creating the high affinity of Pi transporters to take up Pi at low concentration or by producing organic acids and protons into the external environment to solubilize Pi bound to calcium (Schnug & De Kok, 2016). They can also modify their root systems to access wide soil volume by increasing root length and decreasing root diameter, by increasing root hair length or by establishing a symbiosis with mycorrhizal fungi (Jansa *et al*., 2011; Richardson *et al*., 2011).

2.4.2 **Soils phosphorus availability to crops**

Phosphorous is a basic component of several chemical compounds. It is an unsubstituted element for plant survival and it cannot be replaced by another element. It also is involved in regulating of essential enzyme reactions and metabolic pathways (Theodorou & Plaxton, 1993; Schachtman *et al*., 1998)**.** However the phosphorus deficiency is often known as one of the most limiting factor of plant production (De Vries, 1998). The soil phosphorus is generally existent in forms that are not accessible or in the rhizosphere, limiting crops P uptake. As a consequence nearly 30% of the world‘s farming land require the application of phosphorus fertilizers for cropping (Vance *et al*., 2003). Natural reserves of phosphorous fertilizer in the world have been reduced and significant increase in price has occurred which, negatively affects sorghum production by small scale farmers who are financially challenged. Phosphorus reserves are projected to last between 50-130 years based on the current pressure on the world's phosphorus natural reserves (Cooper *et al*.,2011; Cordell & White, 2013; Schnug & De Kok, 2016). The application rate of fertilizer in West Africa is very low, about 5 kg/ha (Obersteiner *et al*., 2013). When P is limited, it leads to harmful consequences for the plant by reducing the plant growth and crop grain biomass yield and delay maturity (Rossiter, 1978; Fageria *et al*.,1988; Atlin *et al*., 1989; Wissuwa & Ae, 2001; Turk *et al*., 2003; Chen *et al*., 2008; Cichy *et al*., 2009; Parentoni *et al*., 2010; Leiser *et al*., 2012, 2015)**.** In particular the shoot and root growth are reduced, which leads to less root mass to reach nutrients and water. The flowering, fruits and seeds are significantly reduced due to P-deficiency (Osman, 2012). Thus, phosphorus is essential for any farming system to achieve food security. The fertilizer is applied in a form that is mainly adsorbed by the soil, which is not immediately available to plants (Balemi & Negisho, 2012). Most of the farming systems in which the application of phosphorus is necessary for plant productivity, the recovery of applied P in the growing season is very low, about 80% of P become immobile and unavailable for P uptake due to adsorption and precipitation (Holford, 1997; Schachtman *et al.*, 1998), which lead to low productivity. Thus the good agronomic practices for P application in the field is crucial for P uptake.

2.4.3 **Grain yield component and related traits**

In general crop yield is the primary interest of producers as well as for breeders, however grain yield is complex trait that is influenced by many environmental factors (Heinrich *et al.*, 1983; Quarrie *et al.*, 2006). The genetic basis of grain yield has been widely investigated compared to others traits, but many unknown genetic mechanisms remain ambiguous (Shi *et al.*, 2009). Sorghum grain yield is also affected directly by its components (grain weight and grain number, panicle number per plant and number of plants harvested) and also by grain yield related traits such as maturity, harvest index, low adaptation to biotic and abiotic stresses including low P fertility (Shi *et al.*, 2009; Sukumaran *et al.*, 2015). The number of grain per panicle and grain weight were report to be yield components that contribute the most to the total grains yield (Heinrich, 19983) the grain number were reported more heritable than the grain weight (Cisse & Ejeta, 2003). Yang *et al.* (2009) reported that most of the genetic variation influencing grain weight in sorghum appeared to be additive. In general the sorghum grain yield heritability appears to be moderate, in particular it excepted to lower under low input field condition (Atlin & Frey, 1990; Ceccarelli, 1989) due to the high residual error (heterogeneity) under low phosphorus condition.

Furthermore studies have reported the significantly low heritability under contrasting P levels (Atlin & Frey, 1989) however in contrast slightly higher estimate values of heritability were detected under Low P. compared to high P. (Leiser *et al*., 2012). Maturity is an essential parameter for plant adaptation (Madhusudhana, 2015). Several studies have also reported the importance of adaptive trait under low P condition such as plant maturity and plant height, which are affected by the delay of maturity and reduction of plant (Rossiter, 1978; Fageria *et al*., 1988; Atlin & Frey, 1989; Wissuwa & Ae, 2001; Turk *et al*., 2003; Chen *et al*., 2008; Cichy *et al*., 2009; Parentoni *et al*., 2010; Leiser *et al*., 2012, 2015).

2.4.4 Breeding for phosphorus deficiency and tolerance

Nitrogen is the most frequently limiting soil substance in the world followed by phosphorus. Sorghum is generally grown in WA under low input farming systems with erratic rainfall (D'amato & Lebel, 1998; Sivakumar, 1988; vom Brocke *et al*., 2010), specifically under LP field conditions. Many soil scientists have reported that the most limiting factor to crop production in the soils of Africa including the Sahel is P availability (Bationo *et al*., 1989; Doumbia *et al*., 1998; Manu *et al*, 1991; Payne *et al*., 1992; Hafner *et al*., 1993; Doumbia *et al*., 2003). There is no doubt about the importance of the response of crops to phosphorus. The majority of farmers do not have access to fertilizer because of the high cost or non-availability (Trolove *et al*., 2003). Breeding for P use efficiency could be an important solution to raise the productivity of crops thereby minimizing the food insecurity problem in Africa. Breeding targeting low input soil fertility specifically under LP was reported feasible (Mahamane, 2008; Leiser *et al*., 2012). Studies have also reported that direct selection under LP is more effective than indirect selection (Atlin & Frey, 1989; Leiser *et al*., 2012). Sorghum local germplasm in WA exhibited a wide genetic variation for P uptake and P utilization traits (Leiser *et al*., 2014; Parentoni *et al*., 2010). The Guinea race is the major sorghum type produced in the Sudan and Savannah zones of Africa (Deu *et al*., 2008; Sagnard *et al*., 2011). West Africa (WA) sorghum germplasm is an important source of diversity available for breeding under low input soil fertility. Guinea and durra races exhibited specific adaptation to LP conditions and the caudatum race was more to adapted to HP condition (Leiser *et al*.,2014). Leiser *et al*. (2015) reported, less delay in heading by photoperiod sensitive genotypes and higher P uptake rate compared to no photoperiod sensitive genotype.

2.5 Genotyping by sequencing (GBS)

Genotyping by sequencing (GBS) is a highly-multiplexed system that has been created in the Bucker lab by Rob Elshire, Cornell University. GBS was constructed to reduce the libraries representation for the Illumina next-generation sequencing platform. This approach is robust over a series of species and able to produce thousands of molecular markers(Elshire *et al*., 2011; Poland *et al*., 2011). The main advantages of the GBS system are reduction of sample manipulation, less PCR and purification stages, no size fractionation and inexpensive barcoding. It reduces the genome complexity and prevents repetitive segments of the genome by using the restriction enzymes. GBS has numerous applications, such as breeding, population genetics studies, germplasm characterization, and trait mapping in diverse organisms. It accomplishes marker discovery and genotyping completely at the same time which is an important strength of this approach. Another important advantage of GBS datasets is the dynamic of the raw data that enables the possibility to reanalyze the raw data obtained from GBS and uncovering further information. The frequent use of genomic selection in plant breeding has become possible because the cost of obtaining molecular marker information, particularly SNPs. Next-generation sequencing technologies have improved output and made possible sequencing of multiple samples at the same time. Sequencing-based high-throughput genotyping combines the advantages of cost-effectiveness, less time, and dense marker data.

Sorghum is one of the cereals with small and low duplication genome size which makes it amenable for genotyping and sequencing. The low gene and copy duplication of sorghum makes it responsive to routine genotyping hence several studies have implemented GBS. Hugo *et al.* (2017) evaluated the genetic and phenotypic diversity of the Ethiopian sorghum core collection using 148,476 SNP markers to cover the entire sorghum genome using GBS. They clustered and separated 11 different sub populations from this core collection. Leiser *et al*. (2014) studied how sensitivity to aluminum toxicity and P-deficiency influenced grain yield with a set of 187 sorghum accessions genotyped by GBS. They identified a single genotypic segment on chromosome 3 with a SNP panel of 220,934 SNPs associated to grain yield. Another application of GBS relates to marker discovery and genotyping. Fadoul (2017) investigated the different protein profiles associated to stress response in sorghum using GBS data from twenty sorghum accessions. They identified SNP markers that were similar to previous findings and these SNPs closely localized with heat and drought tolerance genes. These markers are useful for marker assisted breeding for abiotic stress assessment of promising sorghum lines.

2.6 Principle of QTL mapping and P use efficiency QTL

QTL mapping is based on the principle of identification and association between phenotypic and genotypic markers (Collard *et al*., 2005). Its aim is to identify significant markers that are close to the genes controlling complex quantitative traits (Ross-Ibarra *et al*., 2007). QTL analysis is a linkage-based method for QTL identification in a bi-parental population (Dudley, 1993). It requires a mapping population of 50 to 250 individuals (Mohan *et al*., 1997). A large population size is required for high resolution mapping of segregating trait of interest under different environmental conditions. The polymorphic DNA markers that differentiate the parental genotype segregate among the progenies in a mapping population. The genotypic data generated allows the construction of the genetic map of the population, which represents the order and the position of different markers along the linkage groups. QTLs are detected based on the association of genotypic and phenotypic scores of the progenies in the population. The genetic background, the size of the mapping population and the number of markers loci used determine the precision of QTL mapping which influences the accuracy of mapping (Abdurakhmonov & Abdukarimov, 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)**.** Many studies have been conducted to map QTL for P efficiency by using different populations in rice (Ni *et al*., 1998; Wissuwa & Ae, 2001) to identify major QTL for P efficiency. Under LP growth conditions, near isogenic lines (NILs) for the major QTL had higher P uptake compared with the P inefficient parent whereas the NIL representing the minor QTL showed higher P uptake. These studies suggest that P use efficiency is a complex trait.

2.7 Association Mapping (AM)

An important goal in plant breeding is to connect genotype to phenotype (Botstein & Risch, 2003). The aim of association mapping (AM) is to identify the specific loci linked to phenotypic differences in the trait, to facilitate detection of genotypes that closely resemble the phenotypes (Madhusudhana *et al*., 2015). It refers to significant association of molecular marker with a phenotypic trait. In plants, it gives a powerful complementary approach to existing conventional

QTL mapping and cloning with bi-parental populations, mutational dissection, and transgenic approaches. For candidate gene association it has been adopted in nearly all major crop species for QTL validation, and to underline the genetic basis of quantitative traits (Zhu *et al*., 2008). It has led to the establishment of common community resources in important crops such as maize, rice, sorghum, soybean, and barley. The major benefit of AM is the diversity captured across several different traits. One of the main successes of AM is the capture of diversity across several different traits. Contrary to specific bi-parental populations of which some trait differences exist, most of the AM panels assembled can be used to study numerous traits, thus enabling the study of several aspects of such as plant architecture, development, agronomic performance and adaptive characteristics (Atwell *et al*., 2010; Flint-Garcia *et al*., 2003)**.** Nevertheless AM has some limitations, it requires a large number of molecular markers and confounds effects of population structure (Zhao *et al*., 2006; Yu *et al*., 2008), AM has low power to detect rare alleles in populations and requires more statistical assessment to investigate the relatedness of genotypes and the overall population.

Chen *et al.* (2017) investigated the genetic basis of grain yield and quality reduction in sorghum in the light of heat stress using an association mapping panel. Their GWAS study revealed that 14 SNPs played an important role in heat stress responsiveness in sorghum leaves. Leiser *et al*. (2014) investigating the underlying factors limiting phosphorus use efficiency in West Africa identified a single major genomic segment associated to grain yield using GWAS. They further reported significant genotype by phosphorus interactions under both low and high P conditions in their association mapping study. Morris *et al*. (2013) studied the genetic basis underlying several agro-climatic traits using GWAS and mapped several QTL for plant height and inflorescence architecture in sorghum.

2.8 Nested association Mapping (NAM)

NAM strategies combine the advantages of high recombination resolution, using a large number of genotypes having a common parent, and high allelic diversity. NAM combines the advantages of both linkage analysis and association mapping with main goal decorticate complex trait in maize (Yu *et al*., 2008). NAM takes advantage of its low sensitivity to genetic heterogeneity and its high efficiency in using genome sequence while still conserving high allelic richness due to diverse founder parents. The main goal of designing NAM populations was to capture genetic diversity, exploit historical recombination, develop mapping populations that can be assessed for agronomics traits at field locations of temperate regions, and has power to identify several QTLs, and provide the resources that will enable a large range of community effort and databases for scientists (Yu *et al*., 2008). NAM strategy was created to overcome some limitations of association mapping analysis such as the crypto-relatedness (Yu *et al*., 2008) and unequal linkage phase across founders (Lin *et al*., 2003), NAM populations can address this issue by recoding the genotypic matrix to characterize haplotypes (Xavier *et al*., 2017). Bouchet *et al.* (2017) studied quantitative variation for plant height and flowering using a NAM sorghum population derived from 10 global founder lines. They found several large effect QTL and genomic segments associated to these traits with a 90,000 SNP panel covering 70% of the known SNP global variation. Marcus (2017) characterized genetic bases of leaf erectness, leaf width, and stem diameter using a nested association mapping strategy. They identified important loci associated to foliar vegetation processes with moderate effects in sorghum. (Rusike *et al*., 2006)

A Nested association mapping RIL population of 248 sorghum lines with two founders was used to study grain yield and stay green traits under favorable and drought stress conditions during 2008 and 2009 (Sukumaran, 2012). This study identified a major QTL for grain yield on chromosome

8 and another QTL for flowering time on chromosome 9 under favorable conditions. While under drought stress conditions, three major QTL on chromosome 1, 6 and 8 were closely associated to grain yield and two genomic segments on chromosome 1 were closely linked to flowering time. Sukumaran (2012) also reported six different QTLs related to stay green under drought stress conditions on chromosomes 5, 6, 4,7 and 10 for different stay green traits.

2.9 Current status of QTL analysis on sorghum grain yield and related traits

The last two decades were very important in increasing the understanding of the quantitative grain yield related traits in sorghum. Numerous studies have been conducted on sorghum to identify QTL for different traits such as grain yield and panicle traits with the aim to improve sorghum molecular selection (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). Most of these studies were focused on bi-parental population mapping under favorable soils field conditions which has low power resolution compared to association mapping. for sorghum maturity (Childs *et al*., 1997; Crasta *et al*., 1999; Rooney & Aydin, 1999; Chantereau *et al*., 2001; Hart *et al*., 2001; Brown *et al*., 2006; Mace & Jordan, 2010), stay-green drought tolerance (Tuinstra *et al*., 1997; Crasta *et al*., 1999; Kebede *et al*., 2001; Subudhi *et al*., 2000; Wu *et al*., 2000; Tao *et al*., 2000; Sanchez *et al*., 2002; Haussmann *et al*., 2002; Harris *et al*., 2006), fertility restoration (Klein *et al*., 2001; Mace & Jordan, 2010), aluminum tolerance (Magalhaes, 2004), and biotic stress resistance (Klein *et al*., 2001; Tao *et al*., 2003; Mohan *et al*., 2009; Ramasamy *et al*., 2009) and photoperiod (Chantereau *et al*., 2001; Murphy *et al*., 2011), and plant height (Lin *et al*., 1995; Pereira *et al*., 1995; Rami *et al*., 1998; Hart *et al*., 2001; Brown *et al*., 2006; Feltus *et al*., 2006; Klein *et al*., 2008; Srinivas *et al*., 2009; Mace & Jordan, 2011). Few studies have been undertake on sorghum to identify QTL under low phosphorus deficiency condition, Mace *et al*. (2013) have successfully developed a backcross (BC) nested association mapping (NAM) population on sorghum to map and validate QTL with better resolution, therefore this approach provide an interesting opportunity to identify QTL related to grain quality and panicle traits and grain yield related traits in this study under contrasting P levels. However many QTLs have been reported related to low phosphorus efficiency in cereal crops such as maize (Chen., 2009; Li *et al*., 2010; Zhang *et al*., 2014), rice (Ni *et al*., 1998; Wissuwa & Ae, 2001; Gamuyao *et al*., 2012) millet (Gemenet *et al*., 2015). In sorghum Leiser *et al*. (2014) reported QTL related to aluminum tolerance and grain yield in low phosphorus conditions.

2.10 Application of molecular marker in plant breeding

QTL analysis has become a major tool for breeder to select for desirable genotypes. Selection using markers in plant breeding is known as marker assisted selection (MAS). Although transferring knowledge from QTL studies for use in application such as MAS is never an easy one, a few success stories using this breeding approach have been reported in crops (Bangbol, 2013). Major QTL or genes for complex flowering time in plants such as *Arabidopsis* (El-Assal *et al*., 2001; Masle *et al*., 2005; Werner *et al*., 2005), rice (Doebley *et al*., 1997; Yano *et al*., 1997; Yamamoto *et al*., 1998; Lin *et al*., 2003), soybean (Watanabe *et al*., 2004; Yamanaka *et al*., 2005) and *Brassica* (Österberg *et al*., 2002) have been subsequently cloned, making the linking of QTL analysis to MAS realistic. The success of MAS depends on the strength of marker-trait associations for given trait. The success of MAS depends on the strength of the marker trait associations established for a given trait, therefore, before starting MAS program, there is need to identify, validate, and establish a stable marker-trait association. This is done by using conventional QTL detection methods in bi-parental population or through association mapping approach (Madhusudhana *et al*., 2015). Marker assisted selection was successfully applied in sorghum breeding using plant breeding procedures like backcross method, gene pyramiding, and marker assisted recurrent selection. The potential efficiency of adopting MAS in sorghum breeding will depend on markers tightly linked to genomic regions of trait, heritability of the trait, proportion of genetic variance explained by the markers and the selection method. In other words, detailed understanding of the genomic regions regulating a trait will determine the possibility of applying MAS for this trait. Therefore, there is need to enhance understanding of the genetic control of panicle traits and grain yield related trait under diverse phosphorus field conditions, since most sorghum are produced under low P conditions.

CHAPTER THREE

3.0. ASSESSMENT OF GRAIN AND PANICLE TRAIT PREFERENCES OF FARMERS FOR SORGHUM VARIETIES IN TWO AREAS OF MALI

3.1 Introduction

Sorghum, is a staple food crop for millions of Malian smallholder farmers and thus plays an important role in achieving food security. Across the semi-arid zone of West Africa, sorghum production and improvement is greatly influenced by environmental variability and regional variations in producer and consumer preferences and the low levels of adoption which is about 32% of improved varieties by farmers (Smale *et al*., 2016). Low adoption levels of improved varieties contribute to low sorghum productivity and food insecurity in West Africa. Participatory plant breeding (PPB) and associated methods of learning from and interacting with farmers have been used to improve the suitability of varieties and thus improve adoption (Smale *et al*., 2016). In the Sudanian zone of Mali, PPB has been used to develop improved sorghum varieties that are environmentally adapted and meet farmer's needs (Christinck *et al*., 2005b; Weltzien *et al*., 2006; vom Brocke *et al*., 2010; Kante *et al*., 2017). The success and the potential adoption of future varieties depend on understanding farmers' needs when defining breeding objectives. Local guinea race germplasm, introduced caudatum varieties and crosses of the two have diverse features that may impact adoption. Sorghum breeders are faced with the challenge of identifying and then incorporating these traits into varieties acceptable to farmers. Four major sorghum races are grown in Mali; Guinea, Durra, Caudatum and Bicolor (Touré *et al*., 1998) and each have distinct morphological features. Guinea is the most dominant race grown in Dioïla and Mande but some intermediate guinea-caudatum, caudatum, bicolor (sweet stem), and durra types are also grown

(Siart, 2008). The guinea race has symmetrical grain placement and the panicle which is loosely branched. There is extensive morphological variation for panicle architecture in this race. The caudatum race has asymmetrical grain and its panicles are cylindrical where the length of the primary branch fluctuates within narrow limits from node to node. The durra race is well adapted to drought conditions, sandy soils and residual moisture regimes. The grain is large and globular and the panicles are compact and often borne on a hooked stalk. The bicolor sorghums tend to be sweet stem sorghums that are not used for grain production (Doggett, 1982).

The morphological features of the different races may influence grain yield, differences in grain quality (grain hardness), glume opening, threshability and panicle traits (laxness). Threshing is affected by the degree of glume opening. The more the glumes are closed, the harder it is to thresh. Grain hardness affects grain mold resistance (Jambunathan *et al.*, 1984), grain storage ability, insect resistance (Bueso *et al*., 2000), milling behavior (Suhendro *et al* 2000), flour particle size, cooking properties(Akingbala & Rooney, 1987; Bettge *et al*., 2000), and parameters such as adhesion, cooked grain texture, alkali gel stiffness (Cagampang & Kirleis, 1984), porridge quality (Akingbala & Rooney, 1987), and production of high-quality couscous granules (Aboubacar & Hamaker, 1999). The objective of this study was to determine farmers preferences for panicle related traits including panicle forms, droopiness, threshability, as well as visually assessable grain yield and grain quality traits that are critical for farmers to adopt new varieties of sorghum.

3.2 Materials and Methods

3.2.1 Study area

The study was conducted in the Sudan Savanna zone of Mali (700-1000 mm rainfall) where sorghum is one of the most important cereals produced. A panicle sorting activity accompanied by focus group discussions and individual interviews were conducted in two areas, Mande region which is 80 km southwest of Bamako, and Dioïla region which is 200 km east from Bamako. Dioïla has more intensified agronomic systems than Mande region. Cotton is the dominant cash crop in Dioïla whereas cotton is marginally produced in Mande. In general, women are involved in all field activities of the family in addition to having their own fields that allow them to support some of their needs. Mande and Dioïla regions were selected due to the importance of sorghum in farmers' agronomic systems and the ICRISAT sorghum programme has partnered with farmers' organizations in PPB activities over multiple years in these two areas.

The study was introduced in each village during parallel sorghum cooking activities through ICRISAT farmer organization partners, ULPC in Dioïla and COPROSEM in Mande. The cooking activities are held in the on-farm testing villages every year by the sorghum programme to assess the culinary aspect of varieties tested by farmers (Weltzien *et al*. 2008). There was overlap in culinary test participants and the panicle sorting study. In addition to the villages with culinary tests, 5 other villages were selected with the assistance of farmer' organization.

3.2.2 **Study description**

3.2.2.1 **Focus group panicle classification**

The farmers were asked to classify eighty panicles into 3 different piles in focus group discussion. The first pile included the panicles that farmers prefer and are willing to grow, the second pile were panicles that were acceptable to farmers but had some disadvantages, and the third pile represented panicles farmers did not prefer and would reject.

3.2.2.2 **Individual farmers 'panicle classification**

The individual panicle classification was similar to the focus group panicle classification but the participants were given more latitude in selecting the number of piles. For the individual classification, it was not necessary to have only three piles. Thus the individuals grouped the panicles according to their preferences.

3.2.2.3 **Discussion groups and interviews**

After the classifications, the discussion were held using a semi-structured questionnaire. The questions included what parameters they used to classify the panicles into different piles, why they sorted them way they did, and if there were specific traits that separated the piles. If comments regarding key topics such as glume, panicle laxness and form, and storability did not arise, more specifically we asked about these traits. Progressively through their answers other questions arose and were posed.

The focus groups and individual interviews were performed with two enumerators taking handwritten notes and the discussions were tape-recorded. The tape recording helped the authors to clarify the handwritten notes and address translation issues between Bambara, French, and English language. At the beginning, the demographic data were collected in groups, but some participants were not comfortable so the data was collected individually after the group discussions.

In each village, the permission of the village chief was sought before commencing activities and presented with informed consent information. Before the beginning of each session, participants were informed of the process, how the information would be used, and that participation was completely voluntary and they could withdraw at any time. Permission was also specifically sought for tape recordings of proceeding and taking photos of participants.

3.2.2.4 **Panicle Classification**

The activities of this study were structured around farmer panicle sorting. In order to identify and understand farmer's trait preferences, about eighty panicles representing panicle diversity present in the breeding material were selected and used for panicle classification exercises. Before the panicle exercises began, the panicles of the eighty accessions were numbered and classified into five groups according to the panicle shape; Group 1: Guinea panicles, the most open panicle, long and peduncle lax branches and panicles that hang down; Group 2: Intermediate Guinea-caudatum, an open panicle, with lax branches and the panicles that hang down; Group 3: Intermediate Caudatum-guinea, with lax branches and erect panicles; Group 4: Caudatum, with erect panicles and erect branches, or semi-compact panicle; and Group 5: Dura race, with compact panicles Fig. 3.1).

Group 1 **Group 2** **Group 3** **Group 4** **Group 5**

Figure 3.1**.** Sorghum groups 1-5: Different panicle groups according their panicle shape/form with description above in text.

There were three main data collection activities with participants: 1) collection of demographic information from participants, 2) a panicle sorting activity, followed by 3) group discussions or interviews. The main target of these activities was to understand farmer knowledge and preferences about sorghum grain and panicle traits.

At the start of the activity, basic demographic information and information about cash crops and sorghum production were collected from each individual. Following this, the group or individuals were asked to sort the panicles into three according to their knowledge and preferences in group, while in individual the number of pile depended to the respondent.

3.2.2.5 Data collection and analysis

All of the data was collected through oral interviews and focus groups with the participants and it was analyzed using qualitative methods including thematic coding based on the research questions, descriptive summaries, and analysis thematic of frequencies (Miles *et al.*, 2013). Both enumerators' handwritten notes were used to make a final summary of notes which were then carefully checked with the tape recordings by the author, who is fluent in all three languages. The final notes were compiled and coded using emergent themes based on the guiding research questions. Themes are patterns that pull together or unify different pieces of data and ideas and are associated to specific research questions (Miles *et al.*, 2013). After all of the data was thematically coded, QDA software was used to retrieve each theme in order to write descriptive summaries. These descriptive summaries examined the words directly spoken by the farmers that were previously coded into themes. Thus, the descriptive summaries are an analysis of the contents of the data. Finally, these summaries were analyzed collectively and the broader interpretations of the underlying meaning of the summaries were extrapolated in the discussion.

QDA and Excel were used to analyze the frequency of themes from the focus group discussions and individual discussions with farmers. Descriptive statistics were used to analyze demographics data. Panicle type preference was calculated by counting the number of panicles in each pile. The overall preference for each panicle type was calculated using the formula below (Christinck *et al.*, 2005a).

Farmers' panicle types preferences %=(N1*0+N2*0.5+N3*1)*100 / (N1+N2+N3)

Where:

N1=Third pile or number of panicle type in the third pile "rejected pile"

N2=Second pile or number of panicle type in the second pile "medium or acceptable"

N3=First pile or number of panicle type in the first pile "farmer preferred or good "

3.3 Results

3.3.1 Socio-economic characteristics

This study covered 175 farmers in 11 villages, 4 villages in Mande and 7 villages in Dioïla. The average age of farmer participants in this study was 41 and ranged from 15 to 68. Sorghum was generally grown as sole crop and the average sorghum area produced by women was 0.89 ha and men was 4.2 ha (Table 3.1) Men and women were generally represented equally in individual interviews, although there were more women in the focus groups than men (Table 3.1). Eighty three percent (83%) of participants selected their own seed from their fields for the next season's sowing. Maize represented the most important cereal crop for the participants followed by sorghum and pearl millet (Table 3.2). Fifteen percent (15%) of participants used their harvested sorghum grain only as food, 7% of participants grew sorghum only for sale and about 63% of participants grew sorghum for both (selling and food).

Table 3.1: Demographics of farmers in focus groups and individual interviews 2015

Designations	Gender	Locality		Nbr of groups or individuals	Number of participants	Age (range)	Area (ha) of sorghum produced (range)
		Dioïla	Siby				
Individual interviews	Male	5	5	10	10	48 (35-67)	4.2 (1-12)
	Female	3	7	10	10	40 (29-48)	0.81 (0.5-1.5)
Focus groups	Male	5	4	9	65	46 (23-68)	3.3 (1-14)
	Female	7	4	11	90	38 (15-60)	0.89 (0.3-2)

Nbr = number

Table 3.2 : Sorghum production and relative importance at Dioïla and Sibi 2015

Grain use		Grown in intercrop or Sole crop		Select panicles for seed		Produce seed for sale		Importance of cereals in the village				
								Farmer RANKING	1	2	3	4
	Percent (%)								Percent (%)			
Food	15.4	IC	31.4	Yes	No	Yes	No	sorghum	36	49	5	0
Market	6.8	SC	61.4	83	17	26	74	maize	55	46	2	0
Food & Market	63	Both	7.1					pearl millet	0	0	69	41
Missing	14.8							Rice	0	5	25	59

IC =inter crop, SC=sole crop

3.3.2 Panicle classification results

The results of the focus group panicle sorting showed that all five panicle groups were represented in all the three piles, except that women did not select Group 5 or compact panicles into the acceptable piles (1 and 2) (Fig. 3.2). Men and women sorted the panicle groups in a similar manner, except the Group 5. Thirty five percent (35%) of the panicle samples were rejected by men, whereas 41% of the panicles sample were rejected by women (Table 3.3). Group 2 (intermediate Guinea-caudatum) was the most preferred group by 61% of men and 54% of women, followed by Group 1 (guinea panicle), Group 4 (semi-compact panicle), Group 3 (intermediate caudatum-guinea) and Group 5 (compact panicle) (Fig. 3.2). Two panicle groups that were the least preferred for both men and women were G3 and G5. Eighty-five percent of G5 panicle samples were rejected by men, while G5 was totally rejected by women. (Fig.3.2). Participants' responses also indicated that G3 was less preferred (33%) (Fig. 3.2), while the darks and red grain color were rejected by farmers in G1, G2 and G4). However, during the interviews and focus groups, farmers did identify uses for G3 as well as G4, in particular, for animal feed and selling grain on the market.

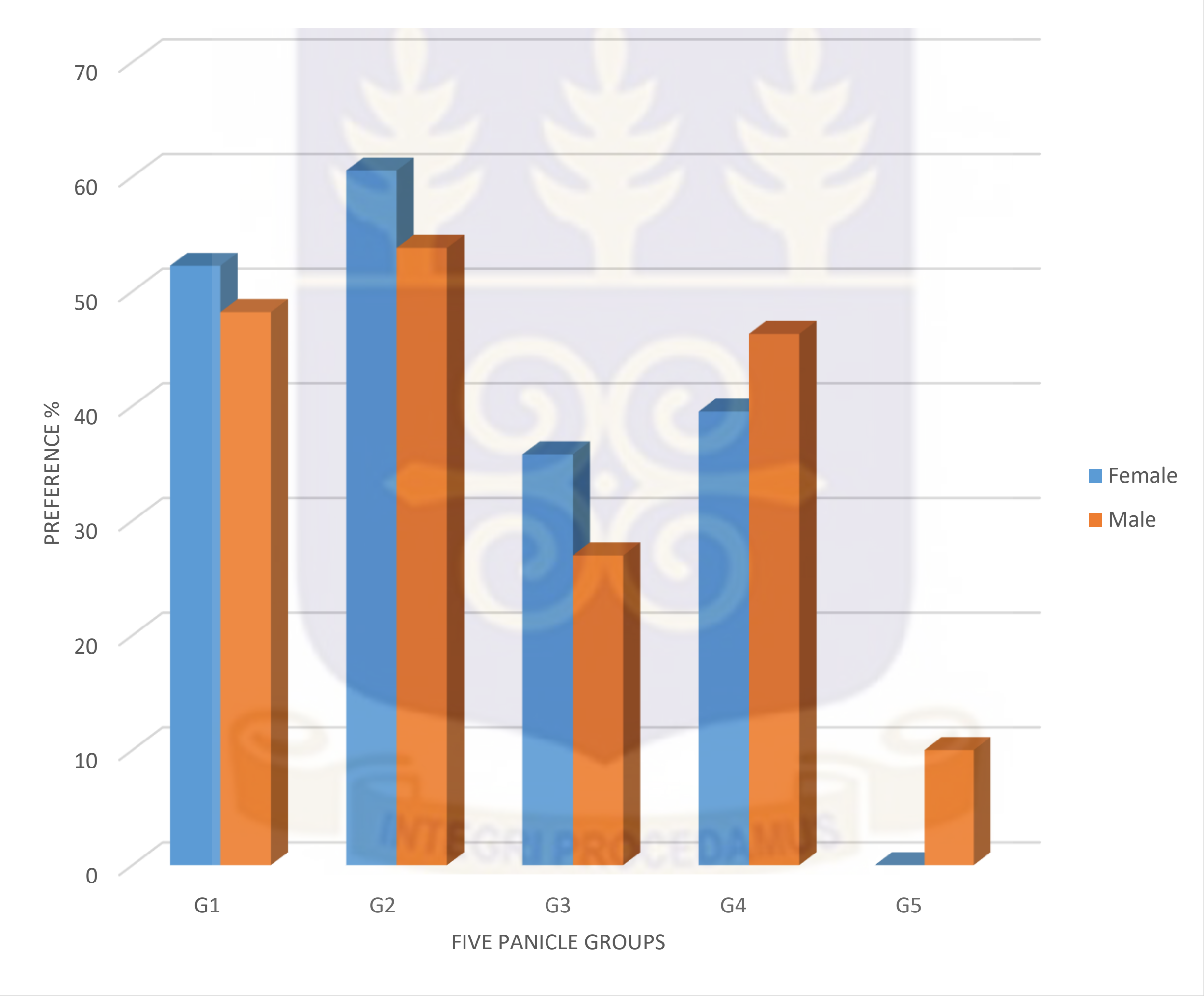

Figure 3.2: Percentage of panicles of each group that farmers (Female and Male) placed in different piles according their panicle preferences.

Where: Group 1(G1): Guineense panicles, Group 2 (G2): Intermediate Guineense-caudatum which has lax branches with its panicle hanging down, Group 3 (G3): Intermediate Caudatum- guineense, with lax branches and erect panicle, Group 4(G4): Erect Panicle with erect branches, or semi-compact panicle, Group 5(G5): are dura race and intermediate with compact panicle.

Table 3.3: Frequencies of respondents citing traits (themes) and percentage of themes in the group discussions.

Themes	Overall mention of themes (n)	Freq. (%) of themes	Themes in groups (n)	Freq. (%) of groups/themes
Hardness and grain storage	22	6.9	14	70
Hardness and food quality	6	1.9	5	25
Hardness pounding	11	3.5	6	30
Hardness and profit	15	4.7	14	70
Hardness yield	9	2.8	6	30
Hardness evaluation	12	3.8	9	45
Hardness /others	5	1.6	4	20
Glume opening/threshing	46	14.5	19	95
Glume and grain lost in the field	23	7.3	10	50
Glume opening environment	1	0.3	1	5
Grain food quality/food tasting	20	6.3	12	60
Yield	74	23.3	20	100
Grain color	22	6.9	14	70
Maturity and adaptation	26	8.2	13	65
Panicle form	22	6.9	20	100
Others	3	0.9	3	15

3.3.3 **Descriptions of emergent themes**

To further understand the attributes farmers preferred within panicle groups G1-G3, the mixed opinions about G4, and what they disliked about G5, we asked in-depth questions in individual interviews and focus groups. The emerging themes and the frequency of observation from these interviews are detailed in Table 3.3. The descriptive summaries reporting what farmers said about each characteristic, are described in detail below.

3.3.3.1 **Panicle form**

The most important morphological trait that differentiated the panicle groups was the panicle form. From G1 to G5, the panicle form shifts from lax and drooping to compact and erect (Fig. 3.2). Across all interviews (individual and group), farmers said they were accustomed to, and preferred, the lax and hanging panicle types (guinea type). Farmers frequently said the lax kind resists bird attack. A handful of participants were associated type of panicle to adaptation to their environment. Farmers say also they only grew the erect type for selling.

3.3.3.2 **Threshability and glume opening**

Threshing ability or glume opening was cited with frequency of 15% by participants as an important attribute, during 95% of the focus group discussions (Table 3.3). They preferred sorghum panicles where at maturity the entire grain is all or almost all visible through the glumes; the grain is easily threshed.

3.3.3.3 **Grain shattering**

Grain loss in the field is a parameter that farmers considered closely in Dioïla and Sibi, with discussion occurring in 50% of the group discussions (Table 3.3). Farmer said they did not prefer some of the panicle type which lose more grain in the field than others during the maturity when the wind blows and during harvest.

3.3.3.4 **Grain color**

Sorghum grain color was reported during this study with frequency of 6.9% of respondents citing the traits (Table 3.3). Farmers preferred white colored grain and more often they associated the color to the “grain quality”. White color was more appreciated for selling. Participants did not prefer the red or dark colored, for them red and dark colors are for animal feeding. However, several farmers said the dark color could be superficial and is only on the pericarp, which can be removed during the pounding.

3.3.3.5 **Grain hardness**

During this study, grain hardness was the most frequently cited with 25% of frequency in 100% of group discussions and it was discussed in-depth with participants. Many aspects of grain hardness were debated and it was clear that for farmers grain hardness was related to several other important factors including grain storage, food quality, and profit. Farmers have specific ways in which they evaluate grain hardness.

Sorghum grain duration in storage, either in traditional or modern storages method, is an important aspect that was frequently discussed by farmers during this study. Participants in the focus groups were unanimous that the duration of grain storage depended on grain hardness; the harder the grain, the longer it lasts in storage. Storage insect attacks are the main problem for farmers (traditional and modern). Farmers also mentioned that insects like soft grain because it is more floury and sweet.

In this study, women in Dioïla and Sibi appreciated harder grain types. The decortication is generally done by hand pounding with a wooden mortar and pestle, but mechanical mills are also available for this process. The women wanted unbroken grain, rather than pieces of grains after decortication. During the decortication process, women use water to clean and wet the pericarp which facilitates the removal of the bran. This process takes a short time for soft grains and more time for hard grains to reach saturation. Women said the amount of the bran after pounding is high for the soft grain a compare to the hard grain, and this is undesirable. However, a few also said it was painful to pound the hard grain and it took more time to remove all of the bran. Farmers in Dioïla and Sibi preferred to have both semolina and flour, but a handful of farmers preferred more semolina than flour. Producers said with hard grain you get enough semolina and flour, whereas with the soft type you will get enough flour but less semolina.

Men and women evaluated the grain hardness together, each using different methods and through consultation with each other. Famers evaluated the grain hardness, especially men, by breaking sorghum grain between the teeth: when it broke into pieces easily they concluded this grain was not hard. On the other hand, women generally determined grain hardness through the pounding process, if it was easily broken into pieces or not. Some men said they got grain hardness information from women after pounding. Women also evaluated grain hardness during the culinary process by gauging how much time it took for the soaking grain to reach water saturation. In general, both men and women agreed that hard grain is heavier than the soft kind in terms of volume and weight.

3.3.3.6 **Sorghum maturity**

Farmers preferred early maturing varieties for multiple reasons. When discussed in further detail, it was found that “early maturity” or “short duration” referred in general to varieties that are adapted to the rainfall. However, according to farmers, the main difficulty with early maturing was the bird damage on the panicle. On the other hand, the main problem with long duration varieties was that when the rainfall stops early, the plants were still not mature. They want varieties that were adapted.

3.3.3.7 **Sorghum variety adoption**

In this study, yield was an important attribute for farmers preferred varieties (Table 3.3), furthermore they considered other traits such as grain quality, threshing ability, panicle shape, and environmental adaptation as important features. Likely, there are trade-offs in these preferences. From the discussions it emerged that participants needed to test varieties in different fields before adoption. At least two years were necessary for them to decide whether to keep growing varieties or reject them. They compared the new varieties with their own variety for at least two seasons on different fields. Farmers wanted to be sure that the new variety would be adapted to the area including soil type, maturity, yield and grain quality and food.

3.4 Discussion

3.4.1 **Farmers perception of sorghum grain yield and food security**

Sorghum grain yield was the most preferred trait for farmers, therefore it plays an important role in sorghum variety adoption. However, farmers' definition of sorghum "grain yield" goes beyond the classical definition that considers the number of panicles, grain weight from the field, and grain weight after threshing. By engaging closely with women about their panicle and grain preferences, this study showed that farmer evaluation of sorghum "grain yield" includes how much of it is useful as food through the "whole" post-harvest process. Participants said that for cooking, you need more soft grain than hard grain if one wants to get the same amount of food, "the hard grain provides more product at the end of the culinary process for breakfast, lunch, and dinner. With soft grain you can't reach all three meals." Likewise, a participant said, "If you took 10 kg with hard grain, you must take 12 kg of soft grain to get the same amount of food. Thus yield is defined in terms of "food yield" and comprised of: threshing (threshing percentage), weight after decortication, efficiency for different food uses, and duration of grain storage without insect attacks for longer-term food security. Yapi *et al.* (1998) reported the reasons for adoption of new sorghum varieties for three regions of Mali were earliness (85%), productivity (67%) and food quality (34%). Our results confirm the findings of Yapi *et al*. (1998) and add definition and depth to the concepts of grain quality and food quality, properties that are essential for adoption.

3.4.2 Combining farmer and researcher knowledge to set breeding objectives

There are multiple attributes that are important to farmers and the identification and the understanding of these attributes are essential for setting breeding objectives and developing materials that are more likely to meet farmers' preferences. Farmers have unique knowledge about traits, and, in many cases, their explanations about traits are supported by scientific studies on the specific traits. For instance participants said grain hardness was related to several other characteristics, including grain storability, food quality, and grain weight. Farmers associated harder grain with longer storability and researchers have found that varieties with increasing grain hardness or increasing thickness of the corneous layer of the endosperm are much less susceptible to the primary grain pests *Sitophilus oryzae, S zeamais, and Sitotroga cerealalla* (Doraiswamy *et al*., 1976; Fadlemula, 1983; Russell, 1966; Wongo & Pedersen, 1990). Farmer's knowledge about grain quality and hardness is aligned with research evidence indicating grain hardness is correlated with milling yield, particle size index, test weight, and kernel density (Reichert *et al*., 1988). Other research found a high positive correlation between grain hardness, grain appreciation and grain productivity (vom Brocke *et al*., 2010). This study revealed that farmers have a unique measure of grain hardness that is principally determined by women when they are pounding the grain.

Farmers associated glume opening with threshing ability, and considered the trait an important grain yield component. Similarly, Adeyanju *et al*., (2015) indicated that genotypes with closed glumes are hard to thresh, and the grain of certain cultivars is tightly attached causing significant reduction in quality which in turn reduces market value and processing ability. Furthermore, they estimated yield by looking at the panicle from the top to the bottom and determining if the glumes were all well-opened, because, glumes can be well-open at the top and progressively closed towards the bottom of the panicle. Finally, sorghum grain color was one reason for farmers to reject panicles during this study. Many previous studies reported that sorghum grain color affects the color of the resulting food, especially foods made with alkali, such as tortilla or alkaline tô, as is the case in Mali (Dicko *et al*., 2002; Hikeezi, 2010; Rooney & Murty, 1982).

The proper understanding of what farmers mean when discussing different traits and identifying the nuances of how farmers value specific traits or a suite of traits is important for scientists to address in order to develop appropriate breeding objectives. Previous studies have shown farmer conceptualization and valuation of their farming systems is holistic, multi-faceted, and often distinct from the scientific community's comprehension (Christinck *et al*., 2005a). Several researchers have been reported that farmers preferred early sorghum varieties (Yapi *et al*., 1998; Christinck *et al*., 2005a). However many early and extra early varieties were developed by breeders. In spite of these efforts, the adoption of these kind of varieties is still low. This study expanded the understanding of sorghum earliness when discussed in detail with farmers, it became clear that earliness was not just short duration, but varieties that were adapted to the environment, or a variety that ends its cycle with the rainfall and overcomes the temporal hydric stress. Usually farmers grow local varieties (guinea types) or improved local varieties that are photoperiod-sensitive. These types of varieties provide flexibility with planting dates and are well-adapted to the variable rainfall patterns in the region (Dingkuhn *et al.*, 2006; Haussmann *et al.*, 2012). Without this in-depth understanding of farmer needs, earliness may have been construed only as short-duration, leading to the development of varieties that were not photoperiod-sensitive

3.4.3 **Association of local sorghum race with key traits**

In addition to the farmer-identified traits that are consistent with research findings, there is strong evidence from this work that farmers also have unique knowledge regarding traits and variety selection. Farmers associated specific panicle types and plant types with a number of traits, because their knowledge about varieties is founded on generations of exposure to locally available germplasm. For example, they believe a lax panicle is associated with heavy grain, bird control, hard grain for storage, and high quality food, whereas erect panicles are associated with soft grain and high fodder quality. This knowledge is generally the basis of their variety selection and likely one of the reasons farmers' associate specific plant forms with adaptation in low-input fields. However, breeders perceive traits as individual units that normally can be separated through selection and specific breeding tools. While breeders may easily conceptualize an erect panicle with hard grain, this is not within the norm for farmers whose knowledge is founded on experiential practice. A challenge for breeders and the social scientists that work with them is, not only to identify farmers' locally adapted materials for crossing and to understand farmer preferences, but also to appreciate how farmers conceptualize group of traits and make trade-offs. This is important for truly understanding what underlies trait preferences so that appropriate varieties are created, and this process of learning can also facilitate improved or innovative approaches to adoption.

3.4.4 Men and women's complementary knowledge for trait preferences

Men and women contributed unique and complementary knowledge to variety selection. This knowledge is based on roles in the household and in their roles in sorghum production and utilization. However, social structures within the family unit enables sharing of this information. For instance, both men and women prefer hard grain for somewhat different reasons, and they share information to determine the actual hardness. While men are in charge of grain and seed storage and desire hard grain in order to reduce loss from insect damage, women are in charge of processing and desire hard grain because it is easy to process and renders more food. Collectively, they determined the grain hardness: men evaluate it based on storage duration and how easily the grain breaks with the fingernail, but they also rely heavily on women's experience pounding the grain. The way the grain breaks and the difficulty of pounding determines the hardness.

3.5 Conclusion:

. Inclusion of men and women in this research process was essential to fully identify the types of varieties that are suitable for farmers. Sorghum grain yield was the most important farmers' preferred trait, but in addition some of these trait are essential to them for adoption such as early maturity, which is important for sorghum varieties to overcome some biotic and abiotic constraints. They also preferred open sorghum panicles with hard grains, high density of grains on the panicle, and good threshability. Results of this study show the relevance of an in-depth approach to identify and understand sorghum attributes that are important to farmers, including grain yield, grain hardness, panicle shape, threshing ability, maturity and adaptation. Gender roles and social structures influence farmer preferences and improve the chances of adoption.

CHAPTER FOUR

4.0. VARIATION IN GENETIC ARCHITECTURE OF FARMER PREFERRED GRAIN AND PANICLE TRAITS UNDER P DEFICIENCY FIELD CONDITION

4.1 Introduction

The majority of sorghum in West and Central Africa (WCA) is produced under a low input farming system (vom Brocke *et al*., 2010). Mainly in low phosphorus field conditions (Buerkert *et al*., 2001; Leiser *et al*., 2012). Much effort has been made by breeders to improve sorghum varieties in Sudan Savannah of Mali as well as in WCA. Some progress has been made for agronomic performance, such as grain yield, maturity, *striga hermonthica* resistance and stay green. However there is additional new diversity including panicle architecture and grain quality that varies within and between sorghum races. Due to these efforts the level of adoption of new varieties rising low (Smale *et al*., 2016). As presented in chapter 3, it is important that the described panicle and grain quality traits are maintained at the level of local varieties (Chapter 3)

Most of these traits are simply inherited, and thus can be selected for during the early stages of a selection program. The traits are actually numerous, and thus can pose a serious challenge as rejections of new varieties can occur even in the stage of variety testing, even with lines that have been developed from broad based guinea race germplasm. This represents loss of efficiency for achieving genetic gains for productivity.

To summarize, these traits are glume opening and free threshing, grain quality (vitrosity, grain hardness, white grain color, storability) good exsertion and lax, drooping panicles. No studies have been undertaken to identify the underlying genetics of these traits under low phosphorus field conditions. Some studies have identified QTL for traits like glume structure, grain hardness, panicle compactness, (Rami *et al*., 1998), panicle exertion (Feltus *et al*., 2006; Rajkumar *et al*., 2013; Zhao *et al*., 2016) under high fertility conditions, and with breeding material that does not represent the sorghum germplasm grown and used in Mali and other West African countries.

For this study a nested association mapping (NAM) population was used to combine single QTL analysis with association analysis (GWAS) to better capture and describe allelic diversity. The traditional bi-parental population mapping captures the genetic recombination between two different genotypes to identify regions of interest with poor mapping resolution. The association mapping captures more allelic diversity, has high resolution and more recombination events (Platt *et al*., 2010; Brachi *et al*., 2011;). These two mapping tools are complementary and give insights into markers being specific to certain genetic backgrounds, thus giving important information for implementing a MAS breeding programme. This enables the breeder to better characterize the diversity of the genetic material for new population development. NAM analysis uses multiple families connected by a single common parent (Yu *et al*., 2008). It was successfully applied to dissect complex traits in maize and other cereals, and a few studies have been conducted using NAM population on sorghum (Mace & Jordan, 2011) mostly as BC1-NAM. More effective early generation identification of the full set of these traits for culling of progenies with undesirable grain /glume/panicle traits for applied sorghum breeding would be highly beneficial for sorghum improvement in Mali. If undesirable progenies can be removed from the breeding populations before yield evaluations start, selection efficiencies for grain yield are expected to increase. Also genetic gain for yielding ability under low phosphorous availability conditions is expected to increase. Marker assisted selection could be used to eliminate progenies with undesirable and unacceptable panicle and grain traits in early generation breeding. This helps breeders to identify progenies for grain yield evaluation with farmer desirable traits. To assess the opportunities for achieving such gain in breeding efficiency, the following specific objectives were pursued: to characterize the feasibility of effectively evaluating the various farmer-preferred panicle and grain quality traits, under both high and low soil (P) fertility conditions, to estimate population genetic parameters from a set of 13 bi-parental BC1F5 NAM populations, to identify QTL for farmer preferred grain quality and panicle traits under phosphorus deficiency field condition using bi-parental QTL and GWAS approaches.

4.2 Materials and Methods

4.2.1 **Plant Materials**

The study included 13 bi-parental populations composed of 1083 BC1F5 progenies, which were developed by backcross nested association mapping (BC-MAN) from 2009 to 2012 by ICRISAT-Mali. The individual population size varied from 70 to 102 BC1F4 progenies (Table 4.1). The recurrent parent Lata is an elite variety from Mali, with open and dropping panicles. The donors were chosen based on their adaptation or tolerance to specific biotic and abiotic production constraints and based on their genetic distinctness from the recurrent parent Lata. (Table 4.1).

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Table 4.1: List of donor parents, number of progenies by population and specific advantage of each donor parent including days to flowering.

	Variety	Race	Pop N°	Pop Names	Origin/ country	Maturity	Nbr / Prog	Specific advantages
1	**Gnossiconi**	Guinea	BC55	55_Gnoss	Burkina Faso	85	71	Grain and panicle traits
2	**Ribdahu**	Caudatum	BC60	60_Ribda	Nigeria	110	80	Midge resistant
3	**Sambalma**	Guinea-Cons	BC61	61_Samba	Nigeria	110	102	Grain and panicle traits, Al-tolerance
4	**N'golofing**	Guinea	BC59	59_N'golo	Mali	88	80	grain and panicle traits
5	Douadjè	Guinea	BC53	53_Douad	Mali	90	80	Low P adaptation and also allele for Al-tolerance.
6	**Grinkan**	Intermediate	BC50	50_Grinka	Mali	90	100	Productivity, Stover quality
7	**Framida**	Caudatum	BC54	54_Fram	Burkina Faso	80	80	Striga tolerance
8	**Fara-Fara**	Guinea	BC58	58_Fara	Nigeria	120	80	Diversity
9	**SK 5912**	Caudatum	BC52	*52_SK591*	Nigeria	105	80	Diversity
10	**SC566-14**	Caudatum	BC62	62_Sc566	Brazil	60	80	Aluminum tolerance
11	**IS15401**	Guinea-Cons	BC56	56_Soumb	Cameroon	110	101	Grain quality, Striga resistant, al tolerant, LP specific adaptation, midge resistance
	IS 23540	Caudatum	BC57	57_IS235	Ethiopia	80	80	Sweet stem
13	**IS23645**	Guinea-Marg	BC51	51_Hafid	Gambia	95	75	vitreous grain

Pop=population, Nbr/Prog= number of progenies, Cons= Conspicuum, Marg= Margaritiferum ,

NB: IS15401=Soumbaleba, IS23645=Hafidjeka

4.2.2 Phenotyping

Phenotyping of the 1083 BC1F5 progenies, from the 13 BC-NAM population was conducted at Samanko ICRISAT station under high P (HP) and low P (LP) in the rainy season 2013. ICRISAT station lies on 12^0 31’ N, 8^0 4’ W (Figure 4.1). The average annual rainfall ranges from 800 to 1100 mm, but the average in 2013 rainfall was 1180 mm. The Sudan-savanna zone of southern West Africa is one of the least predictable climatic regions of the world, with highly variable rainfall.

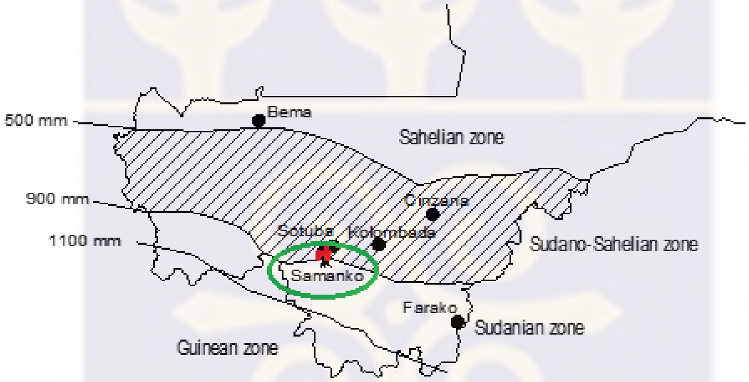

Figure 4.1: Location Samanko, the Sudano-Sahelian zone (800-1100 mm)

An Alpha lattice design was used with two replications and one row per plot. The HP fields were fertilized with diammonium phosphate (18-46-0) at rate of 100 kg ha^{-1} as basal fertilized and urea (50 kg ha-1) (46-0-0) as topdressing. The P fields were fertilized only with the equivalent amount of N as the HP fields but no phosphorous. ICRISAT-Mali has specific plot for LP that was used to conduct this study. Soil samples were taken in 2013 at 0-15 and 0-30 cm depths and sent to soil laboratory for soil chemical analysis during the implementation of the trial but no results were received. Nevertheless other soil samples were taken after in 2016 for soil chemical properties analysis. The result of soil chemical properties analysis in 2016 revealed that the soil Bray-1 P in LP was 3.3 mg/kg soil while it was 14.32 mg/kg soil under HP (appendix 5) which confirmed the status of the LP field (Manu *et al*., 1991). The traits that were measured or scored are described in the (Table 4.2). In particular farmer the threshing ability scoring and grain hardness were done by farmer's threshabilty (men) and grain hardness (women)

Table 4.2: Description of different parameters collected, units and the abbreviated names.

Traits	**Abbreviation**	**Description of parameters**
Panicle exertion (cm)	PEX	Distance of the panicle from the flag leaf sheath.
Panicle laxness (score)	PaLa	Visual assessment of panicle laxness score (1= compact to 9 = very lax)
Glume opening (score)	Glop	Visual appreciation of glumes opening (score 1= complete closed, 2= 25 degrees, 3 = 45 degrees, 4 = 68 degrees, 5 = 90 degrees).
Threshability (score)	Thrsc_F	The thresh ability of the panicle, either difficult to thresh, freely thresh ability or partly thresh ability score (1-5) 1=poor threshing to 5=free threshing. By farmers
Grain hardness appreciation (score)	GrHrds_F	Appreciation of hardness by breaking sorghum grain with the between teeth by farmer, score (1=soft grain to 5=hard grain).
Grain vitrosity/ Vitreousness (score)	VIT	Visual assessment of percentage of vitreous endosperm on a grain section score (1=completely starchy to 9=) after longitudinally dissected 5 grains.

4.2.3 Phenotypic statistical analysis:

4.2.3.1 Individual trial analysis

Each single environment was analyzed for different traits using; GenStat for correlation analysis and ANOVA, BMS to obtain the Best Linear Unbiased Estimate (BLUEs) and the Best Linear Unbiased Predictions (BLUPs) "R" was used for graphics like box plots and two models were automatically fitted to run the analysis. In the first model (Model 1) the genotype were taken as random effects.

$$\text{(Model 1)}\ \ Y_{ijk} = \mu + G_i + R_j + B_k + B(R)_l + E_{ijkl}$$

In second model (Model2) the genotype were taken as fixed effects.

$$\text{(Model 2)}\ \ Y_{ijkl} = \mu + G_i + R_j + B_k + B(R)_l + E_{ijkl}$$

Where Y_{ijk} is the observed value; μ is the population mean; G_i is the effect due to the $_i$-th genotype test; R_j is the effect due to the $_j$-th replication; B_k is the effect due to the $_k$-th block; B(R)=block within the replication; E_{ijk} is the effect due to the random error.

The random model was used to estimate variance components for estimating repeatability (Model 3), Repeatability was calculated with an adjusted formula for unbalanced data sets (Piepho & Mohring, 2007). Finally, Best Linear Unbiased Predictions (BLUP) for genotype performance were obtained with Model 1. In the second analysis, genotypes were treated as fixed effects. The Best Linear Unbiased Estimates (BLUE) obtained from Model 1 were used in GxE and QTL mapping analysis. The model 2 assumes block ~N $(0, \sigma^2_b)$, and error ~N $(0, \sigma^2)$).

Model (3)

Repeatability:

$$w^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{V}{2}}$$

Where; σ_g^2 *= genotypic variance, V is mean variance of difference between treatment mean.*

4.2.3.2 **Combined analysis across environments**

The two trials (LP and HP) were used for combined analysis using the following linear model (model 4) for.

Model (4) $Y_{ijkl} = \mu + G_i + L_j + GL_{ij} + R(L)_k + B(R(L))_l + E_{ijl}$

Where, $Y_{\mathbf{ijk}}$ is the observed value; μ is the population mean; $G_{\mathbf{i}}$ is the effect due to the $_{\mathbf{i}}$-th genotype t; $L_{\mathbf{i}}$ is effect of the j-th location; $GL_{\mathbf{ij}}$ is interaction of the i-th genotype in the j-th location; $R(L)_{\mathbf{k}}$ is the effect due to the replication within location; $B(R(L))_l$ is the effect due to the block within the replication and the replication within location; $E_{\mathbf{ijk}}$ is the effect due to the random error. (Model 5)

- **Broad sense heritability**

$$h^2 = \frac{\sigma_g^2}{(\sigma_g^2 + \frac{\sigma_{gl}^2}{l}) + (\frac{\sigma_e^2}{rl})}$$

Where, σ_g^2 is genotype variance of components, σ_{gl}^2 variance components of interaction genotype and P-levels, σ_e^2 variance component errors, l is environments, r is replications.

4.2.4 Genotyping

The DNA extraction was done by CIRAD, Montpellier France in 2013. After extraction the DNA was sent for genotyping at Geno Toul regional sequencing platform in Toulouse, France. Genotype by Sequencing (GBS) was conducted with the restricted enzyme ApeKI following a protocol of (Elshire *et al*. (2011), using a 384-plexing sequencing approach. CIRAD filtered the detected SNP markers based on sequencing depth, call rate and minor allele frequency, including imputed data for missing SNPs. The genotyping data were obtained for 969 BC1F4 out of 1083 BC1F4 progenies including donor parents where available for QTLs analysis. A total of 2000 to 3000 SNPs were obtained for bi-parental population mapping and 51545 SNP for genome wide association mapping analysis (GWAS).

4.2.5 QTLs mapping methodology

Two different approaches were used to detect QTL for different parameters collected during this study and the BLUEs values were used to perform QTLs analysis.

4.2.5.1 Single families mapping

The bi-parental QTL analysis for each of the 13 populations was conducted by single interval mapping using R/QTL package(Broman & Sen, 2009), using the regression methods described by Haley *et al*. (1992), to take account of missing genotype data at a putative QTL, in which one detect the presence of a single QTL and considers each point on a dense gird across the genome one at time as location of the putative QTL. A thousand conservative permutation threshold was applied to determine at the 0.05 significance threshold to consider the significant level of identified QTL (Churchill & Doerge, 1994) for each trait.

The percentage of phenotypic variance and the additive and dominance effects attributable to an individual QTL were estimated using multi-QTL model involving all QTL detected for a given trait, using the function "fitqtl" of the R/QTL package.

According to R/QTL documentation the "additive effect is derived from the coding sheme-1/0/+1 for genotype AA/AB/BB, and so is half the difference between the phenotype average for the two homozygotes". For a given QTL, if the additive effect is positive, the allele for increasing came from donor parents; if negative the allele for increasing came from recurrent parent (Lata).

4.2.5.2 **Multiple-families:**

Association mapping (GWAS) using a mixed model marker trait association analysis was performed on all 13 populations using the NAM package under "R" (Xavier *et al*., 2017). The package NAM can relax the linkage assumption of existing methods, by enabling operator to consider prior information concerning population structure (Xavier *et al*.,2015). NAM increase its resolution by taking markers as random effect and use a sliding window approach to increase power and avoid double fitting markers into the model (Xavier *et al*., 2015). The model is fitted using the Eigen decomposition (Zhou & Stephens, 2012) and evaluated with the likelihood ratio test.

4.3 Results

4.3.1 Performances of progenies under HP and LP field conditions

Significant genotypic variance was observed under different P-levels for all traits examined. The highly variability were found within the population and between population for different traits observed under HP and LP (Tables 4.3, 4.4). The different parameters were distinctly different across both environments (Table 4.4). The estimated values of repeatability across P-levels varied from 0.59 to 0.89. Grain vitrosity had the highest repeatability value (0.89) while grain hardness had the lowest value of repeatability 0.59. In general, almost all parameters were slightly more repeatable under HP than LP except for panicle exsertion (PEX). The average mean value of these traits were almost the same across both P levels, but the mean value of PEX were higher under LP than HP (Table 4.3).

Table 4.3: Genetic variation (σ²G), standard error (s.e), minimum, maximum, mean and repeatability for different farmer panicle preference traits for 1083 BCNAM progenies evaluated under LP and HP soil conditions.

	HP		**LP**		**Minimum**		**Maximum**		**Mean**		**Repeatability**	
traits	**σ²G**	**s.e**	**σ²G**	**s.e**	**LP**	**HP**	**LP**	**HP**	**LP**	**HP**	**LP**	**HP**
PEX	23.81***	1.25	29.76***	1.50	-14	-10	34	26	8	6	0.85	0.83
LaPa	0.40***	0.03	0.22***	0.02	3	2	8	8	5	5	0.67	0.70
Glop	0.69***	0.04	0.71***	0.04	1	1	5	5	3	4	0.77	0.82
ThrSc_F	0.63***	0.03	0.68***	0.04	1	1	5	5	3	4	0.79	0.80
VIT	2.86***	0.14	1.60***	0.10	1.5	1	10	10	7	7	0.74	0.89
GrHrds_F	0.35***	0.02	0.28***	0.02	1	1	4	4	3	3	0.59	0.79

HP= high P, LP= low P, PEX= panicle exsertion, LaPa=panicle laxness, Glop=glume opening, ThrSc_F=threshability, VIT=grain vitrosity, GrHrds_F=grain harness, G=genotype, *=significance at (P<0.05), **= (P<0.01), *** (P<0.001)

Table 4.4: Family means and ranges minimum (Min), maximum (Max) and recurrent parent (LATA) mean for examined traits evaluated under HP and low LP soil conditions of 1083 BC1F5 progenies.

	PEX		LaPa		Glop		ThrSc_F		VIT		GrHrds_F	
Material	**HP**	**LP**	**HP**	**LP**	**HP**	**LP**	**HP**	**LP**	**HP**	**LP**	**HP**	**LP**
LATA - Mean	3.7	8.7	5.0	5.3	3.8	3.9	4.0	4.1	7.8	6.3	3.1	3.8
Grinkan - Mean	1.9	3.6	5.5	5.5	3.5	3.5	4.0	4.0	8.1	6.6	1.0	2.0
BC-50 - Min; Max	-4 - 15	-5 - 22	3 - 6	4 - 6	1 - 5	1 - 5	1 - 5	1 - 5	4 - 10	4 - 10	2 - 4	2 - 4
IS23645 - Mean	14.8	12.6	6.5	6.5	5.0	5.0	4.5	4.5	9.5	9.1	2.5	3.0
BC-51 - Min; Max	-8 - 14	-12 - 19	4 - 8	4 - 8	2 - 5	1 - 5	1 - 5	2 - 5	4 - 10	4 - 10	1 - 4	1 - 4
SK 5912 - Mean	-1.1	0.2	3.9	4.5	-	-	-	-	3.1	-	1.0	1.0
BC-52 - Min;Max	-4 - 21	-4 - 21	2 - 7	3 - 6	1 - 5	1 - 5	1 - 4	1 - 5	4 - 9	4 - 10	1 - 4	1 - 4
Douadjé - Mean	19.8	31.1	4.9	5.0	5.0	5.0	4.9	5.1	9.8	10.0	4.0	4.0
BC-53 - Min;Max	-5 - 25	-7 - 22	4 - 7	5 - 6	2 - 5	2 - 5	2 - 5	1 - 5	4 - 10	3 - 10	2 - 4	2 - 4
Framida - Mean	1.2	7.8	2.5	3.0	5.0	3.0	3.4	4.0	1.1	3.0	1.1	2.0
BC-54 - Min;Max	-3 - 20	-5 - 19	3 - 7	3 - 6	1 - 5	1 - 5	1 - 5	1 - 5	1 - 10	3 - 9	1 - 4	1 - 4
Gnossiconi- Mean	22.8	21.2	5.1	5.0	5.0	4.9	5.0	4.8	4.3	5.2	3.0	3.5
BC-55 - Min;Max	-1 - 24	0 - 27	4 - 6	4 - 6	2 - 5	2 - 5	3 - 5	2 - 5	3 - 10	4 - 10	2 - 4	2 - 4
IS15401 - Mean	8.0	13.7	3.5	3.5	4.0	4.4	4.0	4.4	5.9	5.3	2.5	2.5
BC-56 - Min;Max	-7 - 21	-7 - 25	2 - 6	3 - 6	1 - 5	1 - 5	1 - 5	1 - 5	2 - 10	3 - 10	1 - 4	2 - 4
IS23540 - Mean	8.6	17.2	4.4	3.5	4.0	3.1	4.0	3.6	3.8	3.8	2.0	2.0
BC-57 - Min;Max	-6 - 22	-8 - 22	3 - 8	3 - 7	1 - 5	1 - 5	1 - 5	1 - 4	2 - 9	3 - 9	1 - 3	1 - 4
IS24887 - Mean	-	-	5.0	6.0	-	3.6	-	2.9	6.2	7.5	3.0	2.5
BC-58 - Min;Max	-8 - 20	-9 - 21	3 - 6	3 - 6	1 - 5	1 - 5	1 - 5	1 - 5	4 - 10	4 - 10	1 - 4	2 - 4
N'golofing - Mean	17.0	18.6	5.5	6.5	4.5	4.4	5.0	4.4	9.0	8.3	4.0	4.0
BC-59 - Min;Max	-4 - 15	-5 - 22	4 - 7	4 - 6	2 - 5	2 - 5	3 - 5	3 - 5	6 - 10	5 - 10	2 - 4	3 - 4
Ribdahu - Mean	17.5	-	3.1	-	5.0	-	4.5	-	1.9	4.3	2.0	-
BC-60 - Min;Max	-6 - 23	-8 - 24	2 - 6	3 - 7	1 - 5	1 - 5	1 - 5	1 - 5	1 - 9	3 - 10	1 - 4	2 - 4
Sambalma - Mean	-	2.7	5.5	7.0	-	-	-	-	8.3	6.5	2.9	3.1
BC-61 - Min;Max	-7 - 16	-12 - 17	4 - 7	4 - 7	1 - 5	1 - 5	1 - 5	1 - 4	3 - 10	4 - 10	2 - 4	2 - 4
SC566 - Mean	15.8	22.7	5.0	4.0	3.0	4.1	3.5	4.0	3.0	3.5	1.0	2.5
BC-62 - Min;Max	-5 - 23	-5 - 25	3 - 6	3 - 6	1 - 5	1 - 5	1 - 5	1 - 5	3 - 10	4 - 10	2 - 4	2 - 4

HP= high P, LP= low P, PEX= panicle exsertion, LaPa=panicle laxness, Glop=glume opening, ThrSc_F=threshability, VIT=grain vitrosity, GrHrds_F=grain harness, BC=backcross.

Combined analysis over the two P-levels revealed significant genetic variance for traits examined (Table 4.5). High estimated values of broad sense heritability were observed for all traits evaluated across P-levels. Genotype by environment interaction variance components were significant but smaller than 10% of the corresponding genotypic variance components.

Table 4.5: Variance components for Genetic (σ^2G) and standard errors (SE) and broad sense heritabilities (h^2) of panicle, glume and grain traits of BC1F5 progenies evaluated over P-levels at Samanko station in 2013.

Traits	**Combined analysis**				
	σ^2G	**s.e**	**σ^2G x P**	**s.e**	**h^2**
PEX	24.99***	1.22	1.86***	0.33	0.88
LaPa	0.29***	0.02	0.03**	0.01	0.78
Glop	0.63***	0.03	0.07***	0.01	0.84
ThrSc_F	0.62***	0.03	0.04**	0.01	0.87
VIT	1.96***	0.10	0.28***	0.03	0.84
GrHrds_F	0.30***	0.02	0.02*	0.01	0.79

HP= high P, LP= low P, PEX= panicle exsertion, LaPa=panicle laxness, Glop=glume opening, ThrSc_F=threshability, VIT=grain vitrosity, GrHrds_F=grain harness, G=genotype, G x P= interaction genotype by P-levels, *=significance at (P<0.05), **= (P<0.01), *** (P<0.001).

4.3.2 Correlation among parameters

Correlations among traits observed were relatively high between HP and LP levels for Glop, ThrSc_F, GrHards_F, LaPa, PEX and VIT (Table 4.6). Threshing ability score and glume opening were strongly correlated within the same P-level and across contrasting P-levels. Women farmer's score for grain hardness were moderately correlated with the laboratory assessment of vitrosity, with the women's score for grain hardness under LP being somewhat less correlated than under HP conditions (Table 4.6). The boxplots (Fig. 4.2) indicate distribution of the individual family correlations with the mid line, the median, the left and right extremity of the colored area the lower quartile and upper quartile and the left and right whiskers the lower and upper whisker. The Figure

4.2 shows correlations across P-levels conducted within individual families also showed close correspondences for most traits, especially PEX, Glop, ThrSc_F and VIT.

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Table 4.6: Genotypic correlation coefficient and P value among farmer’s panicle preferred traits across P-levels using BLUEs of 1083 BC1F5 progenies.

	Correlation (r)											
Traits	**Glop_HP**	**Glop_LP**	**GrHrds_F_HP**	**GrHrds_F_LP**	**LaPa_HP**	**LaPa_LP**	**PEX_HP**	**PEX_LP**	**ThrSc_F_HP**	**ThrSc_F_LP**	**VIT_HP**	**VIT_LP**
Glop_HP	-											
Glop_LP	0.72***	-										
GrHrds_F_HP	0.03ns	0.12***	-									
GrHrds_F_LP	-0.05ns	0.11***	0.61***	-								
LaPa_HP	0.16***	0.10***	0.13***	0.10**	-							
LaPa_LP	0.15***	0.12***	0.19***	0.12***	0.65***	-						
PEX_HP	0.08*	0.03ns	-0.05ns	0.01ns	0ns	0.01ns	-					
PEX_LP	0.12***	0.04ns	0.04ns	0.09**	0.06*	0.03ns	0.79***	-				
ThrSc_F_HP	0.77***	0.72***	0.12***	0.04ns	0.08**	0.09**	0.02ns	0.08*	-			
ThrSc_F_LP	0.69***	0.83***	0.15***	0.12***	0.11***	0.12***	0.08*	0.11***	0.76***	-		
VIT_HP	0.05 ns	0.13***	0.57***	0.41***	0.14***	0.19***	-0.05ns	-0.01ns	0.15***	0.16***	-	
VIT_LP	0.02 ns	0.07*	0.52***	0.44***	0.11***	0.15***	-0.02ns	0.02ns	0.11***	0.16***	0.74***	-

HP= high P, LP= low P, Glop=glume opening, GrHrds_F=grain harness, LaPa=panicle laxness, PEX= panicle exsertion, ThrSc_F=threshability, VIT=grain vitrosity,*=significance at ($P<0.05$), **= ($P<0.01$), *** ($P<0.001$), ns=no significant

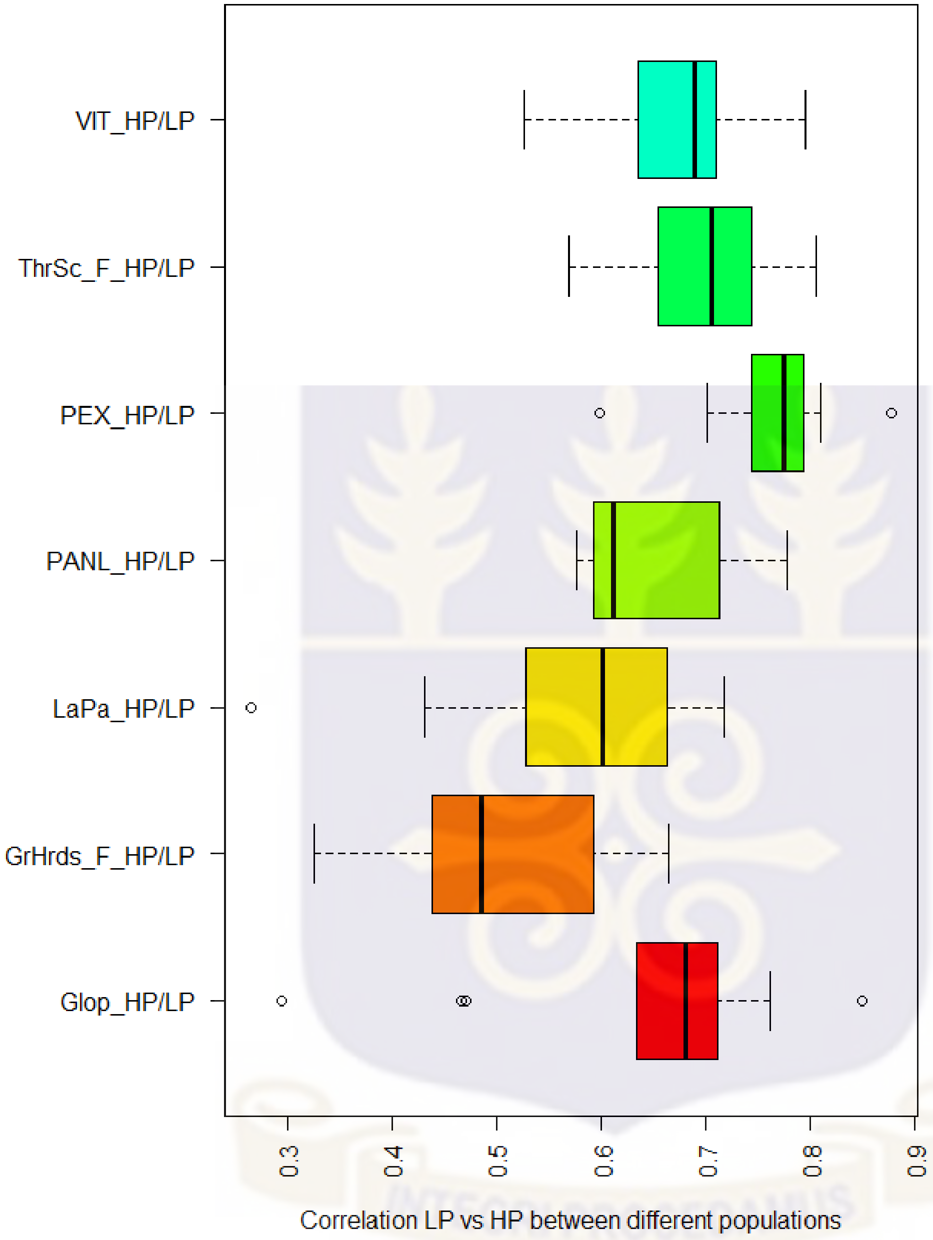

Figure 4.2: Genotypic correlations between individual populations in high P and low P field condition, for grain, glume and panicle traits.

4.3.3 Detection of QTLs.

Single QTLs were identified for panicle exsertion on chromosome (linkage groups (LGs)) 3, 4, 7 and 9, each in just one of the 13 populations except BC50 (Table 4.7). The QTL on LGs 7 and 9 were detected under both HP and LP conditions, and accounted for approximately 40% and 20% of phenotype variation (PVE) in those populations with confidence intervals of approximately 11 and 60 cM, respectively. Three of the 5 detected QTL were detected consistently across P-levels on LGs 4, 7 and 9. Among them two QTL were detected in the same population BC50 and BC60 (Table 4.7). The last QTL in an overlapping region was also detected in the populations BC59 (59_N'golo) HP and BC50 (50_Grinka), each in just a single P-level. Among them three were detected under HP and all five were mapped under LP. (Table 4.7). In almost all cases, the donor parents increased the panicle exsertion from 5 to 10 cm except for BC56. In general QTL detected in HP explained more PVE than QTL in LP while the interval of confidence was almost the same over both P-levels. GWAS for panicle exertion across all populations with a total of 969 BC1F4 progenies, identified 51,545 SNPs. Highly significant peaks were found in both P-levels on LG 7 (Fig. 4.3) of which the significant markers explain about 3% to 1% of PVE; the same LG as for the QTL in the 60_Ribda population with 37 to 45% PVE. Peaks were also identified on LG 6 for which no QTLs were identified, but the peaks were smaller and exceeded the threshold value only in LP, explaining 3% of PVE.

Table 4.7: QTLs identified for panicle exertion within separate P-levels at Samanko.

Env	**Pop**	**Locus**	**LG**	**pos**	**ci.low**	**ci.high**	**lod**	**R2**	**a**	**d**	**a(P)**
LP	56_Soumb	S3_10739017	3	49.17	37.28	63.05	5.16	23.89	-2.96	-1.89	R
LP	50_Grinka	S4_67086719	4	141.78	120.80	143.46	3.96	12.06	2.13	-0.54	**D**
HP	59_N'golo	S4_62839727	4	119.24	106.45	120.52	4.25	23.79	2.44	-2.34	**D**
HP	60_Ribda	S7_55960363	7	80.66	71.61	82.81	9.22	45.00	4.96	6.39	**D**
LP	60_Ribda	S7_55960363	7	80.66	72.60	82.81	7.01	36.53	5.04	5.27	**D**
HP	50_Grinka	S9_6444684	9	44.79	26.81	88.79	5.63	22.90	2.58	2.35	**D**
LP	50_Grinka	S9_6444941	9	45.99	40.88	92.79	4.61	14.68	2.55	1.42	**D**

HP= high P, LP= low P, LG=linkage group number, pos=position, ci=confidence, interval, lod=lod score, R^2=% of phenotypic variation explain by individual QTL, a=additivity d=dominance= a(P)=additivity effect positive parent, R= recurrent parent, D=donors parents.

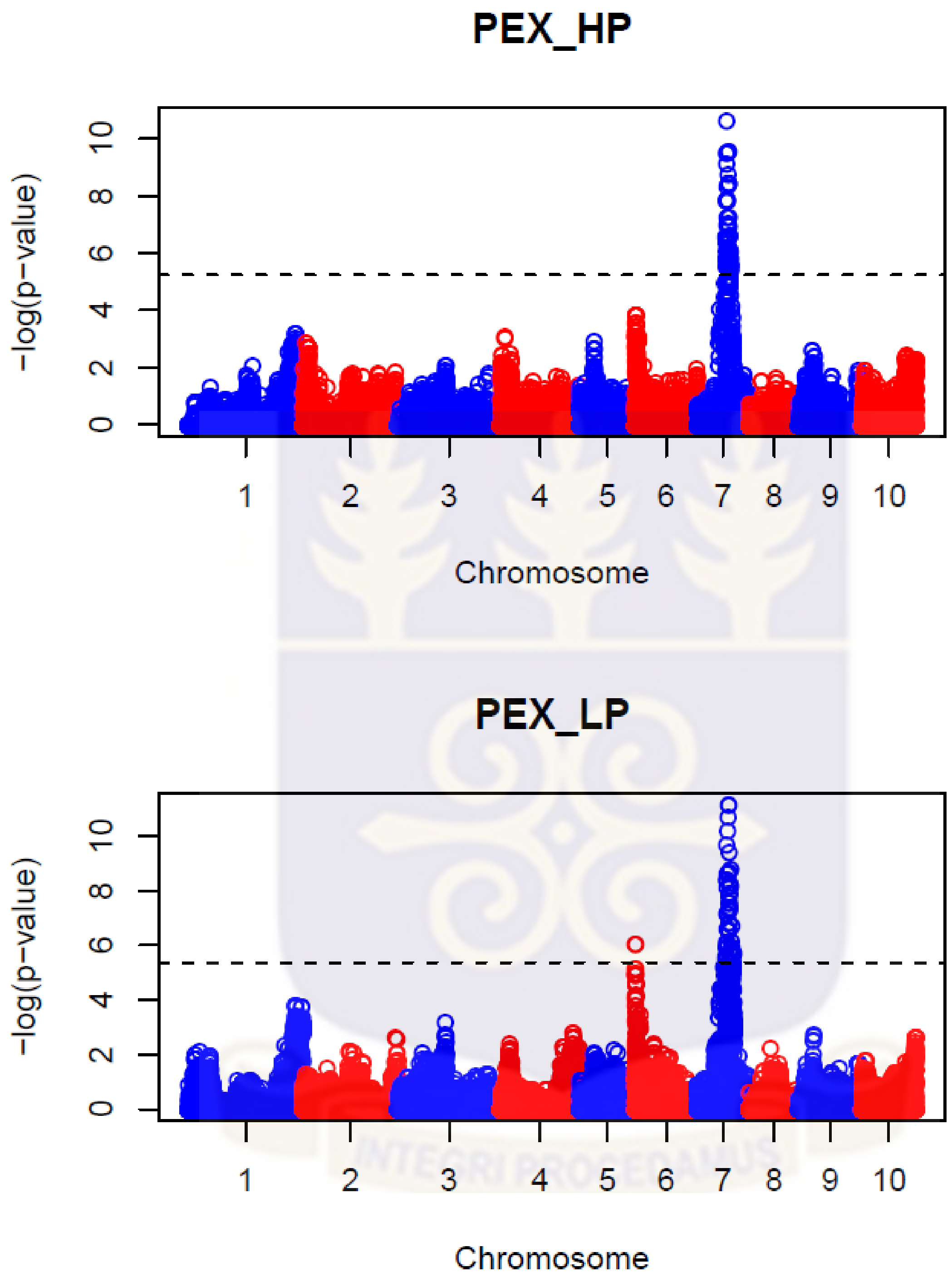

Figure 4.3**:** Manhattan plots displaying genome wide association results for panicle exsertion under high P (PEX_HP) and under low P (PEX_LP).

Six QTL were mapped for panicle laxness or panicle compactness, among them 5 QTL were detected under LP and 4 under HP. Three QTL were mapped across both environment, of which one was on LG 2 in the same population BC51 (*51_Hafid*) and two others were found on chromosome 3 and 6 with different populations (Table 4.8). The Manhattans plots show QTL across P level on chromosomes 2, 4 and 6 (Fig 4.4), the SNPs explained about 2 to 12% of PVE. These confirmed QTL detected by bi-parental populations mapping, did not find significant QTL on chromosome 3 in GWAS analysis. In almost all cases the recurrent parent increased the panicle laxness except for QTL identified in the populations BC51, BC55 and BC61.

Table 4.8: QTLs identified for panicle laxness within separate P-levels 2013.

Env	**Pop**	**Locus**	**LG**	**pos**	**ci.low**	**ci.high**	**lod**	**R2**	**a**	**d**	**a(P)**
HP	51_Hafid	S2_62583939	2	121.03	106.62	137.28	6.66	44.58	0.76	-0.01	**D**
LP	51_Hafid	S2_62583939	2	121.03	82.33	126.00	5.52	38.68	0.62	0.19	**D**
LP	54_Fram	S3_11829089	3	52.98	45.94	61.59	4.13	27.53	-0.30	0.79	R
HP	56_Soumb	S3_17959543	3	64.43	62.99	76.43	8.69	36.86	-0.45	0.61	R
HP	50_Grinka	S3_26650611	3	71.21	59.21	79.17	3.91	17.60	-0.32	0.46	R
LP	56_Soumb	S3_51932074	3	79.32	77.53	80.36	5.11	12.56	-0.18	0.34	R
LP	53_Douad	S3_71036847	3	149.63	144.05	158.24	3.93	28.49	-0.19	-0.26	R
HP	52_SK591	S4_22671930	4	60.10	44.00	61.69	4.54	24.60	-0.60	0.54	R
HP	55_Gnoss	S6_50776134	6	84.56	82.47	104.56	4.93	20.81	0.17	0.24	**D**
LP	61_Samba	S6_53559346	6	86.26	82.26	94.26	5.49	20.35	0.28	0.00	**D**
LP	56_Soumb	S8_1641919	8	12.39	3.04	91.55	4.34	9.39	-0.21	0.10	R

HP= high P, LP= low P, LG=linkage group number, pos=position, ci=confidence, interval, lod=lod score, R^2=% of phenotypic variation explain by individual QTL, a=additivity d=dominance= a(P)=additivity effect positive parent, R= recurrent parent, D=donors parents.

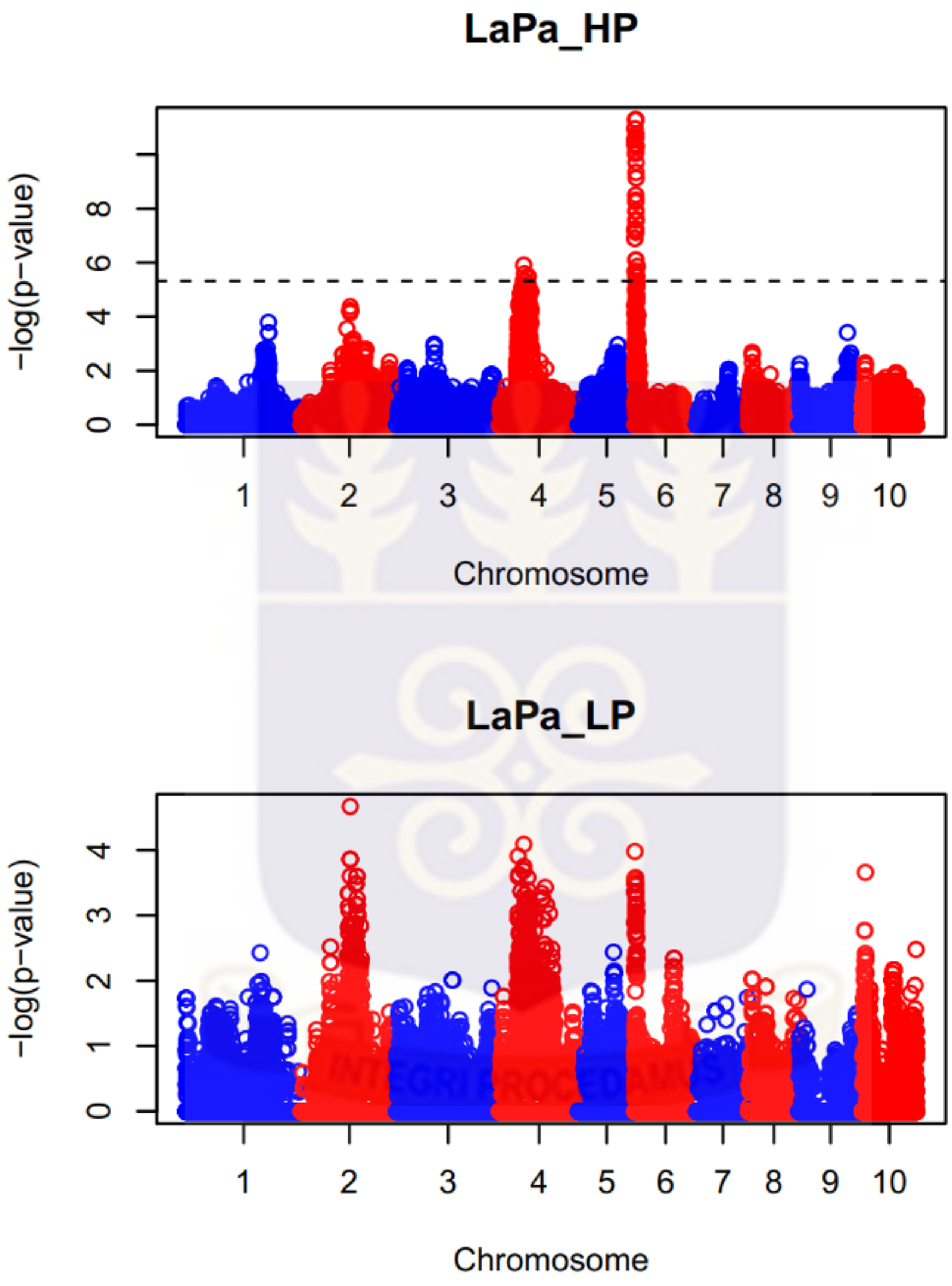

Figure 4.4 **:** Manhattan plot of panicle laxness across high P at top size and low P at bottom side

The bi-parental population mapping for glume opening revealed six significant QTLs, each on a different LGs in a different population (Table 4.9). All the QTLs were identified under HP condition. The QTLs explained about 22 to 34% of PVE with confidence intervals varying from 1 to 26 cM. The GWAS analysis of glume opening in the HP environment revealed peaks that exceeded the threshold on LGs 1, 3 and 8 (Fig 4.5) with 51545 SNPs identified, over 969 progenies BC1F5 under HP. The different SNPs associated with this explained 3 to 7% of PVE. No significant QTLs were mapped also under LP in GWAS, but peaks were found under Bonferroni threshold.

Table 4.9**:** QTLs identified for Glume opening within separate P-levels in 2013 at Samanko.

Env	**Pop**	**Locus**	**LG**	**pos**	**ci.low**	**ci.high**	**lod**	**R2**	**a**	**d**	**a(P)**
HP	54_Fram	S1_55814318	1	117.62	111.74	119.66	5.17	33.66	0.57	0.53	**D**
HP	50_Grinka	S2_61612251	2	115.88	100.98	124.74	6.04	25.84	0.50	0.19	**D**
HP	57_IS235	S3_70278117	3	146.43	134.43	160.70	4.44	26.62	-0.30	1.24	R
HP	59_N'golo	S4_2509720	4	17.65	15.65	22.62	5.42	29.28	-0.45	0.06	R
HP	56_Soumb	S8_792062	8	4.68	3.04	12.13	6.89	31.16	-0.74	0.31	R
HP	61_Samba	S10_50201644	10	81.40	69.66	85.31	4.57	21.70	0.30	0.68	**D**

HP= high P, LP= low P, LG=linkage group number, pos=position, ci=confident, interval, lod=lod score, R^2=% of phenotypic variation explain by individual QTL, a=additivity d=dominance= a(P)=additivity effect positive parent, R= recurrent parent, D=donors parents.

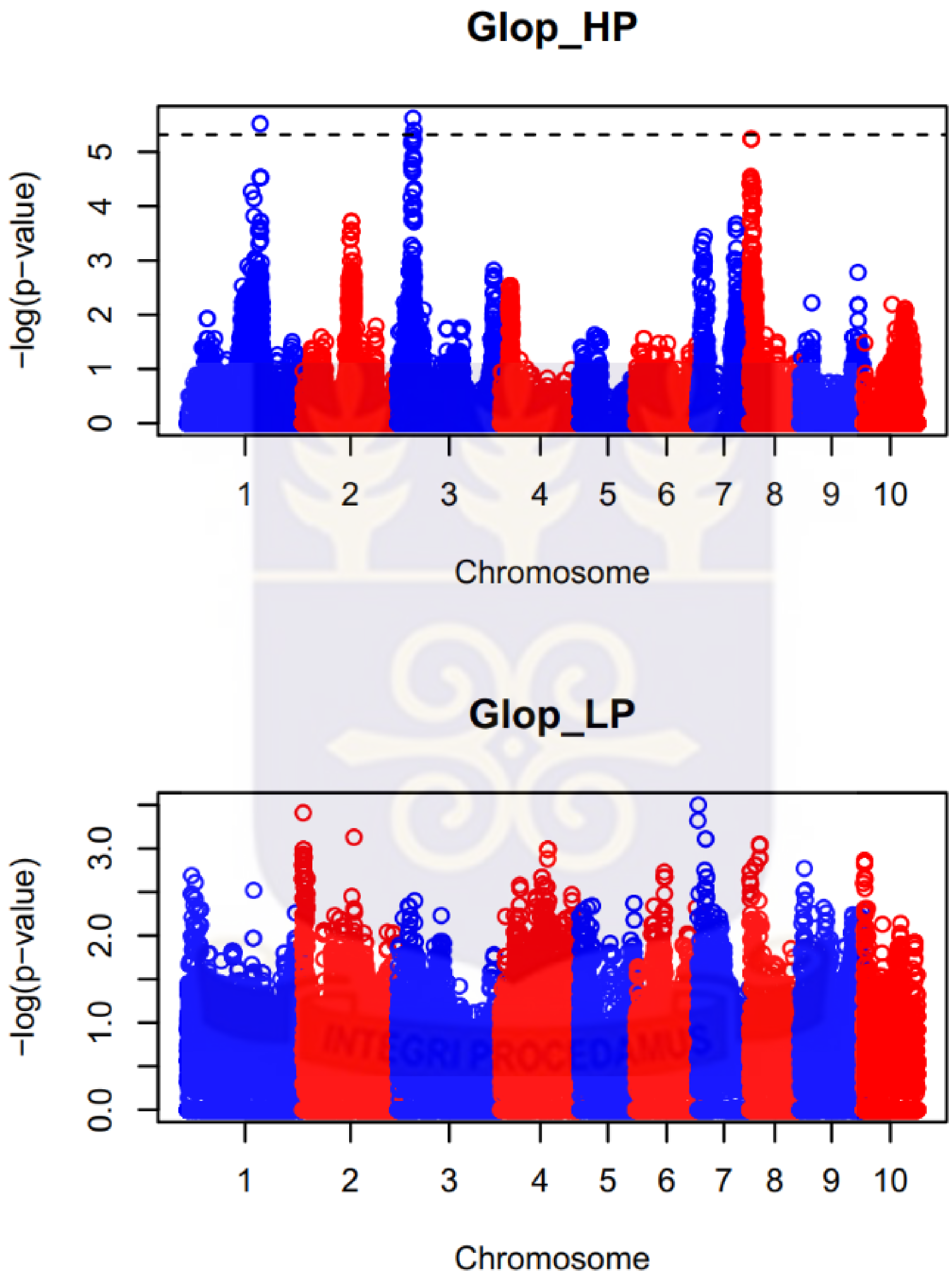

Figure 4.5**:** Manhattan plot of glume opening under high P at top and low P at bottom.

Eight QTLs were detected for threshing ability, each one on a different LG, and each in only one P-level, (Table 4.10). Four of the 8 QTL were detected in the population (BC59), with the favorable allele coming from the donor in two of the cases (LGs 1 and 5). The QTLs accounting for more of the total variation (20 to 25%) were on LGs 2, 5, 7, 8, and 10). Three of these QTLs (on LGs 2, 8 and 10), along with the one on LG 4 are in corresponding regions to significant QTLs for Glume Opening (Table 4.10) and were all identified in the same populations except for LG10. Association analyses revealed the presence of 3 QTLs including 2 QTLs detected across P-levels on chromosomes 3 and 7 and a third QTL identified on chromosome 8 under HP (Figure 4.6), accounting for 2 to 4% of PVE. QTLs on LGs 7 and 8 were confirmed by single population mapping (Table 4.10). In addition, two important peaks were detected below the Bonferroni threshold on chromosomes 2 and 4 under HP (Fig. 4.6). Almost all QTLs detected by both methods for glume opening were co-located with QTLs detected for threshing ability score on LGs 2, 3, 4, 8, and 10 except for QTLs on LGs 1 and 7. GWAS analysis detected an important QTLs below the threshold under HP for Glop.

Table 4.10: QTLs identified for threshing ability within separate P-levels at Samanko.

Env	Pop	Locus	LG	pos	ci.low	ci.high	lod	R2	a	d	a(P)
HP	59_N'golo	S1_15259857	1	48.22	35.97	49.39	3.91	6.51	0.12	-0.52	D
HP	50_Grinka	S2_59725771	2	102.84	99.13	121.34	4.67	20.63	0.52	0.25	D
HP	59_N'golo	S4_4177975	4	15.89	15.46	20.17	5.22	8.87	-0.23	-0.04	R
LP	59_N'golo	S5_3075297	5	16.19	7.91	25.74	3.79	21.54	0.31	-0.39	D
HP	59_N'golo	S6_61610509	6	116.21	115.21	116.60	4.68	9.53	-0.07	-0.41	R
LP	58_Fara	S7_2001441	7	12.39	6.54	32.54	4.43	23.00	-0.52	0.05	R
HP	56_Soumb	S8_792062	8	4.68	3.04	16.78	4.64	22.23	-0.68	0.20	R
LP	62_Sc566	S10_53206543	10	89.77	87.44	93.63	4.23	24.60	-0.03	-1.07	R

HP= high P, LP= low P, LG=linkage group number, pos=position, ci=confidence, interval, lod=lod score, R^2=% of phenotypic variation explain by individual QTL, a=additivity d=dominance= a(P)=additivity effect positive parent, R= recurrent parent, D=donors parents.

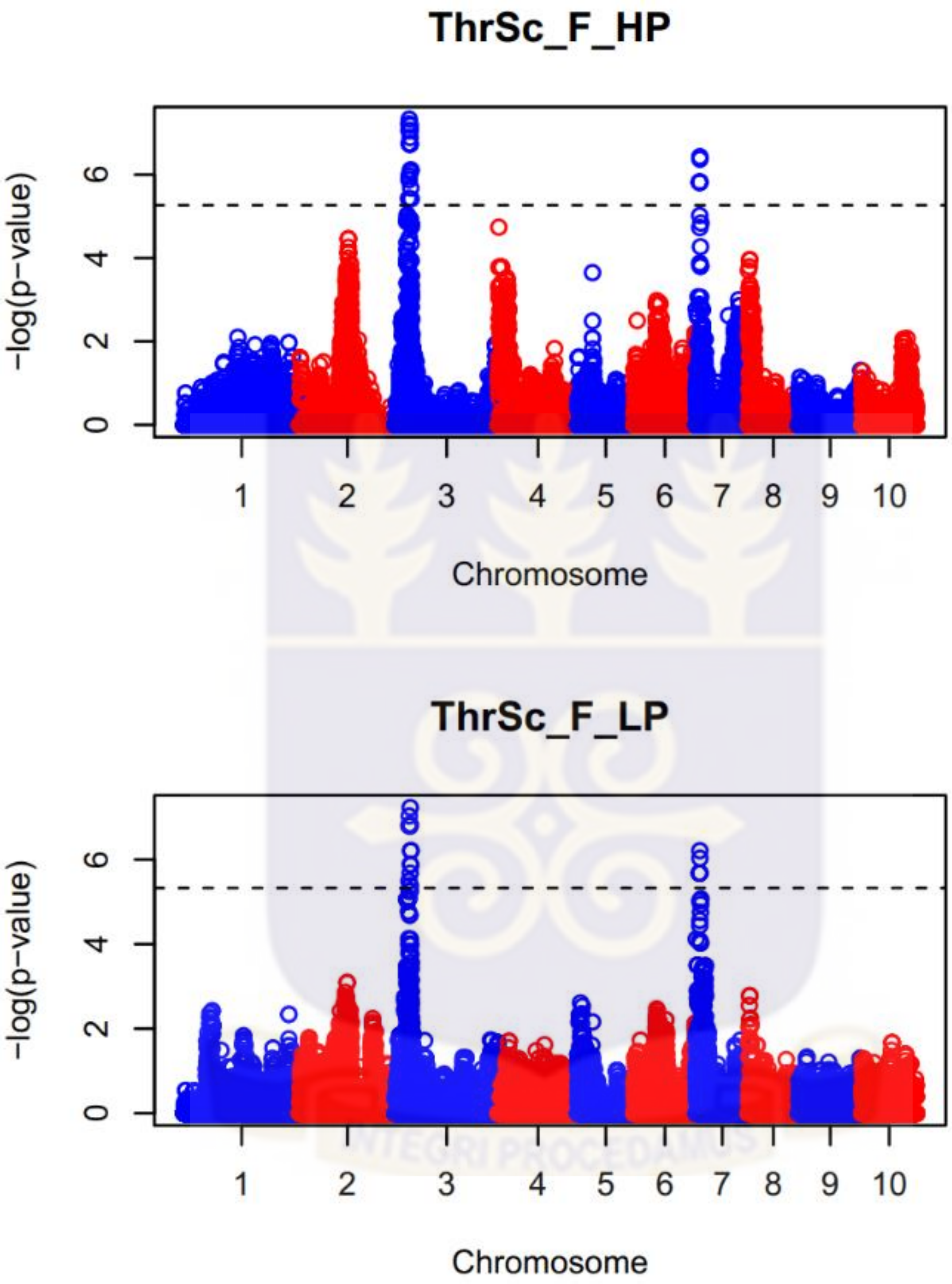

Figure 4.6**:** Manhattan plot of threshing ability score at top side high P and low P at bottom size.

A total of 10 QTLs were mapped under HP and 7 under LP. Six pairs of QTLs were identified in common regions across both LP and HP (Table 4.11), among them three involving the same populations on LGs 4, 8, and 10, and three others involving different populations on LGs 3, 6 and 9. The favorable alleles came from the recurrent parent (Lata) for most of the QTLs. Half of all QTLs were identified in Ribdahu population, with the favorable allele always coming from Lata. Favorable alleles coming from donor parents were identified in only two populations: the 51_Hafid Population on LG 6, and in Soumalemba Populations on LG7 and 8 (Table 4.11). The QTL identified in the 51_Hafid Population accounted for 39% of total variation in that population under LP. This QTL however was not identified in this population under HP, although it was identified in another population 62_Sc588, which also accounted for large PVE (24%) but with the favorable allele coming from the recurrent parent. GWAS was conducted with 669 BC-NAM progenies and 51545 SNPs for VIT. Only a single significant peak on LG 9 under HP (fig. 4.7), the significant SNPs accounting for 5 to 6% of PVE. No significant peak was found under LP for grain vitrosity.

Table 4.11**:** QTLs identified for grain vitrosity across P-levels**.**

Env	**Pop**	**Locus**	**LG**	**pos**	**ci.low**	**ci.high**	**lod**	**R2**	**a**	**d**	**a(P)**
HP	56_Soumb	S1_353098	1	1.19	1.19	141.36	4.39	14.71	-0.06	1.48	R
HP	55_Gnoss	S1_6385525	1	17.00	14.75	34.27	4.12	10.24	-0.61	-0.72	R
HP	60_Ribda	S1_72667724	1	176.57	154.24	178.03	4.54	1.49	-0.36	-0.35	R
LP	60_Ribda	S2_3867520	2	20.69	14.91	45.65	5.34	7.57	-0.19	1.29	R
HP	60_Ribda	S2_68481771	2	137.65	12.50	174.11	4.97	2.73	-0.36	0.99	R
HP	55_Gnoss	S3_57251183	3	102.20	96.43	119.85	4.11	10.21	-0.19	-1.42	R
HP	60_Ribda	S3_59011808	3	112.24	83.29	124.24	4.92	2.01	-0.51	0.33	R
LP	60_Ribda	S4_53626650	4	85.44	14.69	88.25	4.85	3.52	-0.22	-0.61	R
HP	60_Ribda	S4_53641412	4	85.50	44.25	88.25	6.64	0.33	-0.12	-0.28	R
HP	60_Ribda	S5_8287975	5	45.84	0.79	61.60	4.91	1.49	-0.20	-0.63	R
LP	51_Hafid	S6_44559270	6	52.05	51.30	55.30	5.57	38.92	1.04	-1.30	**D**
HP	62_Sc566	S6_48674055	6	67.44	6.80	93.58	4.14	24.15	-1.10	0.48	R
HP	60_Ribda	S6_957024	6	3.47	0.36	102.94	5.77	1.86	-0.31	-0.51	R
LP	56_Soumb	S7_56860054	7	85.17	83.47	100.37	4.89	14.09	0.75	0.69	**D**
HP	60_Ribda	S7_62548183	7	117.56	116.27	119.32	4.98	0.88	-0.31	0.24	R
HP	*56_Soumb*	*S8_1548626*	8	11.55	7.04	43.04	5.08	17.52	0.67	0.57	**D**
LP	*56_Soumb*	*S8_1548626*	8	11.55	9.66	21.04	4.75	13.54	0.64	0.16	**D**
HP	60_Ribda	S9_1155517	9	6.13	0.13	122.78	5.03	2.58	-0.35	0.91	R
LP	60_Ribda	S9_27649	9	0.13	0.13	8.07	4.66	5.09	-0.19	0.90	R
HP	54_Fram	S9_58742088	9	120.79	111.87	122.29	7.15	42.78	-1.34	-1.27	R
HP	60_Ribda	S10_54688005	10	99.09	56.15	101.28	4.76	1.72	-0.35	0.53	R
LP	60_Ribda	S10_54688005	10	99.09	56.15	106.08	4.90	5.20	-0.29	0.95	R
LP	53_Douad	S10_54770688	10	106.29	92.29	120.24	6.20	40.25	-0.72	-1.32	R

HP= high P, LP= low P, LG=linkage group number, pos=position, ci=confidence, interval, lod=lod score, R^2=% of phenotypic variation explain by individual QTL, a=additivity d=dominance= a(P)=additivity effect positive parent, R= recurrent parent, D=donors parents.

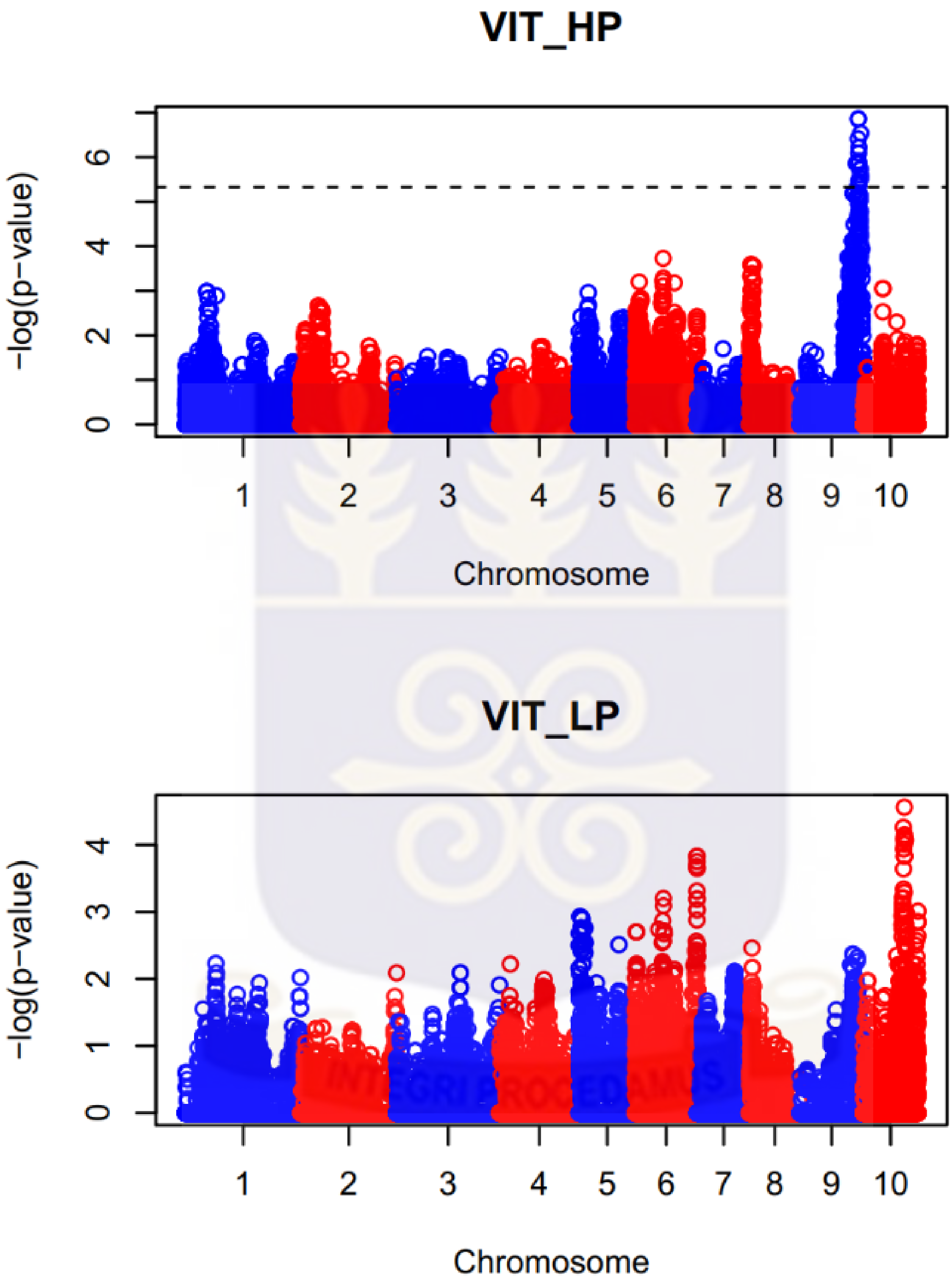

Figure 4.6**:** Manhattan plot of grain vitrosity under high P at top and low P at bottom.

A total of 7 QTL were detected for grain hardness in bi-parental QTL analysis (Table 4.12). Among them five were identified under LP and the remaining 3 were detected under HP. However, a QTL on LG 1 was detected over P-levels in the population 52_SK591 and only under LP in the population 58_Fara. One QTL was mapped under LP in different populations (BC50 and BC61) on LG 2. The QTLs detected for grain hardness explain about 10 to 37% of PVE under LP and 23 to 28% of PVE under HP. The widest confidence interval was under LP compared to HP (Table 4.12). Interestingly the recurrent parent contributed to increase the grain hardness for the different populations. The association analysis revealed only one QTL in LG 2 under LP which was previously detected with bi-parental population mapping under LP and accounted for 3 to 5% of PVE, but two important peaks were found on LGs 4 and 7 under LP below the Bonferroni threshold (Figure 4.8).

Table 4.12**:** QTL identified for grain hardness appreciation by farmers within separate P-levels at Samanko**.**

Env	**Pop**	**Locus**	**LG**	**pos**	**ci.low**	**ci.high**	**lod**	**R2**	**a**	**d**	**a(P)**
LP	54_Fram	S1_42060590	1	81.74	69.74	137.36	4.96	9.53	-0.33	0.43	R
HP	52_SK591	S1_52891916	1	104.59	90.82	108.79	5.54	25.58	-0.38	0.02	R
LP	52_SK591	S1_56774086	1	124.59	90.82	130.59	4.27	23.34	-0.38	-0.42	R
LP	58_Fara	S1_61130542	1	138.59	120.27	142.52	5.31	26.32	-0.43	0.55	R
LP	61_Samba	S2_56723119	2	86.14	82.37	97.50	6.52	29.47	-0.40	0.35	R
LP	50_Grinka	S2_56850967	2	84.98	73.72	132.32	4.06	18.21	-0.47	0.18	R
HP	57_IS235	S2_7719717	2	42.98	33.03	50.82	4.75	28.21	-0.54	0.02	R
LP	54_Fram	S3_10041325	3	45.94	33.54	52.00	4.92	9.38	-0.34	0.05	R
LP	51_Hafid	S4_62195297	4	115.76	111.29	137.56	5.18	36.77	-0.52	-0.10	R
HP	50_Grinka	S6_58241132	6	105.55	82.17	107.62	5.31	23.11	-0.32	0.12	R

HP= high P, LP= low P, LG=linkage group number, pos=position, ci=confidence, interval, lod=lod score, R^2=% of phenotypic variation explain by individual QTL, a=additivity d=dominance= a(P)=additivity effect positive parent, R= recurrent parent, D=donors parents.

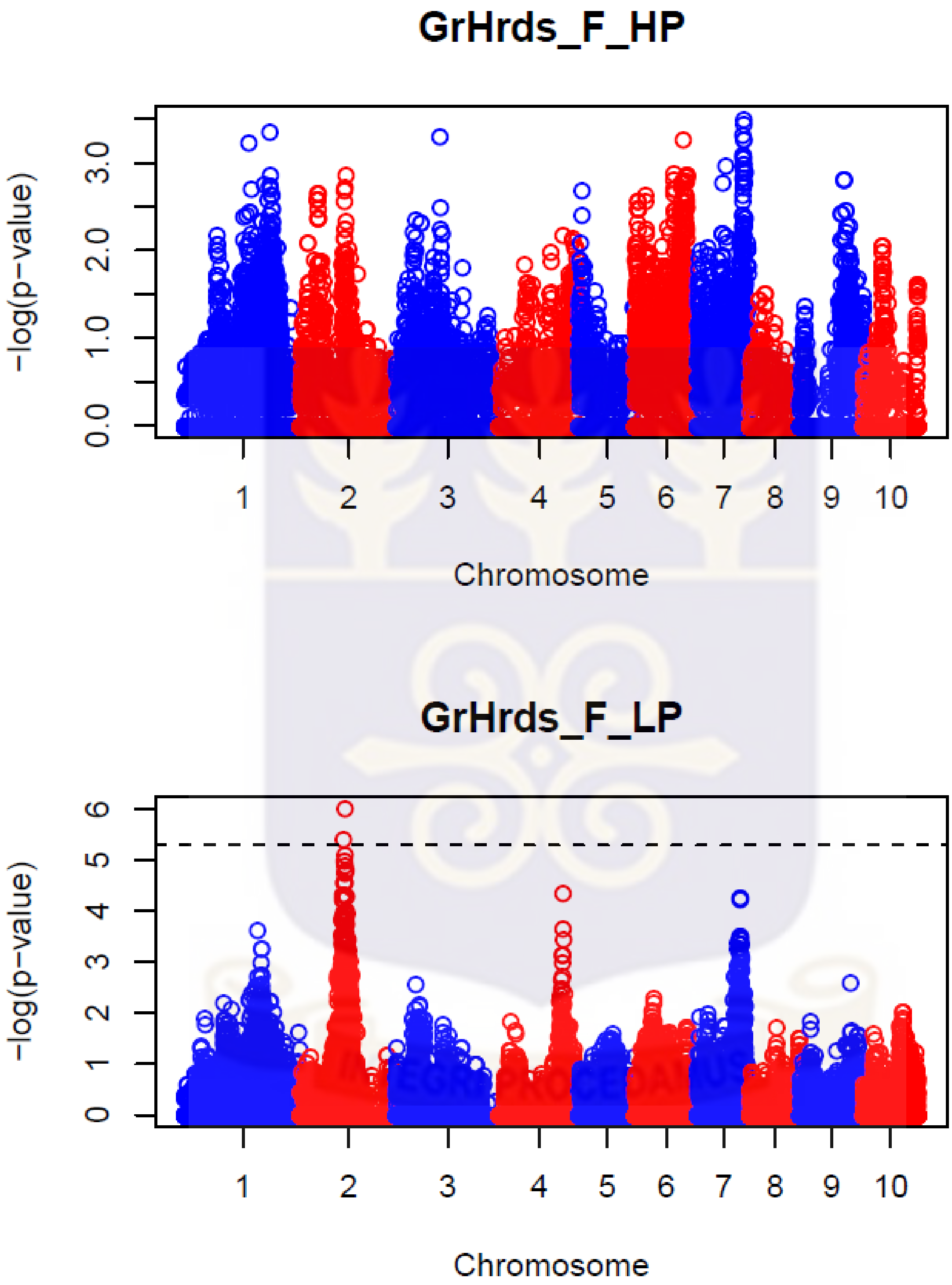

Figure 4.8**:** Manhattan plot of farmer grain hardness appreciation in low P at the bottom and high P at the top.

4.4 Discussion:

4.4.1 Feasibility of phenotyping methods for panicle and grain quality traits

The relatively high repeatability estimates for the grain and panicle traits studied indicated that these traits can be effectively evaluated under simple field trials. The results indicate only two replications with single row plots using an alpha lattice design for potential adjustments at the sub-block level are needed. Further, the acceptable repeatability obtained in the LP trial, often similar to those from the HP trial, and high significant correlation between HP and LP for these traits show that these observations can be done under either LP or HP conditions. This study, by evaluating under both LP and HP conditions, enabled examination of how consistently genotypic differences are maintained under conditions differing for one of the major constraints to sorghum growth in Mali and West and Center of Africa (WCA). The high broad sense heritability estimates for these traits, with highly significant genetic variances and significant but smaller variance components for genotype x P-level interactions, showed that genetic differences were generally maintained over contrasting P-environments. The consistently high genotypic correlations between Low- and High-P conditions within the individual populations showed that over the different populations with highly contrasting donor parents, genetic differences were well maintained over P-levels. No known previous studies have been reported on genotype x P-levels interaction for these traits.

Grain hardness plays an important role for farmers' acceptance of new varieties. High positive significant correlation were detected between grain hardness and grain preferences and grain productivity (vom Brocke *et al*., 2010). In addition the varieties with increased grain hardness or increased thickness of the corneous layer of the endosperm are much less susceptible to the primary grain pests (Doraiswamy *et al*., 1976; Fadlemula, 1983; Russell, 1962; Russell, 1966b;

Wongo & Pedersen, 1990). In this study the grain hardness score appreciation was done by women, because in general in WCA women are in charge of all culinary processes, therefore they have more knowledge about grain qualities. This appreciation was moderately correlated with grain vitrosity and has similar heritability estimate compared to grain vitrosity which is time consuming and often associated with costly laboratory procedures. As grain hardness is a sorghum variety attribute important for smallholder farmers, it is critical that it is evaluated early in the breeding cycle. The results show that involving experienced rural women in the visual evaluation of this traits is highly efficient. Involving women evaluators is thus a recommendable practice, and has the added advantage that the women may highlight specific outliers for specific reasons, and thus advance the common understanding of the diversity of these traits. Usually sorghum grain hardness is assessed in laboratory by using near-infrared spectroscope (NIRS) instrumentation either the ground grain is used or the whole grain (Dykes *et al.*, 2014; Rami *et al.*, 1999), which is not available in many cases to most of breeding programmes. This procedure of assessment for grain hardness could be improved for its future use in breeding.

4.4.2 **Molecular markers for panicle traits and grain quality**

Thirteen NAM populations which represent an important source of diversity were used to map QTL for panicle traits and grain quality including glume types, grain characteristics, and panicle laxness using bi-parental population mapping and genome-wide association mapping under P-deficient field conditions**.** These approaches are complementary and give insights into markers being specific under certain genetic backgrounds, hence providing important information for implementation of MAS breeding programme**.** A total of 42 QTLs were mapped using both methods over P-levels. Among them 41 were mapped with single population mapping and 13 were detected in GWAS analysis. The confidence interval for QTLs detected in single population mapping varied from 1 to 162 cM, however the majority were mapped less than 20 cM. They explained about 1 to 89% of PVE. The large number of QTL detected in bi-parental QTL mapping was expected. In bi-parental population mapping one can also detect genetic background specific QTL while in GWAS one can only find QTL which are existing across all families. Further, in bi-parental mapping, the detected QTL might be of little value, since the effect is often overestimated. Most of the major QTL identified in this study were mapped by previous studies, which provide more robustness to the results reported in this study. A major QTL was considered consistent if it was mapped across P-Levels and having strong correlation between both environments. About 74% of QTL detected, were mapped under HP compared to 57% in LP, with several peaks under the threshold, suggesting HP was more favorable for QTL detection for these traits than its correspondent LP except for PEX which indicated also the slightly low repeatability of these traits than LP, which meant also the LP influence on power of QTLs detection. Sixteen major QTLs were identified consistently across P-levels for different traits; PEX, LaPa, ThrSc_F, VIT, and GrHrds_F for both methods, except for Glop, suggesting the widest variation of the correlation between population in HP and LP for Glop (Fig.4.2). More than half of the QTLs detected for grain vitrosity and panicle laxness, panicle exsertion and threshing ability were detected across P-levels, suggesting that these traits shared similar genetic bases across P-levels. Furthermore a single QTL was detected with different populations either in the HP or in the LP or across P-levels. In spite of the highly positive correlations for most of the traits under both P-levels, and the similar repeatability between P-levels there was indication of the interactions of QTL x P-levels. The detection of QTL on LG 6 in GWAS analysis that was not detected in bi-parental analysis can be explained by the detection of peaks in many individual populations mapping that were below the Bonferroni threshold.

The QTL detected for panicle exsertion were reported previously (Feltus *et al*., 2006; Felderhoff *et al.*, 2012; Rajkumar *et al.*, 2013; Zhao *et al.*, 2016)**.** The donor parents contributed to increase panicle exsertion for most detected QTL except for QTL mapped in the population BC56 (56_Soumb) on LG 3. This suggests more options for breeders to introgress this trait into elite materials after future validation. In addition the genomic regions detected on LGs 6, 7 and 9 have been severally reported as having major maturity and plant height genes and gene for grain yield suggesting possible pleiotropic effects for these QTLs.

Sorghum panicle laxness or compactness is important to prevent against damage caused by insect pest (Sharma *et al*., 1994). It is associated with insect damage and susceptibility to grain mold according to farmers. As a consequence farmers preferred more open panicles. A total of five QTLs were mapped for LaPa, A few studies have been reported on panicle laxness or panicle compactness and based on existing knowledge, none of these QTLs have been previously reported for LaPa, except for QTL on LG 6 (Rami *et al*., 1998). These may be newly identified loci regulating panicle laxness. The recurrent parent contributed to increase LaPa in almost all identified QTLs, except for QTLs identified in the populations BC51, BC55 and BC61. The donor BC51 is *Guinea margariferum* characterized by its highest grain vitrosity and it smallest grain, while BC61donor's is *Guinea conspicuum* which has high grain quality and is tolerant to aluminum toxicity and the BC55 is a guinea with good grain quality. These donor parents are interesting and valuable for breeders in Mali as well as in WA, being guinea varieties which are generally adapted to low soil fertility including low phosphorus (Leiser *et al.*, 2012).

Threshing ability is a serious problem for sorghum and other crops, such as rice and pearl millet (Kumar & Sharma, 1982; Yadav, 1994; Dut *et al*., 2002; Okubo *et al*., 2012) and is essential for variety adoption by smallholder farmers (Asante, 2013). Nearly all QTLs detected by both methods for glume opening were found in overlapping regions with QTL detected for threshing ability score under HP except for QTL on LGs 1 and 7. This indicated that threshing ability score across P-levels shared similar genetic basis with glume opening under HP. Additionally in GWAS analysis, only QTL was detected across P-levels for threshability and shared most the QTL detected under HP. These suggest an indication of QTL by P-levels interaction since QTL were detected specifically in a single environment. Of the QTLs detected 4 QTLs were co-located by previous studies for glumes on LGs 1, 3, 4, 6 and 7 (Hart *et al*., 2001; Feltus *et al*., 2006; Murray *et al*., 2008). The QTLs on LGs 5, and 10 have not been reported in previous studies to our knowledge, and explain about 7 to 34% of PVE. They may be considered as new loci regulating threshability and glume opening. In addition the threshing score ability was more favorable for QTL than glume opening score detection across contrast P-levels.

The grain hardness and vitreousness are important for farmers in food processing and also for grain storage. Many studies have reported the importance of these traits for food processing (Rooney & Murty, 1982), and grain storage (Doraiswamy *et al*., 1976; Fadlemula, 1983; Russell, 1962). These traits are essential for smallholder farmers to adopt sorghum varieties in West Africa. Several QTLs were mapped for these traits, among them, the QTLs on LGs 2, 3, 4, 6 and 10 were earlier reported (Rami *et al*., 1998) as QTL for grain vitreousness. Interestingly the recurrent parent contributed to increased grain hardness and it also contributed to increased grain vitrosity for almost all QTL detected except for QTL in the populations BC51 and BC56. This indicated that both traits share similar genetic basis across P-levels. Both traits were highly significantly

(P<0.001) correlated, but no common QTL was identified across study these two traits using both methods of QTL mapping which is not expected but they were highly correlated.

4.5 Conclusions:

A similar, but highest estimate value of repeatability was detected between HP and LP and highest heritability over P-levels. These traits can be effectively evaluated in simple field trials with two replications and single row plots using an alpha design. There was high significant correlation between grain hardness and grain vitrosity. A total of 42 QTLs were mapped using both methods over P-levels. Among them 41 were mapped with single population mapping and 13 were detected in GWAS analysis. Most the major QTL identified in this study were mapped by previous studies. Nevertheless some newly identified genomic region were found on: LG 2, 3, 4, and 8 for panicle laxness and QTL on LGs 5, and 10 for ThrSc_F and Glop. Other unique QTL associated to hardness were found to co-localize with QTL for grain vitrosity across contrasting P levels. Nearly all these trait share almost all similar genetic basis over P-levels except for Glop which is also an indication of the interaction of QTL x P-levels. The identified QTL associated to farmer preferred traits like: grain quality, glume opening and panicle laxness could be useful in guiding selection of promising sorghum lines under contrasting P conditions.

CHAPTER FIVE

5.0. SELECTION STRATEGIES FOR IMPROVING GRAIN YIELD AND RELATED TRAITS UNDER P-DEFICIENCY FIELD CONDITIONS

5.1 Introduction

The world sorghum production is estimated at 94 million tons (FAO, 2017), the majority of this production is in Africa and Asia under low inputs with erratic rainfall (Breman, 1998; Rattunde *et al.*, 2016)**.** In Mali, traditionally sorghum production was primarily for subsistence but it is becoming more important in the market economy in the country. Recent studies at a village-level shows that the majority of smallholder producers in Mali are involved in intra-rural grain marketing. Sorghum is a major source of nutrients and income for smallholder farmers in Mali as well as in West Africa and Asia. It is a critical cereal crop for food security in West Central Africa, being produced under low input farming systems (vom Brocke *et al.*, 2010)**,** particularly P-deficiency conditions (Buerkert *et al.*, 2001; Leiser *et al.*, 2012) with high variable rainfall. There is need for sorghum grain yield increases to match increasing population growth, particularly in Africa. Grain yield is a complex trait and it is difficult to improve under low input systems as well as optimal input system because of the variation of soil fertility, and other factors which contribute to rise residual variation. However Leiser *et al.* (2012), by using appropriate field design with spatial adjustment, obtained significant increase heritability. Yield-related traits affect grain yield, indirectly or directly, by affecting yield component traits or other mechanisms that are unknown (Shi *et al.*, 2009). Plant growth and development is affected under low phosphorus conditions usually resulting in reduced growth, vigor and grain yield and delay in maturity time (Leiser *et al.*, 2015; Nord & Lynch, 2008). Therefore, phosphorus is an essential macronutrient for plant survival. Tolerance to P-deficiency is desirable for sorghum adaptation in West Africa. Sorghum breeding under P-limited conditions in West Africa has been shown to be necessary and that it is feasible to obtain superior genotypes (Leiser *et al*., 2012). As these conclusions were drawn based on a set of 70 varieties originating from diverse sources, it is important to examine whether these conclusions also apply to breeding populations targeting specifically variety development for a specific environment, the Sudan Savannah of Mali in this case.

Several bi-parental mapping and association mapping populations have been created with sorghum to study the mechanisms controlling grain yield and related traits such as plant height, date of flag leaf appearance, panicle length and hundred seed weight, under favorable field conditions (HP), and mostly with materials derived from the caudatum and kafir races of sorghum. Only few studies have been conducted to dissect sorghum genetic bases for grain yield and its associated traits under P-limited environments (Leiser *et al*., 2015). No bi-parental population mapping studies have been performed to investigate these traits under contrasting phosphorus conditions but a few association mapping studies were conducted (Leiser *et al*., 2014). Thus, this study was conducted to assess population genetic parameters for sorghum grain yield and related traits under HP and LP conditions of BC1F5 NAM progenies. This study also investigated the relative efficiency of direct selection for grain yield under low and high phosphorus soil conditions.

5.2 Materials and Methods:

Two trials were conducted during this study at ICRISAT-Samanko station under high phosphorus (HP) and low phosphorus (LP) conditions over three rainy seasons from 2013 to 2015.

The first trial was conducted to evaluate, characterize and detect QTL for grain yield and yield component. Thirteen bi-parental NAM populations composed of 1083 BC1F5 progenies were phenotype. The individual population sizes varied from 70 to 102 BC1F4 progenies as shown earlier in Table 4.1. The parameters used during data collection are described in Table 5.1. The experimental design used was an alpha lattice design with two replications and one row per plot. Diammonium phosphate (18-46-0) was applied under HP at rate of 100 kg ha^{-1} as basal fertilized and urea (50 kg ha-1) (46-0-0) as topdressing. The LP fields were fertilized only with the equivalent amount of N as the HP fields but no phosphorous.

Table 5.1**:** Description of different parameters, units and the abbreviated names.

Traits	**Abbreviation**	**Descriptions of parameters**
Seedling vigor (score)	GV	Assessment of the biomass of the plants of the plot at 15 days after sowing score (1-9)
Days to flag leaf appearance (Julian day)	DTFL	Number of days from sowing to flag leaf appearance
Plant height (cm)	PH	Length (cm) of the main stalk recorded on 10 random plants from the base of the stalk to the tip of the panicle (Maturity).
Panicle length (cm)	PANL	Length of the peduncle from flag leaf to panicle basis (Maturity)
Grain yield/m2 (g-m^2)	GYLD	Grain yield per surface unit estimated on a plot (Maturity)
Weight of 100 grains (g)	HGW	Weight of 100 grains with percentage of humidity less or equal 12% (Maturity)

The second trial was conducted to evaluate of 298 BC1F5 back cross nested association mapping (BC-NAM) progenies selected after phenotypic evaluation in 2013. The selections were based on their grain yield performance under low phosphorus (LP) and high phosphorus (HP) field condition. The selected progenies were tested for grain yield for two year in “multi-year confirmation trials”, with the aim to evaluate the genotype by environments interaction. The experimental design was an alpha design with 3 replications with two separated trials under HP and LP at Samanko ICRSAT. The trials were conducted with 300 entries, including the 298 selected progenies with the recurrent parents repeated 2 times. The fertilizer was applied following the same procedures in the first trial.

- Phenotypic statistical analysis

The individual P-level was analyzed for different traits using GenStat for correlation analysis and ANOVA, BMS to obtain the Best Linear Unbiased Estimate (BLUEs) and the Best Linear Unbiased Predictions (BLUPs) "R" was used for graphics like box plots and two models were automatically fitted to run the analysis. In the first model (Model 1) the genotypes were taken as random effects.

$$\textit{(Model 1)}\quad Y_{ijk} = \mu + G_i + R_j + B_k + B(R)_l + E_{ijkl}$$

In second model (Model 2) the genotype were taken as fixed effects.

$$\textit{(Model 2)}\quad Y_{ijkl} = \mu + \mathrm{G}_i + R_j + B_k + B(R)_l + E_{ijkl}$$

Where Y_{ijk} is the observed value; μ is the population mean; G_i is the effect due to the i-th genotype test; R_j is the effect due to the j-th replication; B_k is the effect due to the k-th block; B(R)=block within the replication; E_{ijk} is the effect due to the random error.

The random model was used to estimate variance components for estimating repeatability (Model 3), Repeatability was calculated with an adjusted formula for unbalanced data sets (Piepho & Mohring, 2007). Finally, Best Linear Unbiased Predictions (BLUP) for genotype performance were obtained with Model 2. In the second analysis, genotypes were treated as fixed effects. The Best Linear Unbiased Estimates (BLUE) obtained from Model 1 were used in GxE and QTL mapping analysis. The model 2 assume block ~N $(0, \sigma^2_b)$, and error ~N $(0, \sigma^2)$).

Model (3) **Repeatability:**

$$w^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{V}{2}}$$

Where; σ_g^2 = genotypic variance, V is mean variance of difference between treatment means.

Combined analysis across environments

The two trials (LP and HP) were used for combined analysis using the following linear model (model 4) for.

Model (4) $Y_{ijkl} = \mu + G_i + L_j + GL_{ij} + R(L)_k + B(R(L))_l + E_{ijl}$

Y_{ijk} is the observed value; µ is the population mean; G_i is the effect due to the i-th genotype t; L_i is effect of the j-th location; GL_{ij} is interaction of the i-th genotype in the j-th location; $R(L)_k$ is the effect due to the replication within location; $B(R(L))_l$ is the effect due to the block within the replication and the replication within location; E_{ijk} is the effect due to the random error.

(Model 5)

- **Broad sense heritability**

$$h^2 = \frac{\sigma_g^2}{(\sigma_g^2 + \frac{\sigma_{gl}^2}{l}) + (\frac{\sigma_e^2}{rl})}$$

Where σ_g^2 is genotype variance of components, σ_{gl}^2 variance components of interaction genotype and P-levels, σ_e^2 variance component errors, l is environments, r is replications.

Genetic correlation

Model 6

$$r_G = \frac{r_{\text{phenotpic}}}{\sqrt{(h^2{}_{LP} * h^2{}_{HP})}}$$

Where r_G is genetic correlation coefficient of grain yield between HP and LP, r is a phenotype correlation, h^2 is repeatability under LP and HP as described (Cooper *et al*., 1994).

The effectiveness of indirect (selection under HP for grain yield under LP) relative to direct selection under LP for LP grain yield (R_{id}/R_d) was estimated using the model 7

Model 7

$$R_{id}/R_d = r_G \frac{\sqrt{(h^2{}_{HP})}}{\sqrt{(h^2{}_{LP})}}$$

Where r_G is genetic correlation coefficient of grain yield between HP and LP, and h^2HP and h^2LP are the estimates of repeatability for grain yield under HP and LP conditions, respectively.

5.2.1 Genotyping and QTL detection

Genotype by Sequencing (GBS) was conducted with the restriction enzyme ApeKI following a protocol of Elshire *et al*., (2011), using a 384-plexing sequencing approach. After imputation the genotyping data were obtained for 969 BC1F4 out of 1083 BC1F4 progenies including donor parents were available for QTLs analysis. A total of 2000 to 3000 SNPs were obtained for bi-parental population mapping and 51,545 SNP for genome wide association mapping analysis (GWAS). Two different approaches were used to detect QTL for different parameters collected during this study and the BLUEs values were used to perform QTLs analysis. The bi-parental QTL analysis for each of the 13 populations was conducted by single interval mapping using R/QTL package(Broman & Sen, 2009), using the regression methods (Haley *et al*., 1992). The Association mapping (GWAS) using a mixed model marker trait association analysis was performed on all 13 populations using the NAM package under "R" (Xavier *et al*., 2017).

5.3 Results

5.3.1 Progenies performance for grain yield and related traits

The repeatability estimates for grain yield and yield related traits observed were high to very high, except for seedling vigor, under both LP and HP conditions (Table 5.2). The repeatability estimates were slightly higher in the HP relative to LP except for date to flag leaf appearance and seedling vigor. The mean grain yield level, over all progenies, under HP conditions was considerably higher 276.94 gm^{-2} than under LP conditions 166.15 gm^{-2} (Fig. 5.1). The variation among progenies over all populations for grain yield and agronomic traits was highly significant within each P-level (Table 5.2) as indicated by the large variation among progenies which was also observed within each population for grain yield (Fig. 5.1), flag leaf appearance (Fig. 5.2), plant height (Fig. 5.3) and seedling vigor (Fig 5.4). The boxplots showed considerable differences for grain yield variation among progenies within and between populations. For example, 60_Ridb had a higher population mean than the grand mean and recurrent parent under both P-levels, 51_Hafid having inferior population means, and 62_SC566-14 and Grinkan approximating the mean in HP but showing a superior mean under LP.

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Table 5.2. Variance components (σ²G), standard error, minimum, maximum, mean BLUEs and repeatability for grain yield and related traits of 1083 progenies evaluated under LP and HP soil field conditions at Samanko in 2013.

	HP		**LP**		**Minimum**		**Maximum**		**Mean**		**Repeatability**	
Traits	**σ²G**	**s.e**	**σ²G**	**s.e**	**LP**	**HP**	**LP**	**HP**	**LP**	**HP**	**LP**	**HP**
GV	0.17***	0.03	0.07***	0.02	2	2	8	9	5.17	5.92	0.03	0.00
DTFL	56.51***	2.56	50.94***	2.28	49.5	46.5	129	133.25	82.03	80.14	0.95	0.94
PH	1801.4***	87.70	1185.40***	65.60	102.50	115	420	485	256.34	292.40	0.79	0.88
PANL	11.44***	0.63	9.76***	0.57	16	13.5	45.50	50	30.00	29.26	0.76	0.79
GYLD	4918***	313.00	1801***	135.00	20	40	509	703	166.15	276.94	0.60	0.69
HGW	0.09***	0.00	0.09***	0.00	0.49	0.60	3.19	3.202	2.15	2.31	0.90	0.91

HP= high P, LP= low P, Growth vigor score (GV), Date to flag leaf appearance (DTFL), Plant height in cm (PH), Panicle length in cm (PANL), Grain yield in g m^2(GYLD), Hundred grain weight in g (HGW), G=genotype, s.e=standard error *=significance at (P<0.05), **= (P<0.01), *** (P<0.001)

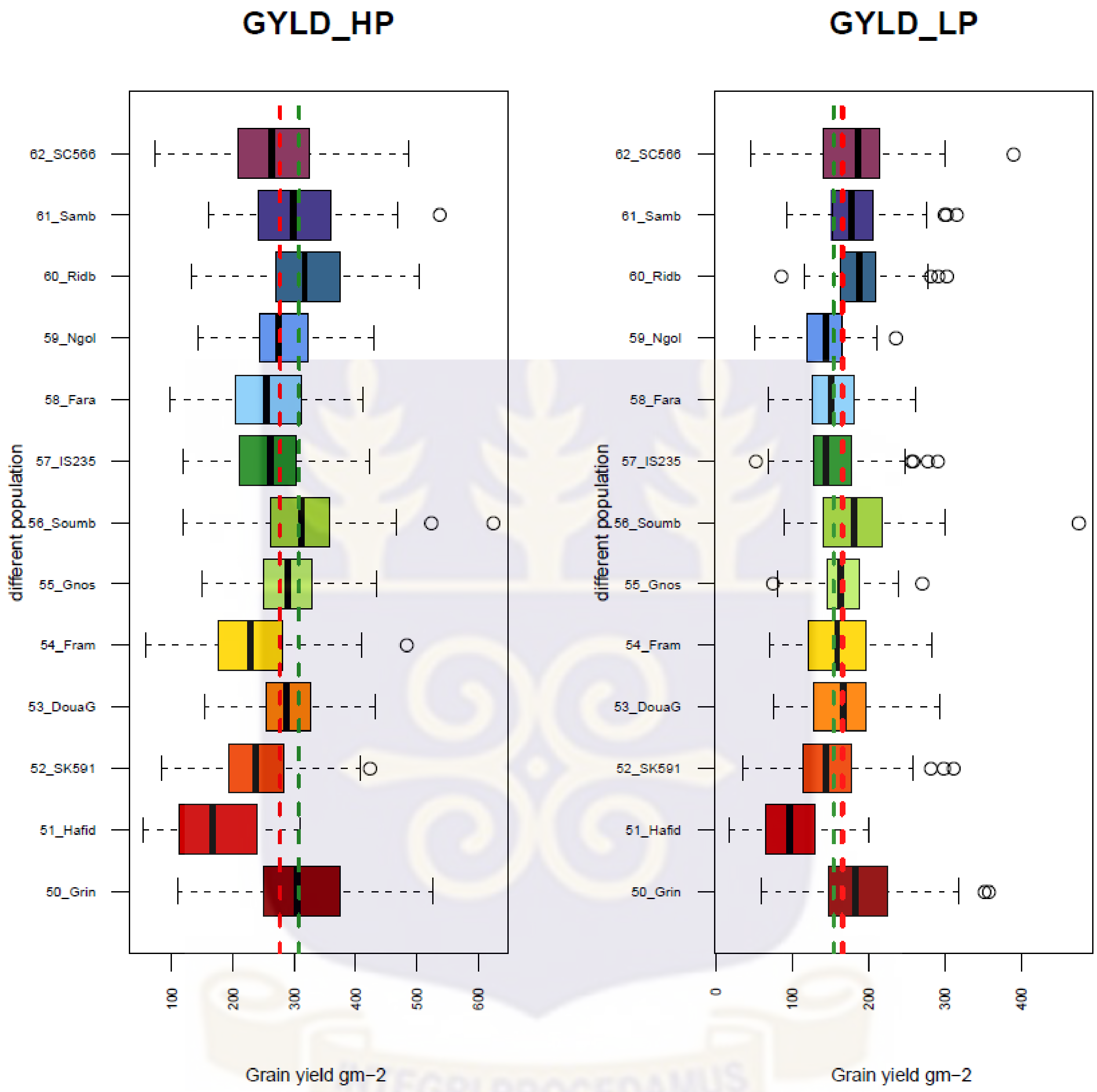

Figure 5.1: Mean values (BLUEs) box-plots of 13 BC1F4 populations for grain yield high P (GYLD_HP) and low P (GYLD_LP) field conditions in 2013, with the grand mean (red dashed line) and mean of recurrent parent Lata3 (green dashed line).

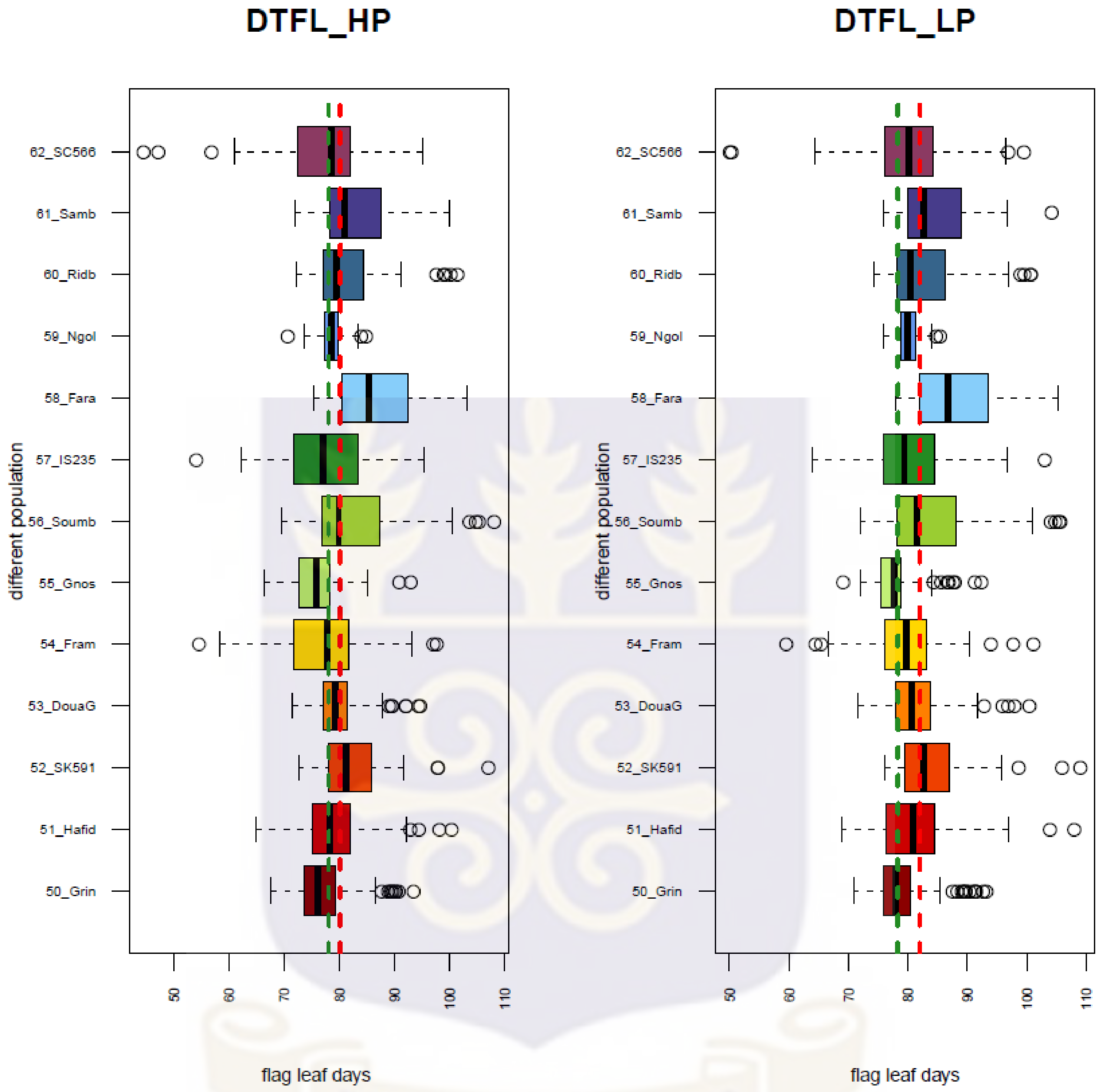

Figure 5.2: Mean values (BLUEs) box-plots of 13 BC1F4 populations of date to flag leaf appearance high P (DTFL_HP) and low P (DTFL_LP) field condition, with the grand mean (red dashed line) and mean of recurrent parent Lata3 (green dashed line).

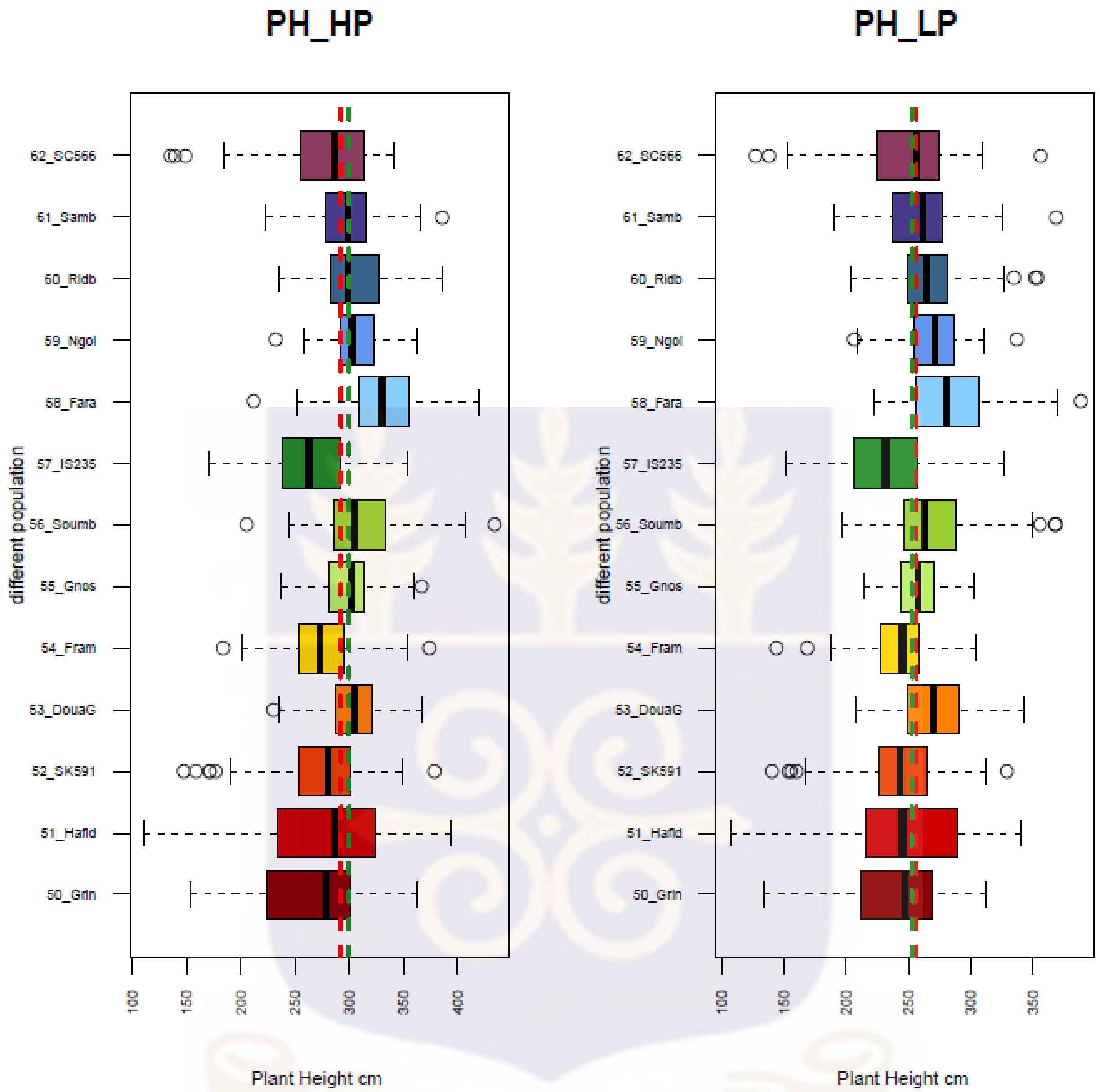

Figure 5.3 Mean values (BLUEs) box-plots of 13 BC1F4 populations of plant height under high P (PH_HP) and low P (PH_LP) field condition, with the grand mean (red dashed line) and mean of recurrent parent Lata3 (green dashed line).

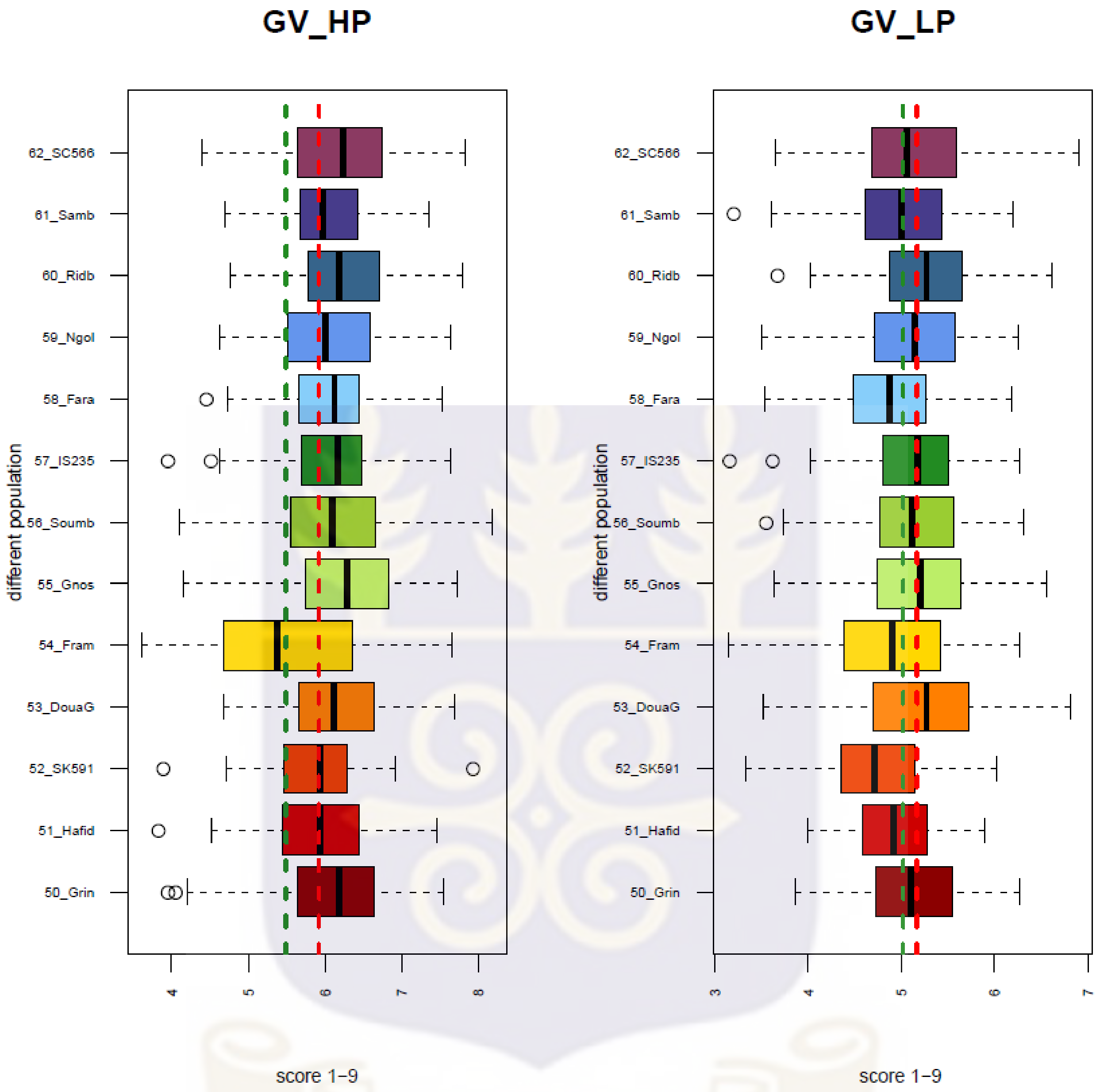

Figure 5.4: Mean values (BLUEs) box-plots of 13 BC1F4 populations of plant growth vigor high P (GV_HP) at left and low P (GV_LP) at right LP field condition, with the grand mean (red dashed line) and mean of recurrent parent Lata3 (green dashed line).

The grain yield and related traits, analyzed over both P-levels in combined analyses, showed highly significant genetic variation (Table 5.3) and high broad sense heritability estimates for all traits except seedling vigor. The genotype x P-level interaction variance components, although significant, were smaller than the genotypic variance components. Weak to high correlations were found across P-levels for different traits examined, while moderate correlation between P levels were detected for grain yield, and high correlations were detected for the remaining traits. A significant but weak correlation was found between PH and GYLD, PH and DTFL, GV and GYLD, and HGW and GYLD across P-levels, but they were moderately correlated under individual P-levels (Table 5.4). However the correlations of progeny (BLUEs) for GYLD between low- and high-P levels within individual families revealed intermediate correlations and considerable differences between families for degree of correlation (Fig. 5.5). In contrast these correlations for W100G were nearly identical across populations (Fig. 5.5)

Table 5.3: Variance components (σ^2) and standard errors (SE), and broad sense heritability estimates (h^2) of grain yield and related trait of 1083 BC1F4 progenies evaluated over P level at Samanko in 2013.

	Combined				
traits	**σ^2G**	**s.e**	**σ^2G x P**	**s.e**	**h^2**
GV	0.10***	0.01	0.02 ns	0.02	0.35
DTFL	52.63***	2.34	1.18***	0.19	0.96
PH	1463.2***	69.60	39***	15.60	0.91
PANL	10.12***	0.51	0.48***	0.18	0.86
GYLD	2460***	171.00	932***	118.00	0.67
HGW	0.08***	0.00	0.01***	0.00	0.91

Growth vigor score (GV), Days to flag leaf appearance (DTFL), Plant height in cm (PH), Panicle length in cm (PANL), Grain yield in g m^2(GYLD), Hundred grain weight in g (HGW), G=genotype, s.e=standard error, G x P=genotype by phosphorus levels *=significance at (P<0.05), **= (P<0.01), *** (P<0.001)

Table 5.4**:** Genotypic correlation coefficient and P value among grain yield components traits across P levels for 1083 progenies evaluated in Samanko in 2013.

	Correlation (r)											
Traits	**DTFL_HP**	**DTFL_LP**	**GV_HP**	**GV_LP**	**GYLD_HP**	**GYLD_LP**	**PANL_HP**	**PANL_LP**	**PH_HP**	**PH_LP**	**HGW_HP**	**HGW_LP**
DTFL_HP												
DTFL_LP	0.91***											
GV_HP	-0.16***	-0.09**										
GV_LP	-0.11***	-0.25***	0.20***									
GYLD_HP	-0.23***	-0.25***	0.44***	0.24***								
GYLD_LP	-0.03 ns	-0.13***	0.12***	0.37***	0.54***							
PANL_HP	0.05 ns	0.03 ns	0.09**	0.06 ns	0.23***	0.06 ns						
PANL_LP	0.19***	0.14***	0.04 ns	0.05 ns	0.12***	0.10**	0.73***					
PH_HP	0.29***	0.28***	0.34***	0.12***	0.31***	0.15***	0.44***	0.37***				
PH_LP	0.27***	0.19***	0.19***	0.32***	0.22***	0.27***	0.36***	0.43***	0.82***			
HGW_HP	-0.13***	-0.14***	0.21***	0.12	0.30***	0.25***	0.06 ns	-0.03 ns	0.13***	0.11***		
HGW_LP	-0.05 ns	-0.07*	0.14***	0.15	0.20***	0.37***	0.07*	0.01 ns	0.18***	0.21***	0.83***	

HP= high P, LP= low P, Growth vigor score (GV), Days to flag leaf appearance (DTFL), Plant height in cm (PH), Panicle length in cm (PANL), Grain yield in g m^2(GYLD), Hundred grain weight in g (HGW),*=significance at (P<0.05), **= (P<0.01), *** (P<0.001), ns=no significant.

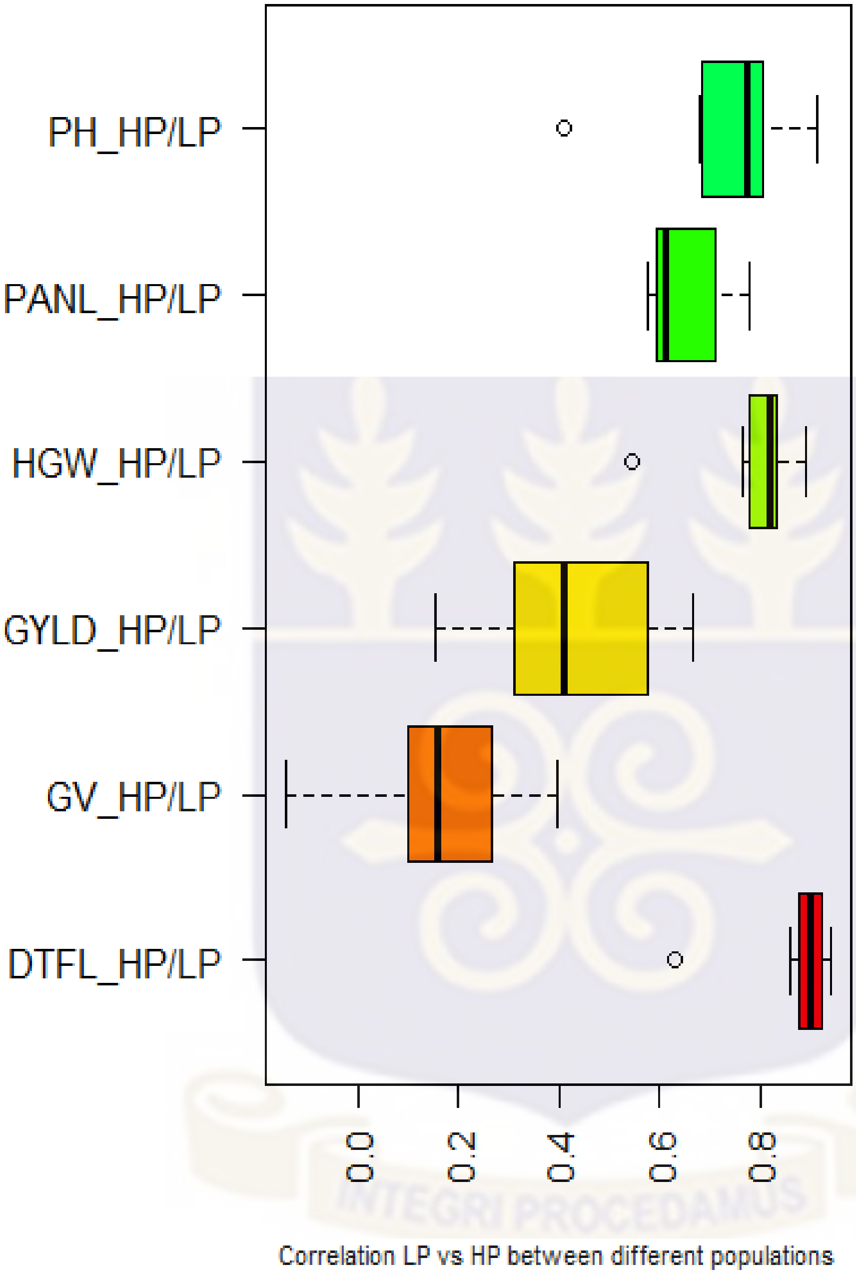

Figure 5.5: Box-plots of the distribution of genotypic correlations between high and low phosphorus field conditions conducted within individual families for grain yield and five related traits, evaluated at Samanko in 2013.

5.3.2 Predicted responses to direct and indirect selection for grain yield under P-limited conditions.

The genetic correlation for grain yield over the two contrasting P-levels (r_G) of 0.81 was somewhat elevated, reflecting the genotypic variance component being relatively larger than that of genotype by P-level interaction. However this genetic correlation was considerably less than 1.00, and when used in Model 2, estimated the relative effectiveness of indirect selection (R_{id}/R_d) for grain yield (selection for grain yield under HP and expected response in LP) to be only (87%) relative to direct selection under LP. Out of the 25 genotypes with the highest grain yields under HP only 8 of them would have been selected under LP condition. (Table 5.5)

Table 5.5. The rank, mean grain yield and corresponding standard error, under LP of the 25 progenies with highest yields under HP in 2013 Samanko.

		HP			LP		
ENTRY	**Pedegree**	**Rank**	**GYLD**	**SE**	**Rank**	**GYLD**	**SE**
185	Lata//IS15401-1-6-1-1	1	625	45.49	1	475	45.49
964	Lata//Samb-5-2-1-1	2	540	45.62	60	255	45.62
215	Lata//IS15401-6-14-1-1	3	532	45.61	9	301	45.61
161	Lata//Grin-8-2-1-1	4	527	45.44	5	319	33.61
872	Lata//Ridb-4-18-1-1	5	503	45.45	229	203	45.45
93	Lata//Grin-1-25-1-1	6	492	45.60	88	239	33.55
28	Lata//Fram-2-1-1-1	7	483	45.47	66	251	33.59
169	Lata//Grin-8-35-1-1	8	483	45.54	7	316	45.54
504	Lata//SC566-7-21-1-1	9	482	45.73	84	242	45.73
91	Lata//Grin-1-22-1-1	10	478	45.57	3	359	33.81
833	Lata//Ridb-1-21-1-1	11	476	45.57	683	142	45.57
227	Lata//IS15401-6-26-1-1	12	470	45.51	307	191	45.51
950	Lata//Samb-4-3-1-1	13	469	45.57	118	227	45.57
133	Lata//Grin-8-21-1-1	14	467	45.52	46	265	33.59
260	Lata//IS15401-7-2-1-1	15	464	45.73	76	245	45.73
240	Lata//IS15401-6-39-1-1	16	464	45.46	40	271	45.46
139	Lata//Grin-8-27-1-1	17	464	45.49	121	226	33.63
985	Lata//Samb-6-15-1-1	18	458	45.56	181	212	45.56
230	Lata//IS15401-6-29-1-1	19	457	45.58	357	184	45.58
216	Lata//IS15401-6-15-1-1	20	456	45.47	56	255	45.47
987	Lata//Samb-6-18-1-1	21	454	45.68	386	180	45.68
204	Lata//IS15401-6-1-1-1	22	454	45.63	22	289	45.63
172	Lata//Grin-8-39-1-1	23	453	45.52	4	357	45.52
887	Lata//Ridb-8-9-1-1	24	453	45.43	11	300	45.43
852	Lata//Ridb-3-9-1-1	25	451	45.44	111	229	45.44
485	Lata//SC566-6-44-1-1	30	443	45.49	2	389	45.49
196	Lata//IS15401-1-19-1-1	42	429	45.59	18	293	45.59
886	Lata//Ridb-8-8-1-1	48	421	45.47	20	292	45.47
999	Lata//Samb-7-4-1-1	51	418	45.62	6	317	45.62
338	Lata//IS23540-9-31-1-1	62	410	45.48	19	292	45.48
920	Lata//Samb-2-13-1-1	65	407	45.50	10	301	45.50
95	Lata//Grin-1-30-1-1	71	404	45.48	21	291	33.62
125	Lata//Grin-8-11-1-1	89	392	45.47	16	296	33.62
512	Lata//SK5912-3-4-1-1	247	336	45.61	14	298	45.61
488	Lata//SC566-6-56-1-1	278	328	45.59	13	299	45.59
174	Lata//Grin-8-46-1-1	302	319	45.49	25	282	45.49
936	Lata//Samb-3-5-1-1	323	315	45.52	12	300	45.52
513	Lata//SK5912-3-5-1-1	337	312	45.55	8	312	45.55
692	Lata//DouaG-5-18-1-1	377	303	45.52	17	294	45.52
233	Lata//IS15401-6-32-1-1	381	302	45.59	15	297	45.59
92	Lata//Grin-1-23-1-1	388	301	45.50	23	284	33.57
78	Lata//Fram-7-10-1-1	830	213	45.46	24	283	33.70

5.3.3 Genotype variation of 298 selected BC1F5 progenies of sorghum in contrasting P-levels (2013-2015)

Significant genetic variation for grain yield was observed in combined analyses over three years under both the LP and the HP conditions (Table 5.6). The estimates of broad sense heritability for grain yield were moderately elevated under both LP and HP conditions, and were nearly identical. The genetic variance for grain yield, from the combined analysis over both P levels, was also highly significant. Although Genotype x P-Levels interaction (GxP) was highly significant it was smaller than genotype variance in contrast to GxY which was greater. The broad sense heritability estimate was actually higher than for the individual P-level analyses. The top 25 progenies with highest grain yield exhibited large change of ranking over two years 2014, 2015 (Appendices 2 and 3)

Table 5.6**:** Variance components estimates and their standard errors (s.e), and broad sense heritability for grain yield of 300 entries under HP and LP field conditions tested over three years at Samanko, separate P-levels and across P-levels.

Env	Sources of variation	σ^2G	s.e.	h^2
HP	G	6319***	1126	0.59
	GxY	11570***	1218	
LP	G	1866***	431	0.57
	GxY	3726***	478	
Combined	G	3997***	661	0.79
	GxP	1202***	370	
	GxY	4515***	608	
	GxPxY	2223***	481	

HP= high P, LP= low P, G=genotype, GxY=genotype by year interaction, GxP= genotype by P-levels, GxPxY=genotype by Plevels by years and *=significant at (P<0.05), **= (P<0.01), *** (P<0.001)

5.3.4 Detection of QTLs for the selected traits using the BC1F4 populations

Two QTLs for grain yield were mapped under LP and 8 QTLs were mapped under HP in individual populations mapping (Table 5.7). Only a single QTL was found on a common, overlapping, region across P-levels on LGs 2 in two different populations, with one being identified in LP and the other in HP (Table 5.7). while 2 QTL were mapped with 2 different populations, the first one on LP and the second under HP. QTLs accounted for 19 to 42% of PVE except for the series of QTLs detected in population 60_Ribda (3 to 6% of PVE). Although the recurrent parent contributed the QTL for increased grain yield in most cases under HP, the QTL for increased yield under LP on LG 9 was from two different donors, and was specific to LP. The GWAS analysis over all populations revealed one single significant association under LP on LG 9 (Fig.5.6) which explained 3 to 6% of PVE. This chromosome region corresponds to the QTL identified in two populations with bi-parental mapping. Nevertheless three important peak where found in GWAS on LGs (3, 6 and 7) below Bonferroni threshold under HP, these peaks correspond to genome regions mapped in bi-parental QTL mapping.

Table 5.7: QTLs identified for grain yield from 13 BC-NAM populations in individual low or high P-level environments, evaluated at Samanko in 2013.

Env	Pop	Locus	LG	pos	ci.low	ci.high	lod	R2	a	d	a(P)
HP	60_Ribda	S1_22008698	1	68.02	66.57	73.80	6.19	6.32	-28.02	-63.19	R
HP	60_Ribda	S2_51297299	2	77.18	43.65	107.65	4.71	5.73	-38.12	8.80	R
LP	55_Gnoss	S2_62912132	2	126.91	78.91	129.96	4.28	25.68	-17.74	39.93	R
HP	52_SK591	S3_22846252	3	67.51	59.49	74.43	4.27	23.92	-37.83	-27.91	R
HP	57_IS235	S3_51657553	3	78.70	52.60	83.52	4.35	19.29	-33.29	2.13	R
HP	61_Samba	S3_71440408	3	151.66	147.49	156.93	4.80	22.66	21.28	70.00	**D**
HP	60_Ribda	S4_12679571	4	56.45	42.47	67.90	5.43	2.87	-16.67	-18.81	R
HP	51_Hafid	S4_62221466	4	115.90	109.44	140.31	5.83	41.56	-61.01	-11.54	R
HP	60_Ribda	S6_1287130	6	4.66	2.00	8.50	5.25	3.10	-12.57	-37.94	R
HP	57_IS235	S6_52354859	6	80.80	78.64	85.31	4.25	18.80	-35.59	24.46	R
LP	50_Grinka	S9_1071308	9	5.60	0.79	14.02	5.17	22.60	34.34	21.49	**D**
LP	58_Fara	S9_1611470	9	8.94	0.40	9.72	4.55	25.88	23.19	-25.08	**D**

HP= high P, LP= low P, LG=linkage group number, pos=position, ci=confidence, interval, lod=lod score, R^2=% of phenotypic variation explain by individual QTL, a=additivity d=dominance= a(P)=additivity effect positive parent, R= recurrent parent, D=donors parents.

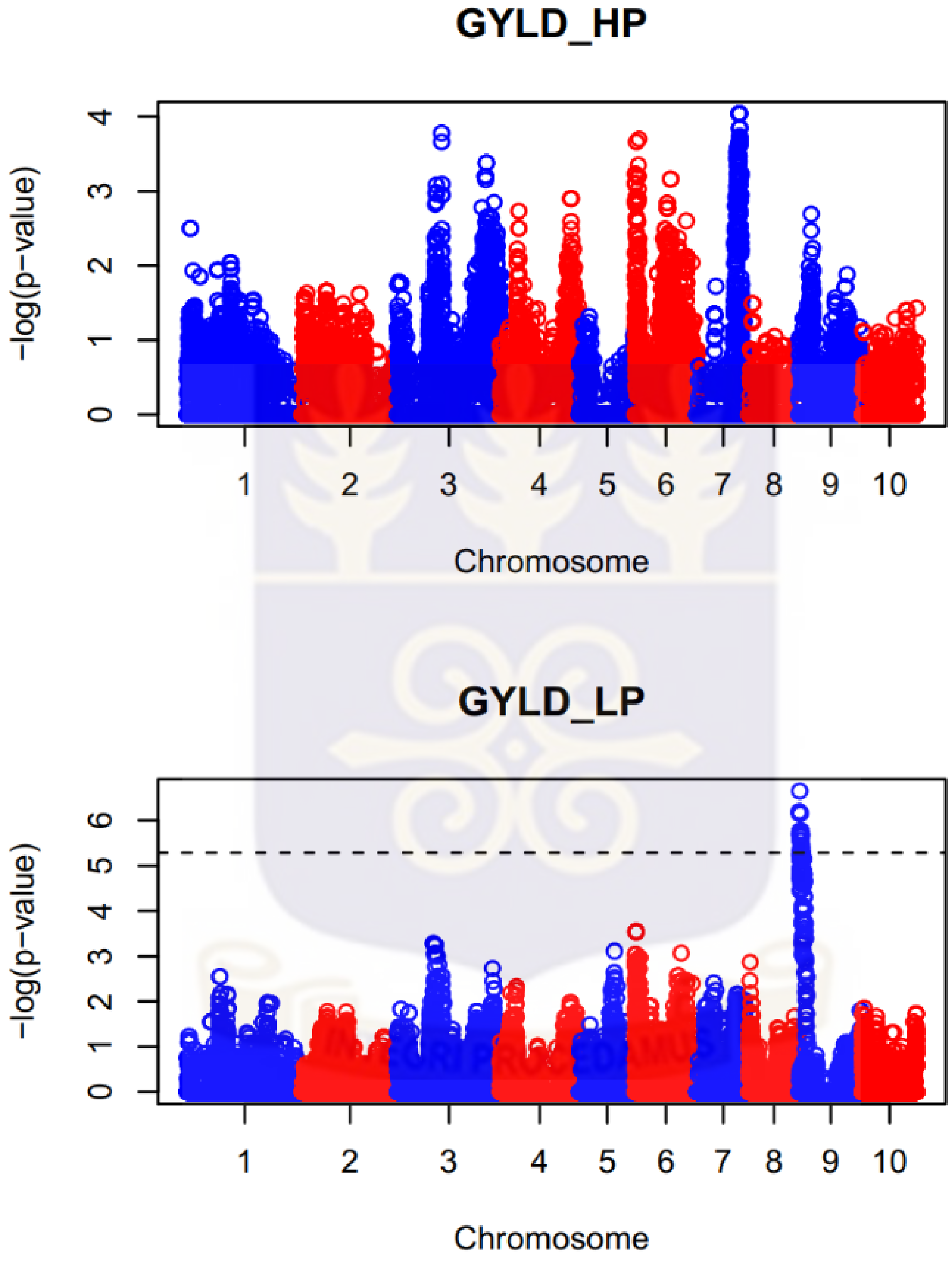

Figure 5.6**:** Manhattan plot of grain yield under low P at bottom and high P at top, Samanko 2013.

Ten QTLs were detected for hundred grains weight over both environments with both methods of QTL analysis on LGs 1, 2, 3, 4 (2QTLs), 5, 6, 7, 8, and 10 (Table 5.8). Seven of these were detected under HP whereas all ten were mapped under LP. Seven QTLs were detected across P-levels. Each QTL was mapped with same the population across P-levels using the single population mapping except for a QTL detected on LG 1 in five different populations (Table 5.8). Only this position was confirmed across P-levels by association mapping and the QTL on LG 4 under HP (Fig.5.7). No other significant peaks were found under Bonferroni threshold on LG 2, 4, 7, and 10 by GWAS analysis. The QTLs detected over P-levels in the single QTL mapping explained about 1 to 41% of PVE, but QTLs detected with GWAS analysis accounted for 3 to 7% of PVE (Fig.5.7). The recurrent parent contributed to increase of HGW with all QTLs, except QTLs detected in the populations 57_IS235 and 60_Ribda. In addition, the QTLs detected under HP explain more phenotypic variation than those under LP (Table 5.8).

Table 5.8: QTLs identified for hundred grains weight from 13 BC1F5 populations in individual low or high P-level environments.

Env	**Pop**	**Locus**	**LG**	**pos**	**ci.low**	**ci.high**	**lod**	**R2**	**a**	**d**	**a(P)**
HP	58_Fara	S1_1336417	1	4.27	0.27	125.58	4.91	24.28	-0.24	0.08	R
LP	56_Soumb	S1_52309945	1	98.62	2.5	102.79	4.38	0.66	-0.04	0.03	R
HP	62_Sc566	S1_653002	1	2.01	0.01	14.01	4.33	25.1	-0.15	-0.01	R
LP	57_IS235	S1_7045029	1	19.35	9.35	29.13	4.26	10.38	0.11	0.19	**D**
HP	59_N'golo	S1_784695	1	2.35	1.05	7.29	5.09	27.8	-0.1	-0.04	R
HP	61_Samba	S1_911521	1	2.67	0.23	154.23	4.57	21.7	-0.14	-0.01	R
HP	51_Hafid	S2_3066264	2	16.05	0.62	29.95	4.74	9.08	-0.07	-0.2	R
LP	51_Hafid	S2_4983425	2	27.15	13.52	31.75	4.61	12.63	-0.06	-0.32	R
HP	54_Fram	S3_52163921	3	79.84	63.45	83.49	4.29	28.45	-0.21	0.02	R
HP	51_Hafid	S3_54789254	3	87.4	84.81	89.2	6.62	6.9	-0.03	-0.23	R
LP	51_Hafid	S3_54789254	3	87.4	83.74	89.2	5.97	3.7	-0.01	-0.19	R
HP	57_IS235	S4_50355442	4	68.47	47.29	80.84	7.57	41.02	0.22	-0.09	**D**
LP	57_IS235	S4_52058452	4	79.36	35.86	80.84	5.18	15.3	0.16	0.04	**D**
HP	51_Hafid	S4_62195297	4	115.76	96.22	119.42	7.01	17.14	-0.2	0.06	R
LP	51_Hafid	S4_62195297	4	115.76	96.22	121.8	6.5	20.27	-0.2	-0.04	R
LP	57_IS235	S5_15796412	5	60.4	49.89	73.52	4.09	10.07	0.13	-0.13	**D**
LP	60_Ribda	S5_53652119	5	85.73	55.17	94.09	4.6	25.78	0.1	0.33	**D**
LP	56_Soumb	S6_1930687	6	6.99	2	9.32	7.91	13.79	-0.16	0.19	R
HP	56_Soumb	S6_553041	6	2.26	0.28	9.32	6.03	17.87	-0.18	0.18	R
LP	56_Soumb	S7_35895690	7	59.7	54.81	77.17	4.5	2.95	-0.08	-0.1	R
LP	52_SK591	S7_59609567	7	103.26	102.48	110.96	4.16	22.79	-0.27	0.38	R
LP	56_Soumb	S8_42667223	8	63.53	46.33	80.57	4.2	7.62	-0.11	0	R
LP	56_Soumb	S10_2450758	10	17.4	11.94	80.09	4.43	4.63	-0.09	-0.02	R
HP	56_Soumb	S10_2919039	10	19.4	13.4	49.4	4.44	11.59	-0.13	-0.03	R

HP= high P, LP= low P, LG=linkage group number, pos=position, ci=confidence, interval, lod=lod score, R^2=% of phenotypic variation explain by individual QTL, a=additivity d=dominance= a(P)=additivity effect positive parent, R= recurrent parent, D=donors parents.

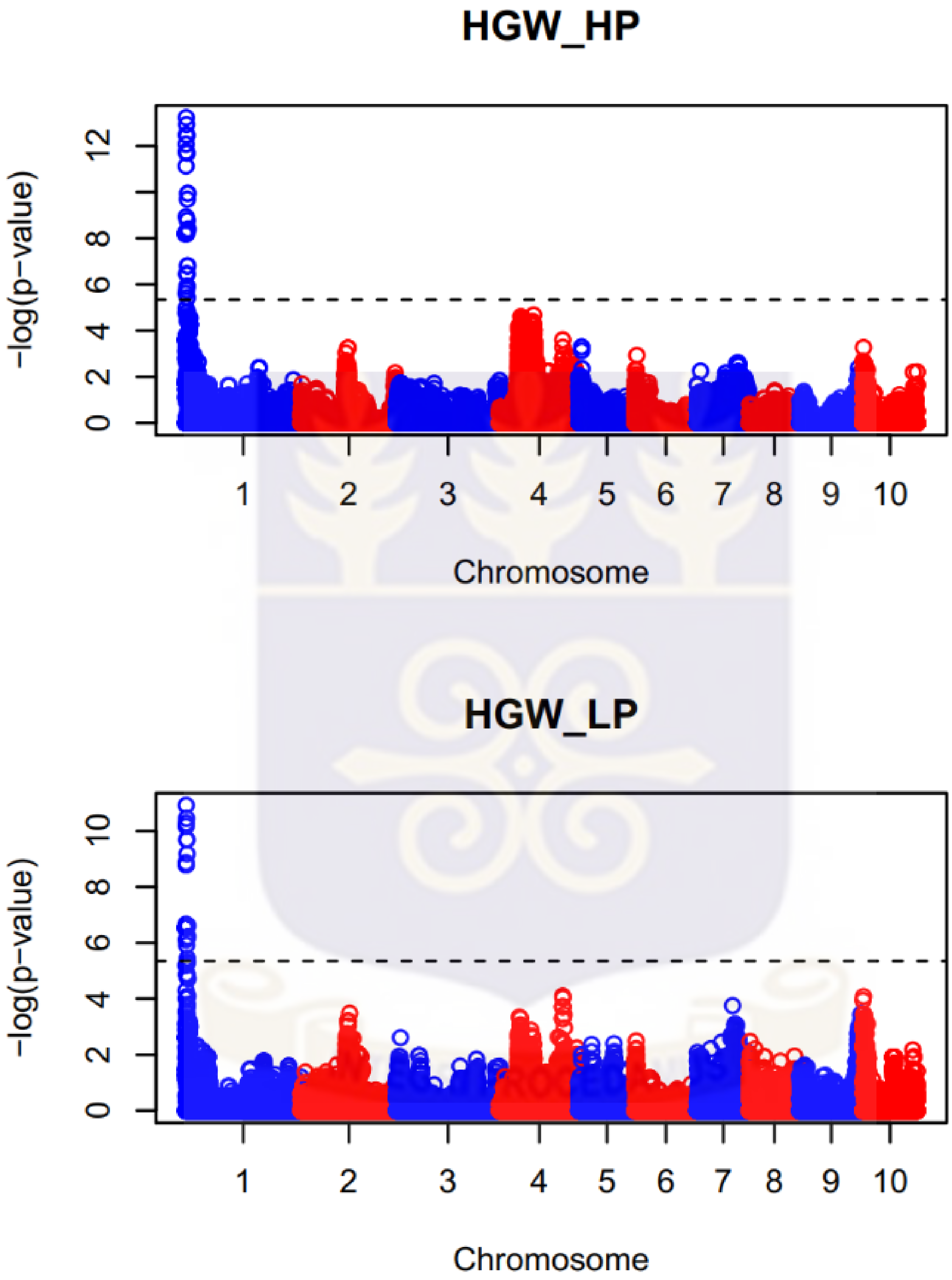

Figure 5.7**.** Manhattan plot of hundred grain weight across P-levels evaluated at Samanko in 2013, high P in top side and low P in bottom side.

No significant QTLs were found with association analysis for panicle length (Table 5.9), while the single population mapping exhibited six significant QTLs on different genomic regions over P-levels on LGs 2, 4, 6(2QTLs), 9 and 10.Among them, two QTLs were specific to HP on LG 6 and 9 and four to LP on LG 2, 4, 6, 10. They accounted for 15 to 32% of PVE and the interval of confidence ranged from 35 to 54 cM under HP and 11 to 28 cM under LP. The recurrent parent contributed to increase the panicle length (Table 5.9).

Table 5.9**:** QTLs identified for panicle length with 13 BC-NAM populations across individual P. levels in Samanko, 2013.

Env	**Pop**	**Locus**	**LG**	**pos**	**ci.low**	**ci.high**	**lod**	**R2**	**a**	**d**	**a(P)**
LP	55_Gnoss	S2_26491692	2	74.21	65.63	77.72	4.64	16.95	-1.46	-1.47	R
LP	50_Grinka	S4_51055585	4	72.99	69.80	80.82	4.79	21.10	-1.95	1.25	R
HP	54_Fram	S6_51609664	6	76.41	25.87	80.23	4.24	27.93	-1.85	1.79	R
LP	55_Gnoss	S6_60456008	6	112.72	88.56	117.14	4.25	14.96	-1.13	-0.43	R
HP	50_Grinka	S9_50001039	9	76.54	47.25	80.79	4.61	20.42	-1.81	2.45	R
LP	54_Fram	S10_6311777	10	36.28	14.39	41.23	4.93	31.92	-2.02	0.66	R

HP= high P, LP= low P, LG=linkage group number, pos=position, ci=confidence, interval, lod=lod score, R^2=% of phenotypic variation explain by individual QTL, a=additivity d=dominance= a(P)=additivity effect positive parent, R= recurrent parent, D=donors parents.

Eight QTLs were detected for date to flag leaf appearance over P-levels with both methods; single population mapping and association mapping (Table 5.10), of which six on LGs 3, 6(2QTLs), 7, 8, and 10 were detected across P-levels and accounted for approximatively 4 to 62% PVE for single population mapping, but most of the detected QTLs accounted for more than 20% of PVE. The specific QTLs were mapped on LGs 2 and 9 under HP in bi-parental population mapping, but in GWAS analysis an important peak was found under LP on LG 9 close to

Bonferroni threshold (Table 5.10 and Fig. 5.8). In addition, QTL on LGs 3 and 6 were detected by bi-parental QTL mapping, with 7 and 5 different populations respectively, and they explained about 21 to 62% of PVE. In association mapping analysis, three QTLs were detected across P-levels accounting for 3 to 49% of PVE, however, three peaks were also found near the Bonferroni threshold across P-levels on LG 1 and only under LP on LG 9. Of the 8 detected QTLs, three were detected consistently on overlapping regions of chromosomes 3 and 6(2QTLs), across P-levels, with both methods. In general, QTL detected under LP explained more PVE than QTL identified under HP (Table 5.10) and also, in most of the identified QTLs, the donor parents contributed to augment DTFL from 1 to 10 days.

Table 5.10**:** QTL identified for date to flag leaf appearance with 13 BC-NAM populations across individual P-levels in Samanko.

Env	**Pop**	**Locus**	**LG**	**pos**	**ci.low**	**ci.high**	**lod**	**R2**	**a**	**d**	**a(P)**
HP	50_Grinka	S2_802671	2	3.72	3.72	8.47	5.28	4.07	1.69	-1.55	**D**
HP	61_Samba	S3_19135249	3	64.24	62.17	83.21	5.69	26.26	4.41	-0.60	**D**
LP	61_Samba	S3_19679240	3	65.37	63.48	66.24	7.51	33.10	4.58	0.00	**D**
HP	60_Ribda	S3_49126119	3	73.02	62.87	79.18	8.71	34.95	4.92	-4.11	**D**
HP	50_Grinka	S3_50713032	3	75.21	72.91	78.88	17.63	38.65	4.73	-3.81	**D**
LP	50_Grinka	S3_50713032	3	75.21	72.91	76.58	21.33	61.99	5.20	-3.36	**D**
LP	60_Ribda	S3_50909626	3	77.02	62.72	79.18	7.21	27.29	4.50	-3.55	**D**
HP	56_Soumb	S3_51359428	3	78.03	77.53	79.32	13.90	33.26	5.96	-4.56	**D**
LP	56_Soumb	S3_51359436	3	78.03	77.53	79.32	17.05	44.94	6.45	-2.98	**D**
HP	57_IS235	S3_51464719	3	78.43	62.20	82.43	4.43	24.23	4.44	-4.13	**D**
LP	57_IS235	S3_51464719	3	78.43	58.27	82.43	5.60	31.31	4.32	-3.76	**D**
HP	52_SK591	S3_53222969	3	82.71	63.04	88.43	5.43	28.67	4.47	-2.71	**D**
LP	52_SK591	S3_53222969	3	82.71	70.89	84.13	7.99	39.20	5.80	-3.06	**D**
LP	58_Fara	S3_53320211	3	82.43	66.50	83.51	6.87	32.50	5.72	-1.25	**D**
HP	58_Fara	S3_53320212	3	83.00	63.92	126.60	4.30	21.94	4.78	-2.04	**D**
HP	60_Ribda	S6_1048796	6	4.36	0.36	10.36	6.24	23.59	2.96	-0.97	**D**
LP	60_Ribda	S6_1048796	6	4.36	2.36	5.32	9.52	31.05	3.28	-2.84	**D**
HP	56_Soumb	S6_1841025	6	6.67	0.26	6.81	10.61	24.14	5.12	-1.72	**D**
HP	55_Gnoss	S6_42525547	6	44.44	26.56	51.63	4.76	21.24	-3.15	5.98	R
LP	54_Fram	S6_540911	6	1.96	0.41	3.41	8.33	47.81	-6.27	1.10	R
LP	62_SC566	S6_553116	6	2.36	0.93	3.76	9.02	45.19	-9.29	4.12	R
LP	56_Soumb	S6_72117	6	0.26	0.26	6.81	9.31	24.42	5.17	-1.89	**D**
HP	62_SC566	S6_781198	6	2.83	0.93	3.76	11.09	52.30	-10.1	2.65	R
HP	57_IS235	S6_838475	6	3.04	1.96	3.51	8.37	42.06	-6.24	-4.41	R
LP	57_IS235	S6_838481	6	3.04	1.96	3.51	5.16	29.22	-4.98	-0.29	R
HP	54_Fram	S6_838601	6	3.04	0.41	3.41	10.13	54.64	-7.49	-3.30	R
HP	51_Hafid	S7_62462436	7	117.17	96.85	124.49	4.43	11.72	3.66	-6.88	**D**
LP	51_Hafid	S7_63984150	7	123.78	106.21	124.49	4.31	17.79	4.94	-6.25	**D**
HP	55_Gnoss	S8_44145522	8	81.85	69.85	89.85	4.27	3.84	0.63	3.51	**D**
LP	51_Hafid	S8_49495163	8	89.23	85.04	110.15	4.30	17.73	-0.29	7.11	R

Env	Pop	Locus	LG	pos	ci.low	ci.high	lod	R2	a	d	a(P)
HP	51_Hafid	S9_54970567	9	105.20	103.80	115.29	4.79	13.89	3.74	-0.56	**D**
HP	55_Gnoss	S10_7567994	10	47.38	25.48	61.38	4.32	7.99	-0.64	4.34	R
LP	55_Gnoss	S10_7567994	10	47.38	25.48	69.38	4.46	14.58	-0.30	5.44	R

HP= high P, LP= low P, LG=linkage group number, pos=position, ci=confidence, interval, lod=lod score, R^2=% of phenotypic variation explain by individual QTL, a=additivity d=dominance= a(P)=additivity effect positive parent, R= recurrent parent, D=donors parents.

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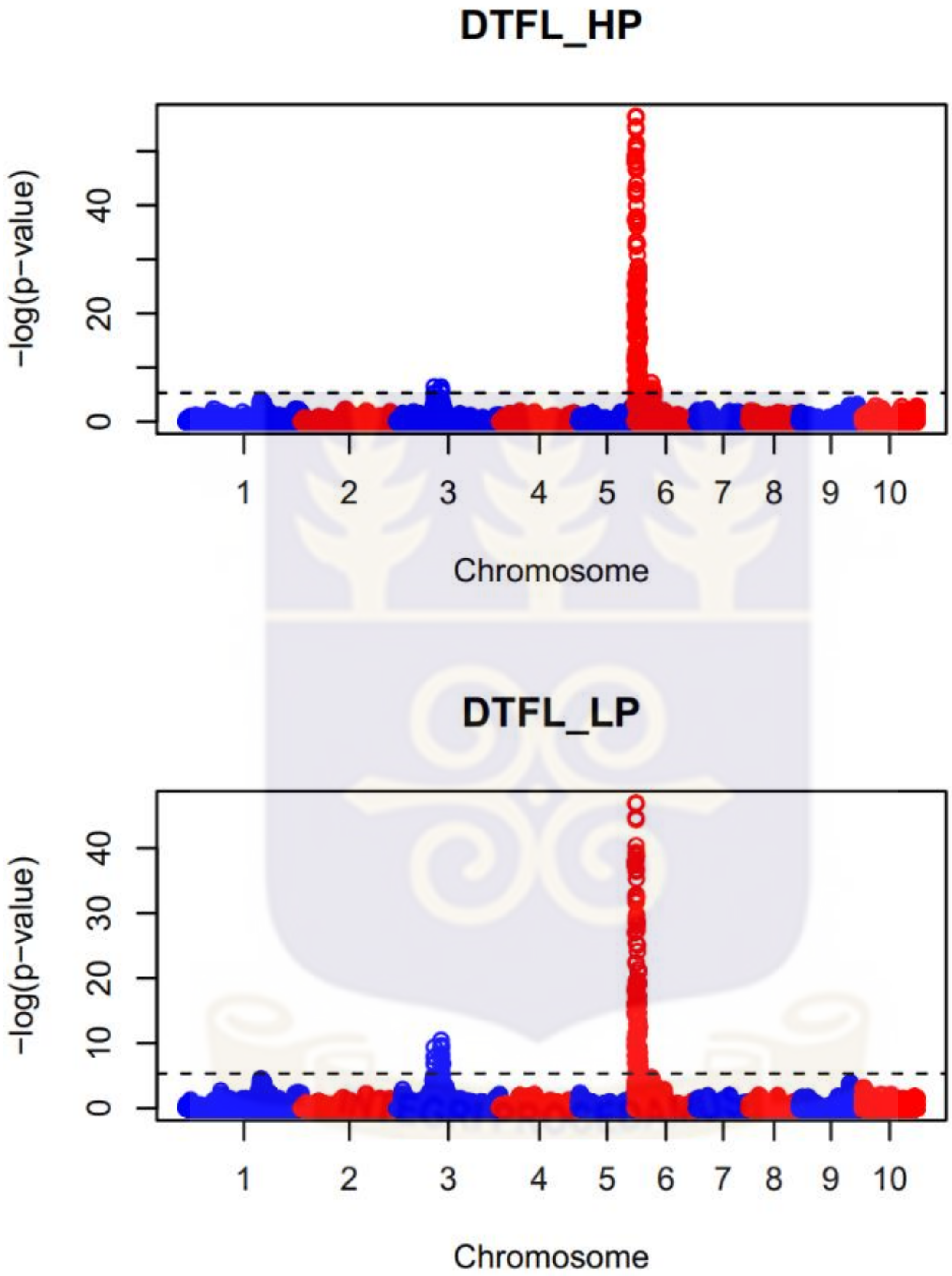


Figure 5.8: Manhattan plots displaying genome wide association results for date to flag leaf appearance, evaluated at Samanko in 2013 under low P (DTFL_LP) and under high P (DTFL _HP).

The QTL analysis showed a total of 6 QTL across both treatments for plant height on chromosomes 1, 3, 4, 7(2QTL) and 9 (Table 5.11). Among them a pair of 4 QTL were found consistent across P-levels (Table 5.11 and Fig. 5.9), each identified with a single population in bi-parental population mapping. They accounted for 21 to 67% of PVE with a confidence interval (ci) that varied from 5 to 29 cM for single population mapping and 4 to 21% in association analysis. The Specific QTL were detected with bi-parental population mapping on LG 1 and 9 under LP and HP respectively, but in GWAS analysis no significant peak was found close to Bonferroni threshold on LG 1 under HP. Additionally the QTL in HP explained more PVE than LP. Two major QTL for plant height were identified consistently with both methods across P-levels on chromosomes 4 and 7 (Table 5.11 and Fig. 5.9).

Table 5.11: QTLs identified for plant height evaluated in Samanko in 2013 with 13 BC-NAM populations across individual P. level.

Env	**Pop**	**Locus**	**LG**	**pos**	**ci.low**	**ci.high**	**lod**	**R2**	**a**	**d**	**a(P)**
LP	**62_SC566**	S1_15280829	1	48.30	20.33	49.76	4.55	26.18	-28.10	9.74	R
HP	53_Douad	S3_71738571	3	153.16	148.75	158.61	5.41	36.95	20.74	4.67	**D**
LP	53_Douad	S3_71738571	3	153.16	142.24	163.50	5.22	35.90	21.82	1.45	**D**
LP	51_Hafid	S4_62662674	4	118.43	116.14	124.67	6.09	41.67	-50.85	33.36	R
HP	51_Hafid	S4_62963538	4	119.91	116.14	142.31	6.53	43.90	-56.62	16.18	R
HP	60_Ribda	S7_51865707	7	66.60	63.51	77.30	6.70	34.45	27.97	19.32	**D**
LP	60_Ribda	S7_51865707	7	66.60	64.60	74.60	6.50	32.42	26.40	12.53	**D**
HP	50_Grinka	S7_58375277	7	96.44	95.19	99.80	22.31	66.87	-48.84	38.08	R
LP	50_Grinka	S7_58375277	7	96.44	95.19	99.80	17.22	57.38	-37.48	23.97	R
HP	55_Gnoss	S9_47958447	9	69.87	64.90	77.87	4.20	20.96	13.73	12.19	**D**

HP= high P, LP= low P, LG=linkage group number, pos=position, ci=confidence, interval, lod=lod score, R^2=% of phenotypic variation explain by individual QTL, a=additivity d=dominance= a(P)=additivity effect positive parent, R= recurrent parent, D=donors parents.

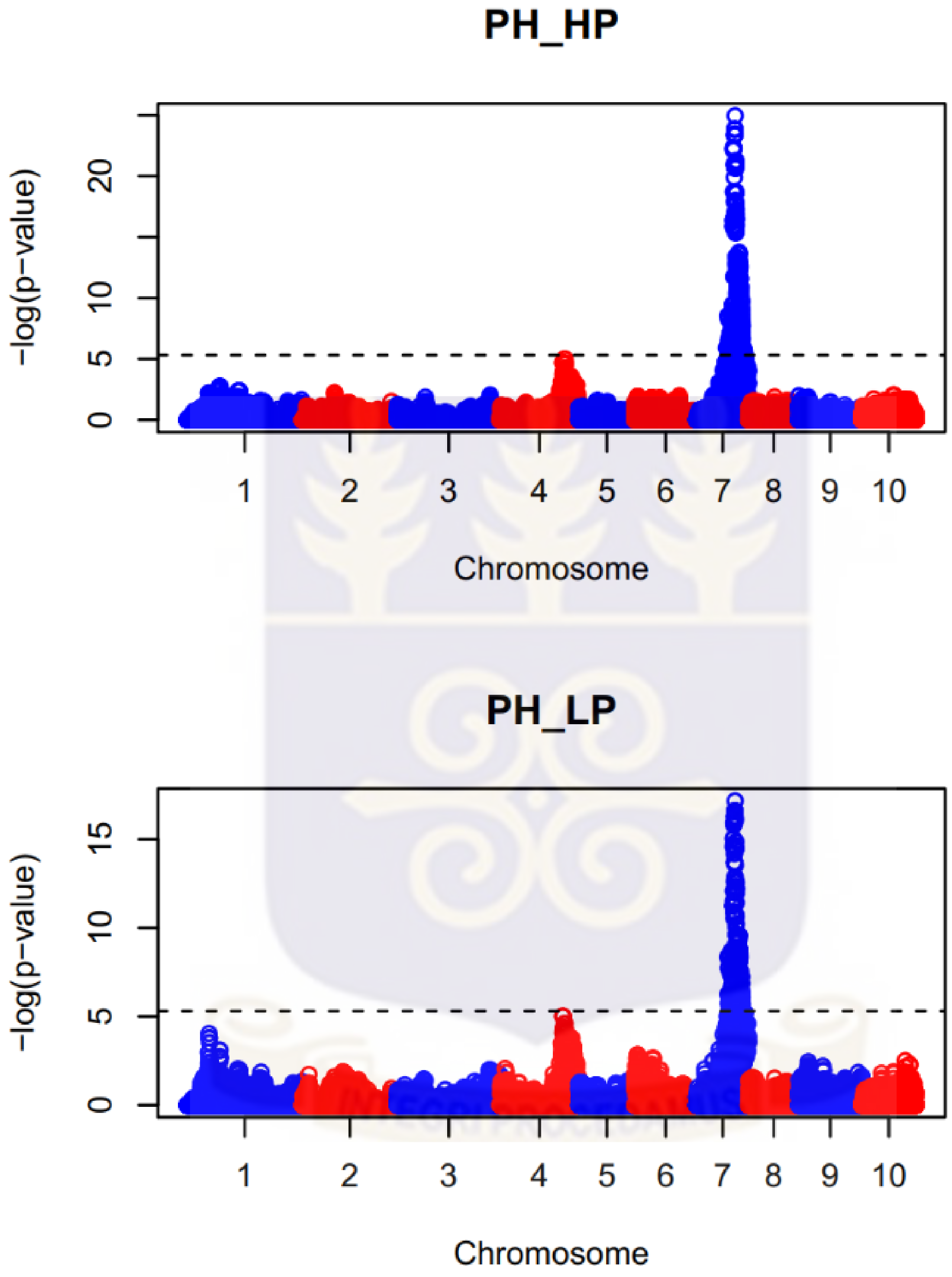


Figure 5.9**:** Manhattan plot of plant height, evaluated at Samanko in 2013 under high P (PH_HP) and under low P (PH_LP).

5.4 Discussion:

The considerable and significant reduction of grain yield and plant height under LP, and the delay in heading under LP conditions relative to HP suggest that the field conditions in this study were appropriate to investigate alternative strategies to select for grain yield under P deficiency. Such yield reductions due to low-P have been previously reported by numerous studies (Chen *et al*., 2008; Cichy *et al*., 2009; 1988; Leiser *et al*., 2012, 2015; Parentoni *et al*., 2010; Rossiter, 1978; 2003; Wissuwa & Ae, 2001)**.** Furthermore, the quite acceptable repeatability estimates for grain yield in LP and HP conditions give confidence in the results obtained in this study.

Broad-sense heritability estimated over multiple years of testing indicated that selection for grain yield of sorghum directly under LP conditions is feasible as earlier reported by Leiser *et al*. (2012). The effectiveness of selection for grain yield under P-deficient conditions relative efficiencies of indirect selection was predicted to be 13% higher. This implied that selection based on grain yield evaluation results under LP would be sufficient compared to selection done based on evaluations under both low and HP conditions. The significant genetic variation for grain yield under LP and HP conditions implied that genetic progress is achievable.

Repeatability estimates for grain yield were observed to take a similar trend and magnitude under both LP and HP conditions which explained the moderate genotypic correlations reported. This implied that the comparative advantage of executing direct selection under LP conditions was higher, more rewarding and cheaper compared to indirect selection under HP conditions.

Similar heritability estimates were found in different studies under contrasting P-levels (Rattunde *et al*., 2016) and nitrogen Levels (Atlin & Frey, 1989; Gelli *et al*., 2016). It has also been reported that direct selection under LP is more effective than indirect selection (Atlin & Frey, 1989; Leiser *et al*., 2012)**.**

The genotype by year interaction was examined with the 298 selected progenies, in both P-levels, genotype by year interactions were greater than the genotype variance components. However, the GxP variance components were smaller than genotype variance components suggesting the importance of year effects on the phenotypic values which is more related to the rainfall events and temperature during the seasons (Appendix 4). This suggests that variety evaluations should be conducted for at least 2 years to ensure the stability of performance of selected new varieties. A strong correlation was detected between LP and HP for grain yield related traits such as DTFL, PH, and HGW. They showed a similar but very high estimated values of repeatability across P-levels in addition to the estimated values of heritability across HP and LP, thus one could be evaluated either in HP or LP with minimal replication.

5.4.1 **Molecular markers for grain yield under contrasting P conditions**

Most of the QTLs for grain yield and panicle length were detected in individual P-levels, suggesting existence of QTL x P-levels interactions while almost all QTL detected for DTFL, PH and HGW were mapped across P-levels indicating they shared similar genetic basis across P-level. The detection of QTLs for grain yield under contrasting P-conditions resulted in fewer QTLs identified under LP (2 QTLs) as compared to HP (9 QTLs). This indicated that the LP conditions were less favorable for detecting yield related QTLs compared to HP. However, a specific QTL detected under LP for grain yield was identified on LG 9 with both QTL mapping methods. This QTL accounted for 23 to 26% of PVE in single QTL mapping and 3 to 6% in GWAS. This QTL was not previously reported, thus it may be considered as a new locus regulating sorghum grain yield under LP condition. The positive QTL for grain yield specifically under LP came from donors Grinkan and Fara Fara that were of highly contrasting origins from Guinea-Caudatum breeding material of IER in Mali and Fara Fara being a landrace from Nigeria respectively. They have very distinct grain types and adaptations. They should be considered as potential candidates for gene discovery. The remaining QTLS on LG 2, 3, 4 and 6 were previously reported (Feltus *et al*., 2006; Sabadin *et al*., 2012; Rajkumar *et al*., 2013; Leiser *et al*., 2014). Another interesting QTL was detected on LG 2 across P-levels with different populations, but always the recurrent parent increased grain yield.

Panicle length and hundred seed weight are important grain yield components (Rami *et al*., 1998) which contribute directly to grain yield improvement. No QTLs were found with GWAS analysis for PANL whereas a total of six QTLs were identified with bi-parental population mapping. However, no QTL were identified under both P-levels for panicle length.

This indicated that difference in P-levels influenced QTL detection for this trait. Interestingly, the recurrent parent contributed to increasing the panicle length from 2 to 4 cm. Nearly all QTLs detected for hundred seed weight were found across P-levels, except for a QTL on LG 8, suggesting a strong association between both environments for QTL detection. Major QTL for HGW previously reported, were also detected in this study on LGs 1, 2, 4, 6 and 10 (Lin *et al*., 1995; Pereira *et al*., 1995; Rami *et al*., 1998; Hart *et al*., 2001; Klein et *al*., 2001; Feltus *et al*., 2006; Srinivas *et al*., 2009). These included QTL on LG 1 that were detected consistently by both methods, GWAS and single population mapping across P-levels. Also another QTL on LG 4 was detected across P-Levels in bi-parental QTL mapping although its peak was found below Bonferroni threshold under HP. The recurrent parent contributed to increase grain weight except for the QTLs detected in the population (57_IS23 and 60_Ribda). Furthermore, three QTLs were mapped in the population (57_IS23) for HGW, therefore these genotypes could be considered as interesting donor candidates to improve this trait. The grain weight with the number of grains per panicle were reported to contribute the most to the total grain yield among the grain yield components (Heinrich, 1983). Therefore, after future validation of these QTLs they can play an important role in sorghum grain yield improvement. Additionally, four QTLs were detected with the population BC56 in the bi-parental QTL mapping and this makes it an interesting population for future investigation of HGW in breeding programme since it carries multiple loci for this trait.

Maturity is a key trait for adaptation of plant to its environmental conditions (Madhusudhana *et al*., 2015). In this study almost all identified QTLs for date to flag leaf appearance were previously reported as QTL for sorghum maturity (Childs *et al*., 1997; Mace & Jordan, 2010; Phuong *et al*., 2013b; Murphy *et al*., 2014; Zhao *et al*., 2016). Six major QTLs were identified across P-levels that correspond to the genomic regions of the sorghum maturity genes *Ma1, Ma3, Ma4, Ma5, and Ma6* using both methods. However, only two genes *Ma1 and Ma6* were confirmed by association mapping. The *Ma1* delayed the flowering and had the largest effect on flowering time while *Ma6* is a strong repressor of flowering under long day conditions and increases the photoperiod sensitivity as well as delaying the flowering time. Both genes are important in ensuring adaptation to agro-climatic conditions, specifically to low soil fertility. Furthermore, Leiser *et al*. (2015) reported less delay in heading by photoperiod sensitive genotypes and higher P uptake rate compared to no photoperiod sensitive genotypes.

The genetic control of plant height in sorghum has been characterized as being quantitatively inherited (Quinby & Karper, 1953). The stover is particularly important in smallholder crop-livestock production systems in the semi-arid and tropical zones of the world (Hash *et al*., 2003). The plant height is an important parameter for stover evaluation. Two QTL for plant height were mapped near genomic regions that correspond to 2 major genes for plant height *Dw1*, and *Dw3* (Feltus *et al*., 2006; Hart *et al*., 2001; Klein et al., 2001; Lin *et al*., 1995; Mace &

Jordan, 2010; Murray *et al*., 2009; Pereira *et al*., 1995; Phuong *et al*., 2013; Rami *et al*., 1998; Srinivas *et al*., 2009). A QTL on LG 3 does not correspond to any of the plant height QTLs previously reported. Thus, it may be considered as new loci regulating plant height in this population.

5.4.2 **Implications for breeding**

Farmers in West Africa are predominantly cultivating sorghum under low-fertility, and particularly low-P conditions (Buerkert *et al*., 2001; Leiser *et al*., 2012). Therefore, the results of this study are of vital importance to enable sorghum breeders increase the genetic gains for grain yield for the majority of West African farmers. This study and a previous study (Leiser *et al*., 2012) demonstrate the feasibility of making genetic progress for yield under LP conditions using conventional breeding techniques. Sorghum breeding programmes in West Africa should thus strengthen their breeding capacities by establishing fields with low P availability on their research stations, or working with farmers. Comparisons of conventional direct LP selection for yield versus developing new markers for assisting selection for yield in LP conditions need to consider the relative expense and the turn-around time for obtaining results to enable making selections. Both factors at present would favor emphasis of conventional selection in the near to medium term. However, building capacity for use of molecular tools in support of strong conventional programmes would be desirable for long term progress.

5.5 Conclusions:

The results indicate wide variation over P-levels. The direct selection for grain yield under LP conditions was predicted to be 13% more efficient than the indirect selection under HP for improving LP yield. The results of this study encourage sorghum breeders in Mali to evaluate for grain yield under low-P conditions. The overall goal is to achieve higher genetic gains while targeting less fertile production conditions. A total of 42 significant QTLs were mapped over P-levels using bi-parental population mapping and GWAS analysis. Among them, eighteen pairs of QTLs were identified across P-levels in the bi-parental mapping while 6 were found across with GWAS analysis. Some newly identified QTL have reported such a specific QTL mapped on LG 9 under LP for grain yield and also QTL for plant height on LG 3. Several QTL that have previously been reported for different traits were also mapped in this study for all traits which confirmed the robustness of these results. After future validation, these results could help breeders to quickly move forward to the next stages of selection being assisted by markers.

CHAPTER SIX

6.0. GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 General discussion

6.1.1 Relevance of the studied traits for small-holder farmers

Sorghum (*Sorghum bicolor* L. Moench) is widely cultivated by smallholder farmers in WCA for meeting their food needs, and thus is vital for their food security. Sorghum is extensively grown under low-soil fertility conditions and erratic rainfall (D'amato & Lebel, 1998; Sivakumar, 1988), owing to the widespread nature of these conditions and sorghum's ability to produce grain under these conditions, and to do so more reliably than other major cereals such as maize. Many soil scientists recognize that low P-availability in Sub-Saharan soils is a major limiting factor to staple cereal production in Africa (Bationo *et al.*, 1989; Hafner *et al.*, 1993; Payne *et al.*, 1992). Furthermore the majority of farmers do not have access to fertilizer because of high cost or deficient availability (Trolove *et al.*, 2003). Therefore studying the genetics of sorghum yield under P-limited conditions will help sorghum breeders to design their programs to develop varieties with better yields under these conditions, and thereby contribute to improving food security in Africa.

The adoption of newly bred sorghum varieties is still relatively low in Mali (Smale *et al.*, 2016; Yapi *et al.*, 1998) despite the efforts made by scientists to improve sorghum varieties. The recognition that a farmer's appreciation of a variety is multi-faceted, and that varietal traits preferred by farmers do not necessarily correspond to the scientific community's priorities (Christinck *et al.*, 2005), calls for participatory research approaches to understand farmer's preferences and priorities. Furthermore breeders and social scientists also need to appreciate how farmers conceptualize group of traits and make trade-offs. Therefore this study conducted detailed participatory evaluations of farmers' appreciations of sorghum panicle types, grain and glume traits and processing characteristics; traits that may be critical for adoption of new varieties and what trait combinations farmers perceive as important for varietal adaptation.

6.1.2 Quantitative genetic parameter estimates

For all panicle traits studied the estimates for the genetic component of variance was highly significant and had high values, under both high and low P conditions. As error estimates were generally low, the repeatabilities for all panicle traits were high or very high under both LP and HP conditions. For the panicle, grain and glume traits, identified in collaboration with farmers, this study provides the first estimates of genetic parameters, using simple scoring techniques. In addition, the study showed that the phenotypic correlations between trait observations made under HP and LP conditions was very high. The estimates of genotypic correlations for individual populations tended to vary more widely, possibly in relationship to the difference in trait expression between the two parents studied. The scoring techniques used for these assessments, can easily be used during early generation selections in sorghum breeding programmes targeting southern Mali, or other areas where such types of sorghum varieties are preferred by farmers, possibly in collaboration with women and men farmers, as they are highly heritable, also under low P conditions, it can also be expected that they could be heritable, when evaluated in trials conducted in farmers' fields. It tends to be easier to observe these traits under LP conditions, when plant height is reduced, and thus the panicles of several plants in a row are easily observable.

For grain yield and related traits the mean values of the progenies were negatively affected under low phosphorus field conditions. As plant development was affected by these conditions, the progenies exhibited important reductions of grain yield and plant height and delayed date to flag leaf appearance, as has been previously reported by several studies, also for other cereal crops (Rossiter, 1978; Fageria *et al*., 1988; Atlin & Frey, 1989; Turk *et al*., 2003; Chen *et al*., 2008; Cichy *et al*., 2009; Parentoni *et al*., 2010; Leiser *et al*., 2012). The phenotypic correlations reflect this quite clearly, as the correlations among these traits under LP conditions tended to be higher than under HP conditions, although almost all correlations were highly significant.

The similarly high repeatability estimates for grain yield and almost all grain yield related traits under both LP and HP conditions indicated that these traits can be effectively assessed in field trials, with two to three replications, using alpha designs to facilitate spatial adjustments. The study revealed highly significant genotype x P-level interaction components, however they were smaller than genotype variance components, when analyzed over a three year period. In addition, the study estimated consistently high genotypic correlations for individual traits when observed under HP- and LP conditions. The estimates for the respective genotypic correlations within individual populations varied highly for some traits like GYLD and GV, but much less for DTFL, or PH, possibly reflecting the fact that the populations had been culled to acceptable levels for plant height and DTFL during the line development process.

For GYLD and the yield related traits the study also permitted the estimation of parameters for multiple years. The result that at individual P levels, as well as for the overall analysis the interaction component for genotype by year effects was higher than either the genotype effects or the P effects is very important and has not been reported before for West-

African conditions. It is thus critical for developing effective selection strategies for productivity related traits, that the importance of GxY effects is considered, by ensuring that evaluations are repeated over at least 2 years, in order to identify new varieties with a reliable performance over different years with different rainfall distributions. In terms of identifying varieties with improved performance under LP conditions, the results from this study confirm the recommendation of Leiser *et al.*, (2012), that yield testing and selection under LP conditions is more efficient than under HP conditions. Considering that visual selections are more easily done under LP conditions, and the quantitative assessment of yield and related traits is more efficient when done under low P conditions, the maximum of sorghum breeding efforts for P limited conditions, as found in farmers' fields in Mali and other countries in West Africa should be conducted under low P conditions, especially when operating under resource constraints. HP field condition may be useful for assessing the delay in flowering, reductions in PH or HGW, which could be important selection criteria to be added to a selection index, as proposed by Leiser *et al.* (2015).

In sorghum adaptation traits are very important for sorghum variety stability as well as farmer acceptance of the new varieties. In addition to the importance of improving grain yield, panicle traits such as glume opening or threshing ability, grain hardness, grain vitreousness and panicle exsertion that meet farmer preferences are essential for the future adoption of new sorghum varieties in WCA (Asante, 2013; Rami *et al.*, 1998; Rooney & Murty, 1982; Sharma *et al.*, 1994). These are parameters that influence directly "grain yield useful for farmers", as they are related to the storability of the grain, as well as decortication yields, and improved food quality characteristics.

Breeding sorghum for grain yield under low input field condition is an important challenge that breeders in WCA should take into greater consideration since the most of the sorghum crop is produced under such field conditions. In spite of the higher heterogeneity of soil conditions in such trials fields that contributed to increased estimates for the residuals, which impact negatively the heritability, this study has shown that response to direct selection under LP conditions is advantageous over indirect selection under HP conditions. Other studies have similarly been reported that breeding under such harsh field condition is feasible (Leiser *et al.*, 2012; Mahamane, 2008) who reported that with adequate field designs, the direct selection was advantageous over indirect selection under high fertility conditions. The results of this study confirmed these findings, as with the sub-block adjustments of the alpha design, for grain yield the repeatabilities were found to be similar in HP compared to LP, and the genetic correlation of r_G=0.81 between HP and LP, and thus the selection direct selection in LP conditions was predicted to be 13% more effective than indirect selection under HP.

6.1.3 **Molecular genetic findings**

Several QTLs were mapped for grain yield, grain quality, panicle and grain yield related traits using both methods, bi-parental mapping and association mapping, over both P-levels. Of the 84 detected QTLs 32 were detected across P-levels by bi-parental QTL mapping, while 11 were detected by association mapping. Most of the QTLs were detected across P-levels for almost all traits except for grain yield, glume opening grain panicle length. Nevertheless some QTL were specifics to the individual P-levels indicating the QTLs by P-level interaction, since they were not detected across P-levels in spite of the strong correlation between both environments for most of these traits. The large number of QTL detected in bi-parental QTL mapping was expected as in bi-parental one can also detect genetic background specific QTLs. With GWAS we can only find QTLs which are existing across all families. In bi-parental populations, however, the detected QTLs might be of little value, since the identified region of interest is mapped with poor resolution. The bi-parental population mapping can be used for variety release and to guide breeders for the future development of new breeding population, while the GWAS can be used for gene discovery. Several major QTLs previously reported were confirmed during this study that provided robustness to these results. The QTLs newly identified in this study need to be validated before their future use in MAS.

6.1.4 Integrating field- based selection with marker-based selection

Nowadays, molecular markers have become an undeniable tool for breeders. It is important for breeders in West Africa to take advantage of this tool to build effective and efficient breeding strategies. The implementation of MAS requires some support like Laboratory facilities, and human resources for data analysis and interpretation, essential for any success in MAS. Thus MAS should be integrated moderately into well-organized breeding schemes, based on detailed analyses of quantitative genetic analyses of the traits targeted.

The results from single QTL mapping could be used in current breeding schemes to characterize the differences between potential donor parents and the recurrent parent, to guide the development of new breeding populations. After validating the QTLs, it should be possible to introgress specific favorable alleles into elite breeding materials using MAS. Another possible use of MAS could be the elimination of unacceptable progenies carrying unfavorable alleles during the early generation selection. This would thus enable breeders to increase the numbers of progenies for yield testing that have traits that meet farmers' preferences. By increasing the number of lines that can be tested and selected upon for grain yield, the selection intensity for grain yield, and thus expected genetic gains will increase.

6.2 CONCLUSION

Farmers in this study preferred open sorghum panicles with hard grains, high density of grains on the panicle, and good threshability. Farmers associated specific panicle types and plant types with several traits, because their knowledge about varieties is founded on generations of exposure to locally available germplasm, whereas breeders are familiar with varieties that stretch beyond these norms. High repeatability estimates were detected for the panicle traits and for almost all grain yield related traits over P-levels. High correlations were found between HP and LP for these traits, these traits were generally slightly more repeatable under HP than LP. Highly significant genetic variance and significant but smaller variance components for genotype x P-level interactions, and the consistently high genotypic correlations between HP- and LP conditions within the individual populations were detected. Similar repeatability was found in the HP compared to LP grain yield and the high genetic correlation (r_G=0.81) between HP and LP .The selection was predicted 13% more effective under direct selection than indirect selection. Several QTLs were mapped for grain yield, grain quality and panicle traits and grain yield related traits using both methods bi-parental mapping and association mapping over P-levels. Of the 84 detected QTLs 34 were detected across P-level by bi-parental QTL mapping, while 11 were by association mapping. Most of the QTLs were detected across P-levels for almost all traits except for grain yield, glume opening, and grain panicle length. Some QTLs were specific to the individual P-levels indicating the QTLs by P-level interaction. Among them an important specific QTL was mapped on LG 9 under LP for grain yield by both QTL mapping methods.

6.3 RECOMMENDATION

A challenge for breeders and the social scientists that work with them is, to identify farmers' locally adapted materials then understand farmer preferences, and also to appreciate how farmers conceptualize suites of traits and make trade-offs. This is important to understand what underlies the trait preferences. There are multiple attributes that are important to farmers and the identification and the understanding of these attributes are very important in setting breeding objectives and developing materials that hopefully will meet farmers' preferences. The BC1F4 progenies constitute an important source of genetic diversity genetic for sorghum breeder in WCA for divers' studies. It is not necessary to evaluate under booth P-levels nearly all traits examined in this study. Even for grain yield, and possibly panicle length and glume opening direct selection under LP is more efficient. The QTLs identified in this study need to be validated before their future use in MAS, the implementation of MAS necessitates some support like laboratory facilities and human resources. Thus MAS should be integrated moderately while organizing current breeding schemes, using quantitative genetic assessments for the traits under consideration, and the research station facilities available. The results of QTL mapping could be used in the current breeding scheme to characterize the different donor parents, and recurrent parent to guide the development of new breeding population.

REFERENCES

Abdurakhmonov, I. Y., & Abdukarimov, A. (2008). Application of association mapping to understanding the genetic diversity of plant germplasm resources. *International Journal of Plant Genomics, 2008*.

Aboubacar, A., & Hamaker, B. R. (1999). Physicochemical properties of flours that relate to sorghum couscous quality. *Cereal Chemistry, 76*(2), 308–313.

Adesina, A. A., & Zinnah, M. M. (1993). Technology characteristics, farmers' perceptions and adoption decisions: A Tobit model application in Sierra Leone. *Agricultural Economics, 9*(4), 297–311.

Adeyanju, A., Perumal, R., & Tesso, T. (2015). Genetic analysis of threshability in grain sorghum [*Sorghum bicolor* (L.) Moench]. *Plant Breeding, 134*(2), 148–155.

Akingbala, J. O., & Rooney, L. W. (1987). Paste properties of sorghum flour and starches. *Journal of Food Processing and Preservation, 11*(1), 13-24.

Asante, D. (2013). Farmer and consumer preferences for rice in the Ashanti region of Ghana: Implications for rice breeding in West Africa. *Journal of Plant Breeding and Crop Science, 5*(12), 229–238.

Ashby, J. (1991). Adopters and adapters: the participation of farmers in on-farm research. *Planned Change in Farming Systems: Progress in on-Farm Research. New York: John Wiley and Sons,* pp. 273-286.

Ashok Kumar, A., Reddy, B. V. S., Sharma, H. C., Hash, C. T., Srinivasa Rao, P., Ramaiah, B., & Reddy, P. S. (2011). Recent advances in sorghum genetic enhancement research at ICRISAT. *American Journal of Plant Sciences, 2*(4), 589–600.

Atlin, G., & Frey, K. (1990). Selecting oat lines for yield in low-productivity environments. *Crop Science, 30*(3), 556–561.

Atlin, G. N., & Frey, K. J. (1989). Predicting the relative effectiveness of direct versus indirect selection for oat yield in three types of stress environments. *Euphytica*, *44*(1), 137–142.

Atwell, S., Huang, Y. S., Vilhjálmsson, B. J., Willems, G., Horton, M., Li, Y., … Jiang, R. (2010). Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. *Nature*, *465*(7298), 627–631.

Balemi, T., & Negisho, K. (2012). Management of soil phosphorus and plant adaptation mechanisms to phosphorus stress for sustainable crop production: a review. *Journal of Soil Science and Plant Nutrition*, *12*(3), 547–562.

Bangbol Sangma, H. (2013). *Genetic characterization of flowering time in sorghum*, University of Queensland, p. 158.

Bationo, A., Christianson, C., & Mokwunye, U. (1989). Soil fertility management of the pearl millet producing sandy soils of Sahelian West Africa: The Niger experience. In *International Workshop on Soil, Crop, and Water Management Systems for Rainfed Agriculture in the Sudano-Sahelian Zone, Niamey (Niger), 11-16 Jan 1987*. ICRISAT.

Bettge, A. ., Giroux, M. J., & Morris, C. F. (2000). Susceptibility of waxy starch granules to mechanical damage, *Cereal chemistry*, *77*(6), 750-753.

Botstein, D., & Risch, N. (2003). Discovering genotypes underlying human phenotypes: past successes for Mendelian disease, future approaches for complex disease. *Nature Genetics*, *33*(3s), 228.

Bouchet, S., Olatoye, M. O., Marla, S. R., Perumal, R., Tesso, T., Yu, J., … Morris, G. P. (2017). Increased Power To Dissect Adaptive Traits in Global Sorghum Diversity Using a Nested Association Mapping Population. *Genetics*, *206*(2), 573–585.

Boyles, R. E., Cooper, E. A., Myers, M. T., Brenton, Z., Rauh, B. L., Morris, G. P., & Kresovich, S. (2016). Genome-Wide Association Studies of Grain Yield Components in Diverse Sorghum Germplasm. *The Plant Genome*, *9*(2), 1-17.

Brachi, B., Morris, G. P., & Borevitz, J. O. (2011). Genome-wide association studies in plants: the missing heritability is in the field. *Genome Biology*, *12*(10), 232.

Breman, H. (1998). Amélioration de la fertilité des sols en Afrique de l'Ouest: Contraintes et perspectives. *Soil Fertility Management in West African Land Use Systems*, 7–20.

Broman, K. W., & Sen, S. (2009). *A Guide to QTL Mapping with R/qtl*. New York: Springer Vol 46, p. 400.

Brown, P. J., Klein, P. E., Bortiri, E., Acharya, C. B., Rooney, W. L., & Kresovich, S. (2006). Inheritance of inflorescence architecture in sorghum. *Theoretical and Applied Genetics*, *113*(5), 931–942.

Buckler, E. S., & Thornsberry, J. M. (2002). Plant molecular diversity and applications to genomics. *Current Opinion in Plant Biology*, *5*(2), 107–111.

Buerkert, A., Bationo, A., & Piepho, H.-P. (2001). Efficient phosphorus application strategies for increased crop production in sub-Saharan West Africa. *Field Crops Research*, *72*(1), 1–15.

Bueso, F. J., Waniska, R. D., Rooney, W. L., & Bejosano, F. P. (2000). Activity of antifungal proteins against mold in sorghum caryopses in the field. *Journal of Agricultural and Food Chemistry*, *48*(3), 810–816.

Cagampang, G. B., & Kirleis, A. W. (1984). Relationship of sorghum grain hardness to selected physical and chemical Measurement of grain quality, *Cereal Chemists*, 61(2), 100-105.

Ceccarelli, S. (1989). Wide adaptation: how wide? *Euphytica*, *40*(3), 197–205.

Celarier R.P. (1959). Cytotaxonomy of the Andropogonea. III. Sub-tribe Sorgheae, genus, sorghum. In *Phenotyping for Plant Breeding*. Springer, Vol. 23, pp. 395–418.

Chambers, R. (2008). PRA, PLA and pluralism: Practice and theory. *The Sage Handbook of Action Research. Participative Inquiry and Practice*, *2*, 297–318.

Chantereau, J., Trouche, G., Rami, J.-F., Deu, M., Barro, C., & Grivet, L. (2001). RFLP mapping of QTLs for photoperiod response in tropical sorghum. *Euphytica*, *120*(2), 183–194.

Chen, J., Xu, L., Cai, Y., & Xu, J. (2008). QTL mapping of phosphorus efficiency and relative biologic characteristics in maize (Zea mays L.) at two sites. *Plant and Soil*, *313*(1-2), 251–266.

Chen, J., Xu, L., Cai, Y., & Xu, J. (2009). Identification of QTLs for phosphorus utilization efficiency in maize (Zea mays L.) across P levels. *Euphytica*, *167*(2), 245–252.

Chen, J., Chopra, R., Hayes, C., Morris, G., Marla, S., Burke, J., … Burow, G. (2017). Genome-Wide Association Study of Developing Leaves' Heat Tolerance during Vegetative Growth Stages in a Sorghum Association Panel. *The Plant Genome*, *10*(2),1-15.

Childs, K. L., Miller, F. R., Cordonnier-Pratt, M.-M., Pratt, L. H., Morgan, P. W., & Mullet, J. E. (1997). The sorghum photoperiod sensitivity gene, Ma3, encodes a phytochrome B. *Plant Physiology*, *113*(2), 611–619.

Christinck, A., Weltzien, E., & Hoffmann, V. (2005b). *Setting breeding objectives and developing seed systems with farmers: a handbook for practical use in participatory plant breeding projects*. Margraf Verlag, p. 187.

Churchill, G. A., & Doerge, R. W. (1994). Empirical threshold values for quantitative trait mapping. *Genetics*, *138*(3), 963–971.

Cichy, K. A., Snapp, S. S., & Blair, M. W. (2009). Plant growth habit, root architecture traits and tolerance to low soil phosphorus in an Andean bean population. *Euphytica*, *165*(2), 257–268.

Cisse, N., & Ejeta, G. (2003). Genetic variation and relationships among seedling vigor traits in sorghum. *Crop Science*, *43*(3), 824–828.

Clayton, W. D., & Renvoize, S. A. (1986). Genera graminum. Grasses of the World. *Genera Graminum. Grasses of the World*, Kew Bulletin Additional Series, vol XII. Royal Botanic Garden, Kew, pp. 338-345.

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*(1-2), 169–196.

Cooper, M., & DeLacy, I. H. (1994). Relationships among analytical methods used to study genotypic variation and genotype-by-environment interaction in plant breeding multi-environment experiments. *Theoretical and Applied Genetics*, *88*(5), 561–572.

Cooper, J., Lombardi, R., Boardman, D., & Carliell-Marquet, C. (2011). The future distribution and production of global phosphate rock reserves. *Resources, Conservation and Recycling*, *57*, 78–86.

Cooper, P., Dimes, J., Rao, K., Shapiro, B., Shiferaw, B., & Twomlow, S. (2008). Coping better with current climatic variability in the rain-fed farming systems of sub-Saharan Africa: An essential first step in adapting to future climate change. *Agriculture, Ecosystems & Environment*, *126*(1), 24–35.

Cooper, M., Messina, C. D., Podlich, D., Totir, L. R., Baumgarten, A., Hausmann, N. J., … Graham, G. (2014). Predicting the future of plant breeding: complementing empirical evaluation with genetic prediction. *Crop and Pasture Science*, *65*(4), 311–336.

Cordell, D., & White, S. (2013). Sustainable phosphorus measures: strategies and technologies for achieving phosphorus security. *Agronomy*, *3*(1), 86–116.

Crasta, O. R., Xu, W. W., Rosenow, D. T., Mullet, J., & Nguyen, H. T. (1999). Mapping of post-flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. *Molecular and General Genetics*, *262*(3), 579–588.

Cuevas, H. E., Rosa-Valentin, G., Hayes, C. M., Rooney, W. L., & Hoffmann, L. (2017). Genomic characterization of a core set of the USDA-NPGS Ethiopian sorghum germplasm collection: implications for germplasm conservation, evaluation, and utilization in crop improvement. *BMC Genomics*, *18*(1), 108.

Dahlberg, J., Berenji, J., Sikora, V., & Latkovic, D. (2011). Assessing sorghum [Sorghum bicolor (L) Moench] germplasm for new traits: food, fuels & unique uses. *Maydica*, *56*(1750), 85–92.

D'amato, N., & Lebel, T. (1998). On the characteristics of the rainfall events in the Sahel with a view to the analysis of climatic variability. *International Journal of Climatology*, *18*(9), 955–974.

Danial, D., Parlevliet, J., Almekinders, C., & Thiele, G. (2007). Farmers' participation and breeding for durable disease resistance in the Andean region. *Euphytica*, *153*(3), 385–396.

Deu, M., Sagnard, F., Chantereau, J., Calatayud, C., Hérault, D., Mariac, C., … Mamadou, A. (2008). Niger-wide assessment of in situ sorghum genetic diversity with microsatellite markers. *Theoretical and Applied Genetics*, *116*(7), 903–913.

De Vries, B. (1998). Umm el-Jimal: A Frontier Town and its Landscape in Northern Jordan, Volume 1: Fieldwork 1972–1981. *Portsmouth: Journal of Roman Archaeology Supplementary Series*, *26*, 33–35.

Dicko, M. H., Hilhorst, R., Gruppen, H., Laane, C., van Berkel, W. J. H., & Voragen, A. G. J. (2002). Zymography of monophenolase and o-diphenolase activities of polyphenol oxidase. *Analytical Biochemistry*, *306*, 336–339.

Dillon, S. L., Shapter, F. M., Henry, R. J., Cordeiro, G., Izquierdo, L., & Lee, L. S. (2007). Domestication to Crop Improvement: Genetic Resources for Sorghum and S accharum (Andropogoneae). *Annals of Botany*, *100*(5), 975–989.

Dingkuhn, M., Singh, B., Clerget, B., Chantereau, J., & Sultan, B. (2006). Past, present and future criteria to breed crops for water-limited environments in West Africa. *Agricultural Water Management*, *80*(1), 241–261.

Doebley, J., Stec, A., & Hubbard, L. (1997). The evolution of apical dominance in maize. *Nature*, *386*(6624), 485.

Doggett, H. (1965). The development of cultivated sorghums. *Crop Plant Evolution. Cambridge Univ. Press, London*, pp. 50-69.

Doggett, H. (1982). *Factors reducing sorghum yields: Striga and birds*. *ICRISAT, Patancheru*, 313–320.

Doraiswamy, V., Subramariam, T. R., & Dakshina murthy, A. (1976). Varietal preference in sorghum for the weevil sitophilus oryzoe L., *Bulletin of Grain Technology*, *14*(2), 107-110.

Doumbia, M. D., Sidibé, A., Bagayoko, A., Diarra, M. A., Bationo, A., Kablan, R. A., … Hons, F. M. (2003). Recommandations specifiques d’engrais: Calibration et validation du module phosphore du modele NuMaSS. *African Crop Science Journal*, *11*(1), 17–25.

Doumbia, M., Hossner, L., & Onken, A. (1998). Sorghum growth in acid soils of West Africa: variations in soil chemical properties. *Arid Land Research and Management*, *12*(2), 179–190.

Dudley, J. (1993). Molecular markers in plant improvement: manipulation of genes affecting quantitative traits. *Crop Science*, *33*(4), 660–668.

Dutt, Y., Bainiwal, C., & Sehrawat, K. (2002). Combining ability analysis for threshing percentage in pearl millet. *Crop Research-hisar*, *24*(2), 378–380.

Dykes, L., Hoffmann, L., Portillo-Rodriguez, O., Rooney, W. L., & Rooney, L. W. (2014). Prediction of total phenols, condensed tannins, and 3-deoxyanthocyanidins in sorghum grain using near-infrared (NIR) spectroscopy. *Journal of Cereal Science*, *60*(1), 138–142.

El-Assal, S. E.-D., Alonso-Blanco, C., Peeters, A. J., Raz, V., & Koornneef, M. (2001). A QTL for flowering time in Arabidopsis reveals a novel allele of CRY2. *Nature Genetics*, *29*(4), 435–440.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species. *PLoS ONE*, *6*(5), e19379.

Fadlemula, A. (1983). *Resistance of sorghum varieties to the rice weevil Sitophilus oryzae (L.) and to the Angoumois grain moth Sitotroga cerealella (Olivier)*. Kansas State University, pp. 1-78.

Fadoul, H. E., Siddig, M. A. El, Wahab, A., Abdalla, H., & Hussein, A. A. El. (2017). Genome-Wide SNPs Identification and Determination of Proteins Associated with Stress Response in Sorghum (Sorghum bicolor L. Monech) Accessions. *American Journal of Plant Sciences*, *8*, 1624–1631.

Fageria, N., Wright, R., & Baligar, V. (1988). Rice cultivar evaluation for phosphorus use efficiency. *Plant and Soil*, *111*(1), 105–109.

FAO. (2016). FAOSTAT. http://www.fao.org/faostat/en/#data/QC

FAO. (2017). FAOSTAT. http://www.fao.org/faostat/en/#data/QC

Felderhoff, T. J., Murray, S. C., Klein, P. E., Sharma, A., Hamblin, M. T., Kresovich, S., … Rooney, W. L. (2012). QTLs for energy-related traits in a sweet x grain sorghum [(L.) Moench] mapping population. *Crop Science*, *52*(5), 2040–2049.

Feltus, F. A., Hart, G. E., Schertz, K. F., Casa, A. M., Kresovich, S., Abraham, S., … Paterson, A. H. (2006). Alignment of genetic maps and QTLs between inter- and intra-specific sorghum populations. *Theoretical and Applied Genetics*, *112*(7), 1295–1305.

Flint-Garcia, S. A., Thornsberry, J. M., & Edward S. Buckler IV. (2003). Structure of linkage disequilibrium in plants. *Annual Review of Plant Biology*, *54*(1), 357–374.

Gamuyao, R., Chin, J. H., Pariasca-Tanaka, J., Pesaresi, P., Catausan, S., Dalid, C., … Heuer, S. (2012). The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. *Nature*, *488*(7412), 535–539.

Garber E.D. (1950). Cytotaxonomic studies in the genus Sorghum. *University of California Publication in Botany*, *23*, 283–361.

Gelli, M., Mitchell, S. E., Liu, K., Clemente, T. E., Weeks, D. P., Zhang, C., … Dweikat, I. M. (2016). Mapping QTLs and association of differentially expressed gene transcripts for multiple agronomic traits under different nitrogen levels in sorghum. *BMC Plant Biology*, *16*(1).

Gemenet, D. C., Leiser, W. L., Beggi, F., Herrmann, L. H., Vadez, V., Rattunde, H. F. W., … Haussmann, B. I. G. (2016). Overcoming Phosphorus Deficiency in West African Pearl

Millet and Sorghum Production Systems: Promising Options for Crop Improvement. *Frontiers in Plant Science*, *7* .1389.

Gemenet, D., Leiser, W., Zangre, R., Angarawai, I., Sanogo, M., Sy, O., … Haussmann, B. (2015). Association analysis of low-phosphorus tolerance in West African pearl millet using DArT markers. *Molecular Breeding*, *35*(8), 171.

Hafner, H., George, E., Bationo, A., & Marschner, H. (1993). Effect of crop residues on root growth and phosphorus acquisition of pearl millet in an acid sandy soil in Niger. *Plant and Soil*, *150*(1), 117–127.

Haley, C. S., & Knott, S. A. (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, *69*(4), 315–324.

Harlan, J. R., & de Wet, J. M. (1971). Toward a rational classification of cultivated plants. *Taxon*, 509–517.

Harris, K., Subudhi, P., Borrell, A., Jordan, D., Rosenow, D., Nguyen, H., … Mullet, J. (2006). Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. *Journal of Experimental Botany*, *58*(2), 327–338.

Hart, G. E., Schertz, K. F., Peng, Y., & Syed, N. H. (2001). Genetic mapping of Sorghum bicolor (L.) Moench QTLs that control variation in tillering and other morphological characters. *TAG Theoretical and Applied Genetics*, *103*(8), 1232–1242.

Hash, C. ., Bhasker Raj, A. ., Lindup, S., Sharma, A., Beniwal, C. ., Folkertsma, R., … Blümmel, M. (2003). Opportunities for marker-assisted selection (MAS) to improve the feed quality of crop residues in pearl millet and sorghum. *Field Crops Research*, *84*(1-2), 79–88.

Haussmann, B. I. G., Fred Rattunde, H., Weltzien-Rattunde, E., Traoré, P. S. C., vom Brocke, K., & Parzies, H. K. (2012). Breeding Strategies for Adaptation of Pearl Millet and

Sorghum to Climate Variability and Change in West Africa. *Journal of Agronomy and Crop Science*, *198*(5), 327–339.

Haussmann, B., Mahalakshmi, V., Reddy, B., Seetharama, N., Hash, C., & Geiger, H. (2002). QTL mapping of stay-green in two sorghum recombinant inbred populations. *Theoretical and Applied Genetics*, *106*(1), 133–142.

Heinrich, G., Francis, C., & Eastin, J. (1983). Stability of grain sorghum yield components across diverse environments. *Crop Science*, *23*(2), 209–212.

Hikeezi, D. M. (2014). *Development of methodologies for end-use quality evaluation*. University of Pretoria, p. 187.

Holford, I. (1997). Soil phosphorus: its measurement, and its uptake by plants. *Soil Research*, *35*(2), 227–240.

Holland, J. B. (2007). Genetic architecture of complex traits in plants. *Current Opinion in Plant Biology*, *10*(2), 156–161.

Jambunathan., R., Singh, U., & Subramanian, V. (1984). Grain quality of sorghum, pearl millet, pigeonpea and chickpea. *In* Interference between agriculture nutrition and food science; Proceedings of a Workshop held at Hyderabad, India, 10-12 November 1981, Achaya, K.T. (Ed.). United Nations University Press, Tokyo, Japan, ISBN: 92-808-0478-2, pp: 47-50.

Jansa, J., Finlay, R., Wallander, H., Smith, F. A., & Smith, S. E. (2011). Role of Mycorrhizal Symbioses in Phosphorus Cycling. In E. Bünemann, A. Oberson, & E. Frossard (Eds.), *Phosphorus in Action* (Vol. 26, pp. 137–168). Berlin, Heidelberg: Springer Berlin Heidelberg.

Kante, M., Rattunde, H. F. W., Leiser, W. L., Nebié, B., Diallo, B., Diallo, A., … Haussmann, B. I. G. (2017). Can Tall Guinea-Race Sorghum Hybrids Deliver Yield Advantage to Smallholder Farmers in West and Central Africa? *Crop Science*, *57*(2), 833.

Kebede, H., Subudhi, P. K., Rosenow, D. T., & Nguyen, H. T. (2001). Quantitative trait loci influencing drought tolerance in grain sorghum (Sorghum bicolor L. Moench). *Theoretical and Applied Genetics*, *103*(2-3), 266–276.

Klein, R. R., Mullet, J. E., Jordan, D. R., Miller, F. R., Rooney, W. L., Menz, M. A., … Klein, P. E. (2008). The Effect of Tropical Sorghum Conversion and Inbred Development on Genome Diversity as Revealed by High-Resolution Genotyping. *Crop Science*, *48*, 12-26.

Klein, R. R., Rodriguez-Herrera, R., Schlueter, J. A., Klein, P. E., Yu, Z. H., & Rooney, W. L. (2001). Identification of genomic regions that affect grain-mould incidence and other traits of agronomic importance in sorghum. *TAG Theoretical and Applied Genetics*, *102*(2), 307–319.

Kumar, I., & Sharma, H. (1982). Inheritance of grain threshability in rice. *Euphytica*, *31*(3), 815–816.

Leiser, W. L., Rattunde, H. F. W., Piepho, H.-P., Weltzien, E., Diallo, A., Melchinger, A. E., … Haussmann, B. I. G. (2012). Selection Strategy for Sorghum Targeting Phosphorus-limited Environments in West Africa: Analysis of Multi-environment Experiments. *Crop Science*, *52*(6), 2517.

Leiser, W. L., Rattunde, H. F. W., Piepho, H.-P., Weltzien, E., Diallo, A., Toure, A., & Haussmann, B. I. G. (2015). Phosphorous Efficiency and Tolerance Traits for Selection of Sorghum for Performance in Phosphorous-Limited Environments. *Crop Science*, *55*(3), 1152.

Leiser, W. L., Rattunde, H. F. W., Weltzien, E., Cisse, N., Abdou, M., Diallo, A., … Haussmann, B. I. (2014). Two in one sweep: aluminum tolerance and grain yield in P-limited soils are associated to the same genomic region in West African sorghum. *BMC Plant Biology*, *14*(1), 206.

Leiser, W. L., Rattunde, H. F. W., Weltzien, E., & Haussmann, B. I. G. (2014). Phosphorus uptake and use efficiency of diverse West and Central African sorghum genotypes under field conditions in Mali. *Plant and Soil*, *377*(1-2), 383–394.

Li, M., Guo, X., Zhang, M., Wang, X., Zhang, G., Tian, Y., & Wang, Z. (2010). Mapping QTLs for grain yield and yield components under high and low phosphorus treatments in maize (Zea mays L.). *Plant Science*, *178*(5), 454–462.

Lin, H., Liang, Z.-W., Sasaki, T., & Yano, M. (2003). Fine mapping and characterization of quantitative trait loci Hd4 and Hd5 controlling heading date in rice. *Breeding Science*, *53*(1), 51–59.

Lin, Y.-R., Schertz, K. F., & Paterson, A. H. (1995). Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. *Genetics*, *141*(1), 391–411.

MacDonald, G. K., Bennett, E. M., Potter, P. A., & Ramankutty, N. (2011). Agronomic phosphorus imbalances across the world's croplands. *Proceedings of the National Academy of Sciences*, *108*(7), 3086–3091.

Mace, E. S., Hunt, C. H., & Jordan, D. R. (2013). Supermodels: sorghum and maize provide mutual insight into the genetics of flowering time. *Theoretical and Applied Genetics*, *126*(5), 1377–1395.

Mace, E. S., & Jordan, D. R. (2010). Location of major effect genes in sorghum (*Sorghum bicolor (L.) Moench*). *Theoretical and Applied Genetics*, *121*(7), 1339–1356.

Mace, E. S., & Jordan, D. R. (2011). Integrating sorghum whole genome sequence information with a compendium of sorghum QTL studies reveals uneven distribution of QTL and of gene-rich regions with significant implications for crop improvement. *Theoretical and Applied Genetics*, *123*(1), 169–191.

Madhusudhana, R., Rajendrakumar, P., & Patil, J. V. (Eds.). (2015). *Sorghum Molecular Breeding*. New Delhi: Springer India, p. 231.

Magalhaes, J. V. (2004). Comparative Mapping of a Major Aluminum Tolerance Gene in Sorghum and Other Species in the Poaceae. *Genetics*, *167*(4), 1905–1914.

Mahamane, S. (2008). *Evaluation of cowpea (Vigna unguicula L. Walp) genotypes for adaptation to low soil phosphorus conditions and to rock phosphate application*. Texas A&M University, p. 141.

Manu, A., Bationo, A., & Geiger, S. (1991). Fertility status of selected millet producing soils of West Africa with emphasis on phosphorus. *Soil Science*, *152*(5), 315–320.

Marcus, O. O. (2017). *Quantitative genomic analysis of agroclimatic traits in sorghum*. Kansas state University.

Masle, J., Gilmore, S. R., & Farquhar, G. D. (2005). The ERECTA gene regulates plant transpiration efficiency in Arabidopsis. *Nature*, *436*(7052), 866–870.

Michels, K., Sivakumar, M., & Allison, B. (1993). Wind erosion in the Southern Sahelian Zone and induced constraints to pearl millet production. *Agricultural and Forest Meteorology*, *67*(1-2), 65–77.

Miles, M. B., Huberman, A. M., & Saldana, J. (2013). *Qualitative data analysis*. Sage, p. 387.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T., Yano, M., Bhatia, C., & Sasaki, T. (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding*, *3*(2), 87–103.

Mohan, S. M., Madhusudhana, R., Mathur, K., Howarth, C. J., Srinivas, G., Satish, K., … Seetharama, N. (2009). Co-localization of quantitative trait loci for foliar disease resistance in sorghum. *Plant Breeding*, *128*(5), 532–535.

Morris, G. P., Ramu, P., Deshpande, S. P., Hash, C. T., Shah, T., Upadhyaya, H. D., … Harriman, J. (2013). Population genomic and genome-wide association studies of agroclimatic traits in sorghum. *Proceedings of the National Academy of Sciences*, *110*(2), 453–458.

Murphy, R. L., Klein, R. R., Morishige, D. T., Brady, J. A., Rooney, W. L., Miller, F. R., … Mullet, J. E. (2011). Coincident light and clock regulation of pseudo-response regulator protein 37 (PRR37) controls photoperiodic flowering in sorghum. *Proceedings of the National Academy of Sciences*, *108*(39), 16469–16474.

Murphy, R. L., Morishige, D. T., Brady, J. A., Rooney, W. L., Yang, S., Klein, P. E., & Mullet, J. E. (2014). () Represses Sorghum Flowering in Long Days: Alleles Enhance Biomass Accumulation and Grain Production. *The Plant Genome*, *7*(2), 1-10.

Murray, S. C., Rooney, W. L., Hamblin, M. T., Mitchell, S. E., & Kresovich, S. (2009). Sweet sorghum genetic diversity and association mapping for brix and height. *The Plant Genome*, *2*(1), 48–62.

Murray, S. C., Sharma, A., Rooney, W. L., Klein, P. E., Mullet, J. E., Mitchell, S. E., & Kresovich, S. (2008). Genetic Improvement of Sorghum as a Biofuel Feedstock: I. QTL for Stem Sugar and Grain Nonstructural Carbohydrates. *Crop Science*, *48*(6), 2165.

Nagaraja Reddy, R., Madhusudhana, R., Murali Mohan, S., Chakravarthi, D. V. N., Mehtre, S. P., Seetharama, N., & Patil, J. V. (2013). Mapping QTL for grain yield and other agronomic traits in post-rainy sorghum [Sorghum bicolor (L.) Moench]. *Theoretical and Applied Genetics*, *126*(8), 1921–1939.

Ni, J., Wu, P., Senadhira, D., & Huang, N. (1998). Mapping QTLs for phosphorus deficiency tolerance in rice (Oryza sativa L.). *TAG Theoretical and Applied Genetics*, *97*(8), 1361–1369.

Nkongolo, K. K., Chinthu, K. K. L., Malusi, M., & Vokhiwa, Z. (2008). Participatory variety selection and characterization of Sorghum (Sorghum bicolor (L.) Moench) elite accessions from Malawian gene pool using farmer and breeder knowledge. *African Journal of Agricultural Research*, *3*(4), 273–283.

Nkonya, E., Schroeder, T., & Norman, D. (1997). Factors affecting adoption of improved maize seed and fertiliser in northern Tanzania. *Journal of Agricultural Economics*, *48*(1-3), 1–12.

Nord, E. A., & Lynch, J. P. (2008). Delayed reproduction in *Arabidopsis thaliana* improves fitness in soil with suboptimal phosphorus availability. *Plant, Cell & Environment*, *31*(10), 1432–1441.

Obersteiner, M., Peñuelas, J., Ciais, P., Van Der Velde, M., & Janssens, I. A. (2013). The phosphorus trilemma. *Nature Geoscience*, *6*(11), ngeo1990.

Odendo, M., De Groote, H., Odongo, O., & Oucho, P. (2002). Participatory rural appraisal of farmers' maize selection criteria and production constraints in the moist mid-altitude Zone of Kenya. IRMA Socio Economic Working Paper No. 02-01. Nairobi, Kenya: CIMMYT and KAR pp 1-30

Okubo, K., Watanabe, T., Miyatake, N., Maeda, S., & Inoue, T. (2012). Evaluation method for threshability of rice varieties by grasping the panicle with hand. *Japanese Journal of Crop Science*, *81*, 201–206.

Osman, K. T. (2012). *Soils: principles, properties and management*. Springer Science & Business Media, p. 263.

Österberg, M. K., Shavorskaya, O., Lascoux, M., & Lagercrantz, U. (2002). Naturally occurring indel variation in the Brassica nigra COL1 gene is associated with variation in flowering time. *Genetics*, *161*(1), 299–306.

Parentoni, S., de Souza Jr, C., de Carvalho Alves, V., Gama, E., Coelho, A., De Oliveira, A., … Meirelles, W. F. (2010). Inheritance and breeding strategies for phosphorous efficiency in tropical maize (Zea mays L). *Maydica*, *55*(1), 1.

Payne, W. A., Malcolm, D. C., Hossner, L. R., Lascao, R. J., Onken, A. B., & Wendt, C. W. (1992). Soil Phosphorus Availability and Pearl Millet Water-Use Efficiency. *Crop Science*, *32*(4), 1010.

Pereira, M. G., Ahnert, D., Lee, M., & Klier, K. (1995). Genetic mapping of quantitative trait loci for panicle characteristic ena seed weigt in sorghum. *Revista Brasileira De Genetica*, *18*, 249-249.

Phuong, N., Stützel, H., & Uptmoor, R. (2013). Quantitative trait loci associated to agronomic traits and yield components in a <i>Sorghum bicolor</i> L. Moench RIL population cultivated under pre-flowering drought and well-watered conditions. *Agricultural Sciences*, *04*(12), 781–791.

Piepho, H.-P., & Mohring, J. (2007). Computing Heritability and Selection Response From Unbalanced Plant Breeding Trials. *Genetics*, *177*(3), 1881–1888.

Platt, A., Vilhjálmsson, B. J., & Nordborg, M. (2010). Conditions under which genome-wide association studies will be positively misleading. *Genetics*, *186*(3), 1045–1052.

Poland, J. A., Bradbury, P. J., Buckler, E. S., & Nelson, R. J. (2011). Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. *Proceedings of the National Academy of Sciences*, *108*(17), 6893–6898.

Quarrie, S., Pekic Quarrie, S., Radosevic, R., Rancic, D., Kaminska, A., Barnes, J., … Dodig, D. (2006). Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. *Journal of Experimental Botany*, *57*(11), 2627–2637.

Quinby, J., & Karper, R. (1953). Inheritance of height in sorghum. *Agronomy Journal, 46*:211-216.

Rajkumar, Fakrudin, B., Kavil, S. P., Girma, Y., Arun, S. S., Dadakhalandar, D., … Kamatar, M. Y. (2013). Molecular mapping of genomic regions harbouring QTLs for root and yield traits in sorghum (Sorghum bicolor L. Moench). *Physiology and Molecular Biology of Plants*, *19*(3), 409–419.

Ramasamy, P., Menz, M. A., Mehta, P. J., Katilé, S., Gutierrez-Rojas, L. A., Klein, R. R., … Magill, C. W. (2009). Molecular mapping of Cg1, a gene for resistance to anthracnose (Colletotrichum sublineolum) in sorghum. *Euphytica*, *165*(3), 597–606.

Rami, J.-F. (1999). *Etude des facteurs génétiques impliqués dans la qualité technologique du grain chez le maïs et le sorgho*. Université Paris Sud, vol. (80), pp. 98

Rami, J.-F., Dufour, P., Trouche, G., Fliedel, G., Mestres, C., Davrieux, F., … Hamon, P. (1998). Quantitative trait loci for grain quality, productivity, morphological and agronomical traits in sorghum (Sorghum bicolor L. Moench). *TAG Theoretical and Applied Genetics*, *97*(4), 605–616.

Ratnadass, A., Butler, D. R., Marley, P. S., Bandyopadhyay, R., Hess, D., & Akintayo, I. (2003). Sorghum head-bugs and grain molds in West and Central Africa: II. Relationships between weather, head-bug and mold damage on sorghum grains. *Crop Protection*, *22*(6), 853–858.

Rattunde, H. F. W., Michel, S., Leiser, W. L., Piepho, H.-P., Diallo, C., Brocke, K. vom, … Weltzien, E. (2016). Farmer Participatory Early-Generation Yield Testing of Sorghum in West Africa: Possibilities to Optimize Genetic Gains for Yield in Farmers' Fields. *Crop Science*, *56*(5), 2493.

Reichert, R. D., Mwasaru, M. A., & Mukuru, S. Z. (1988). Characterization of colored grain sorghum lines and identification of high tannin lines with good dehulling characteristics. *Cereal Chemists*, *65*(3), 165-170.

Reynolds, T. W., Waddington, S. R., Anderson, C. L., Chew, A., True, Z., & Cullen, A. (2015). Environmental impacts and constraints associated with the production of major food crops in Sub-Saharan Africa and South Asia. *Food Security*, *7*(4), 795–822.

Richardson, A. E., Lynch, J. P., Ryan, P. R., Delhaize, E., Smith, F. A., Smith, S. E., …& Oberson, A. (2011). Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant and Soil*, *349*(1-2), 121–156.

Rooney, L. W., & Murty, D. S. (1982). Evaluation of sorghum food qualities. *In Proceedings of the International Symposium on Sorghum; 2-7Nov 81, Patancheru, AP., India. Patancheru, A.P. India ICRISAT*, 571–588.

Rooney, W. L., & Aydin, S. (1999). Genetic control of a photoperiod-sensitive response in Sorghum bicolor (L.) Moench. *Crop Science*, *39*(2), 397–400.

Ross-Ibarra, J., Morrell, P. L., & Gaut, B. S. (2007). Plant domestication, a unique opportunity to identify the genetic basis of adaptation. *Proceedings of the National Academy of Sciences*, *104*(suppl 1), 8641–8648.

Rossiter, R. C. (1978). Phosphorus deficiency and flowering in subterranean clover (T. subterraneum L.). *Annals of Botany*, *42*(2), 325–329.

Rusike, J., Twomlow, S., Freeman, H. A., & Heinrich, G. M. (2006). Does farmer participatory research matter for improved soil fertility technology development and dissemination in Southern Africa? *International Journal of Agricultural Sustainability*, *4*(3), 176–192.

Russell, M. P. (1962). Effects of sorghum varieties on the lesser rice weevil, Sitophilus oryzae (L.) I. Oviposition, immature mortality, and size of adults. *Annals of the Entomological Society of America*, *55*(6), 678–685.

Russell, P. M. (1966). Effects of four sorghum varieties on longevity of the lesser rice weevil Sitophilus oryzae L. *Journal of Stored Products Research*, *2*(1), 75-79.

Sabadin, P. K., Malosetti, M., Boer, M. P., Tardin, F. D., Santos, F. G., Guimarães, C. T., … Magalhaes, J. V. (2012). Studying the genetic basis of drought tolerance in sorghum by managed stress trials and adjustments for phenological and plant height differences. *Theoretical and Applied Genetics*, *124*(8), 1389–1402.

Sagnard, F., Deu, M., Dembélé, D., Leblois, R., Touré, L., Diakité, M., … Togola, S. (2011). Genetic diversity, structure, gene flow and evolutionary relationships within the Sorghum bicolor wild–weedy–crop complex in a western African region. *Theoretical and Applied Genetics*, *123*(7), 1231.

Sako, D. (2013). *Genetic Analysis for Panicle Architecture and Grain Yield in Sorghum [Sorghum bicolor (L.) Moench] in Mali*. University of Ghana, Legon, p. 166.

Sanchez, A. C., Subudhi, P. K., Rosenow, D. T., & Nguyen, H. T. (2002). Mapping QTLs associated with drought resistance in sorghum (Sorghum bicolor L. Moench). *Plant Molecular Biology*, *48*(5), 713–726.

Schachtman, D. P., Reid, R. J., & Ayling, S. M. (1998). Phosphorus uptake by plants: from soil to cell. *Plant Physiology*, *116*(2), 447–453.

Schnug, E., & De Kok, L. J. (Eds.). (2016). *Phosphorus in Agriculture: 100 % Zero*. Dordrecht: Springer Netherlands, p. 357.

Sharma, H. C., Lopez, V. F., & Vidyasagar, P. (1994). Influence of panicle compactness and host plant resistance in sequential plantings on population increase of panicle-feeding insects in *Sorghum bicolor* (L.) Moench. *International Journal of Pest Management*, *40*(2), 216–221.

Shi, J., Li, R., Qiu, D., Jiang, C., Long, Y., Morgan, C., … Meng, J. (2009). Unraveling the Complex Trait of Crop Yield With Quantitative Trait Loci Mapping in Brassica napus. *Genetics*, *182*(3), 851–861.

Siart, S. (2008). *Strengthening local seed systems: Options for enhancing diffusion of variety diversity of sorghum in Southern Mali*. Margraf Publishers, GmbH.

Sibiya, J., Tongoona, P., Derera, J., & Makanda, I. (2013). Farmers' desired traits and selection criteria for maize varieties and their implications for maize breeding: A case study from KwaZulu-Natal Province, South Africa. *Journal of Agriculture and Rural Development in the Tropics and Subtropics (JARTS)*, *114*(1), 39–49.

Sinaj, S., Richner, W., Flisch, R., & Charles, R. (2009). Données de base pour la fumure des grandes cultures et des herbages (DBF-GCH). *Revue Suisse d'Agriculture*, *41*(1), 1–98.

Singh, P., Aggarwal, P., Bhatia, V., Murty, M., Pala, M., Oweis, T., … Wani, S. P. (2009). Yield gap analysis: modelling of achievable yields at farm level. *Rainfed Agriculture: Unlocking the Potential*, *81*.

Sivakumar, M. (1988). Predicting rainy season potential from the onset of rains in Southern Sahelian and Sudanian climatic zones of West Africa. *Agricultural and Forest Meteorology*, *42*(4), 295–305.

Smale, M., Kergna, A., & Diakité, L. (2016). *An Economic Assessment of Sorghum Improvement in Mali*, Impact Assessment Report No. 2, p. 52.

Soleri, D., Smith, S. E., & Cleveland, D. A. (2000). Evaluating the potential for farmer and plant breeder collaboration: a case study of farmer maize selection in Oaxaca, Mexico. *Euphytica*, *116*(1), 41–57.

Srinivas, G., Satish, K., Madhusudhana, R., Nagaraja Reddy, R., Murali Mohan, S., & Seetharama, N. (2009). Identification of quantitative trait loci for agronomically important traits and their association with genic-microsatellite markers in sorghum. *Theoretical and Applied Genetics*, *118*(8), 1439–1454.

Subudhi, P. K., Rosenow, D. T., & Nguyen, H. T. (2000). Quantitative trait loci for the stay green trait in sorghum (Sorghum bicolor L. Moench): consistency across genetic backgrounds and environments. *Theoretical and Applied Genetics*, *101*(5-6), 733–741.

Suhendro, E. L., Kunetz, C. F., McDonough, C. M., Rooney, L. W., & Waniska, R. D. (2000). Cooking characteristics and quality of noodles from food sorghum. *Cereal Chemistry*, *77*(2), 96-100.

Sukumaran, S., Reynolds, M. P., Lopes, M. S., & Crossa, J. (2015). Genome-wide association study for adaptation to agronomic plant density: A component of high yield potential in spring wheat. *Crop Science*, *55*(6), 2609–2619.

Tao, Y. Z., Hardy, A., Drenth, J., Henzell, R. G., Franzmann, B. A., Jordan, D. R., … McIntyre, C. L. (2003). Identifications of two different mechanisms for sorghum midge resistance through QTL mapping. *Theoretical and Applied Genetics*, *107*(1), 116–122.

Tao, Y. Z., Henzell, R. G., Jordan, D. R., Butler, D. G., Kelly, A. M., & McIntyre, C. L. (2000). Identification of genomic regions associated with stay green in sorghum by testing RILs in multiple environments. *TAG Theoretical and Applied Genetics*, *100*(8), 1225–1232.

Taylor, J. R., Schober, T. J., & Bean, S. R. (2006). Novel food and non-food uses for sorghum and millets. *Journal of Cereal Science*, *44*(3), 252–271.

Theodorou, M. E., & Plaxton, W. C. (1993). Metabolic adaptations of plant respiration to nutritional phosphate deprivation. *Plant Physiology*, *101*(2), 339–344.

Touré, A., Traore, K., Bengaly, A., Scheuring, J., Rosenow, D., & Rooney, L. (1998). The potential of local cultivars in sorghum improvement in Mali. *African Crop Science Journal*, *6*(1), 1–7.

Trolove, S. N., Hedley, M. J., Kirk, G. J. D., Bolan, N. S., & Loganathan, P. (2003). Progress in selected areas of rhizosphere research on P acquisition. *Australian Journal of Soil Research*, *41*(3), 471.

Tuinstra, M., Ejeta, G., & Goldsbrough, P. (1998). Evaluation of near-isogenic sorghum lines contrasting for QTL markers associated with drought tolerance. *Crop Science*, *38*(3), 835–842.

Tuinstra, M. R., Grote, E. M., Goldsbrough, P. B., & Ejeta, G. (1997). Genetic analysis of post-flowering drought tolerance and components of grain development in Sorghum bicolor (L.) Moench. *Molecular Breeding*, *3*(6), 439–448.

Turk, M., Tawaha, A., & El-Shatnawi, M. (2003). Response of lentil (Lens culinaris Medik) to plant density, sowing date, phosphorus fertilization and ethephon application in the absence of moisture stress. *Journal of Agronomy and Crop Science*, *189*(1), 1–6.

Upton, M. (1987). *African farm management*. UK, Cambridge University Press, CUP Archive.

vom Brocke, K., Trouche, G., Weltzien, E., Barro-Kondombo, C. P., Gozé, E., & Chantereau, J. (2010). Participatory variety development for sorghum in Burkina Faso: Farmers' selection and farmers' criteria. *Field Crops Research*, *119*(1), 183–194.

Watanabe, S., Tajuddin, T., Yamanaka, N., Hayashi, M., & Harada, K. (2004). Analysis of QTLs for reproductive development and seed quality traits in soybean using recombinant inbred lines. *Breeding Science*, *54*(4), 399–407.

Weltzien, E., Christinck, A., Touré, A., Rattunde, F., Diarra, M., Sangaré, A., & Coulibaly, M. (2006). Enhancing farmers' access to sorghum varieties through scaling-up participatory plant breeding in Mali, West Africa. *AgroSpecial*, *5*, 20006–1002.

Werner, J. D., Borevitz, J. O., Warthmann, N., Trainer, G. T., Ecker, J. R., Chory, J., & Weigel, D. (2005). Quantitative trait locus mapping and DNA array hybridization identify an FLM deletion as a cause for natural flowering-time variation. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(7), 2460–2465.

Wet, J. de, & Huckabay, J. (1967). The origin of Sorghum bicolor. II. Distribution and domestication. *Evolution*, *21*(4), 787–802.

Wissuwa, M., & Ae, N. (2001). Further characterization of two QTLs that increase phosphorus uptake of rice (Oryza sativa L.) under phosphorus deficiency. *Plant and Soil*, *237*(2), 275–286.

Witcombe, J. R., Joshi, A., Joshi, K. D., & Sthapit, B. (1996). Farmer participatory crop improvement. I. Varietal selection and breeding methods and their impact on biodiversity. *Experimental Agriculture*, *32*(4), 445–460.

Wongo, L. E., & Pedersen, J. R. (1990). Effet of threshing different sorghum cultivars on Sitotroga cerealella (Oliv.) (Lepidoptera: Gelechiidae) and Sitophilus oryzae (L.) (Coleoptera: Curculionidae). *Journal of Stored Products Research*, *26*(2), 89-96.

Xavier, A., Muir, W., Rainey, K., Xu, S., Xavier, M. A., & Rcpp, I. (2017). R Package "NAM.", p. 23.

Xavier, A., Xu, S., Muir, W. M., & Rainey, K. M. (2015). NAM: association studies in multiple populations. *Bioinformatics*, *31*(23), 3862–3864.

Xu, W., Subudhi, P. K., Crasta, O. R., Rosenow, D. T., Mullet, J. E., & Nguyen, H. T. (2000). Molecular mapping of QTLs conferring stay-green in grain sorghum (Sorghum bicolor L. Moench). *Genome*, *43*(3), 461–469.

Yadav, O. (1994). Inheritance of threshing percentage in pearl millet. *Euphytica*, *78*(1), 77–80.

Yamamoto, T., Kuboki, Y., Lin, S. Y., Sasaki, T., & Yano, M. (1998). Fine mapping of quantitative trait loci Hd-1, Hd-2 and Hd-3 , controlling heading date of rice, as single Mendelian factors. *TAG Theoretical and Applied Genetics*, *97*(1-2), 37–44.

Yamanaka, N., Watanabe, S., Toda, K., Hayashi, M., Fuchigami, H., Takahashi, R., & Harada, K. (2005). Fine mapping of the FT1 locus for soybean flowering time using a residual heterozygous line derived from a recombinant inbred line. *Theoretical and Applied Genetics*, *110*(4), 634–639.

Yang, Z., Van Oosterom, E. J., Jordan, D. R., & Hammer, G. L. (2009). Pre-anthesis ovary development determines genotypic differences in potential kernel weight in sorghum. *Journal of Experimental Botany*, *60*(4), 1399–1408.

Yano, M., Harushima, Y., Nagamura, Y., Kurata, N., Minobe, Y., & Sasaki, T. (1997). Identification of quantitative trait loci controlling heading date in rice using a high-density linkage map. *TAG Theoretical and Applied Genetics*, *95*(7), 1025–1032.

Yapi, A., Kergna, A., Debrah, S., Sidibe, A., & Sanogo, O. (1998). Impact of sorghum and millet research in Mali (pp. 76–93). Presented at the Assessing joint impacts: proceedings of an International Workshop on Joint impact assessment of NARS/ICRISAT, 2-4 Dec 1996, ICRISAT, Patancheru, India (Bantilan MCS and Joshi PK, eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Yu, J., Holland, J. B., McMullen, M. D., & Buckler, E. S. (2008). Genetic Design and Statistical Power of Nested Association Mapping in Maize. *Genetics*, *178*(1), 539–551.

Yu, J., Pressoir, G., & Briggs, W. (2006). Vroh Bi I, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES: A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics*, *38*(2), 203–208.

Zhang, H., Uddin, M. S., Zou, C., Xie, C., Xu, Y., & Li, W.-X. (2014). Meta-analysis and candidate gene mining of low-phosphorus tolerance in maize. *Journal of Integrative Plant Biology*, *56*(3), 262–270.

Zhao, H., Dekkers, J., Fernando, R., & others. (2006). Power and precision of regression-based linkage disequilibrium mapping of QTL. In *Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Minas Gerais, Brazil, 13-18 August, 2006* (pp. 21–20). Instituto Prociência.

Zhao, J., Mantilla Perez, M. B., Hu, J., & Salas Fernandez, M. G. (2016). Genome-Wide Association Study for Nine Plant Architecture Traits in Sorghum. *The Plant Genome*, *9*(2), 1-10.

Zhou, X., & Stephens, M. (2012). Genome-wide efficient mixed-model analysis for association studies. *Nature Genetics*, *44*(7), 821–824.

Zhu, C., Gore, M., Buckler, E. S., & Yu, J. (2008). Status and Prospects of Association Mapping in Plants. *The Plant Genome Journal*, *1*(1), 5-20.

Zou, G., Zhai, G., Feng, Q., Yan, S., Wang, A., Zhao, Q., … Tao, Y. (2012). Identification of QTLs for eight agronomically important traits using an ultra-high-density map based on SNPs generated from high-throughput sequencing in sorghum under contrasting photoperiods. *Journal of Experimental Botany*, *63*(15), 5451–5462.

APPENDICES

Appendix 1: questionnaire Design for focus and individual discussion

Panicle Selection Activity

1. Date: ___________________
2. Location *(village and district)*: _______________________
3. GROUP or INDIVIDUAL INTERVIEW *(circle one)*
4. Is the group or individual FEMALE or MALE *(circle one)*

5. Members of the group:

	a. Name	b. Age	c. Are they selecting panicles for seed from their field? *Yes/No*	d. Are they producing seed for sale? *Yes/No*	e. HH Grain use a. food b. market c. both d. none	f. Other important uses of sorghum?	g. Area (ha) of sorghum produced last year (2014)	h. Grown in intercrop or sole crop? a. IC b. SC c. both

6. Which cereals do you grow in your village? Rank the cereal crops in order of importance.

1.
2.
3.
4.
5.
6.

Group the panicles according to preference.

7. Number of panicle groups:

8. Which panicles are in each group?

Group 1:	Group 2:	Group 3:	Group 4:
Group 5:	Group 6:	Group 7:	Group 8:
Group 9:	Group 10:	Group 11:	Group 12:

Open-ended discussions questions *Be sure to indicate the panicle group and panicle number when writing notes.*

Panicle Group Spectrum Questions

9. Why did you choose to group the panicles this way? [Understand the spectrum of the groups]

10. Are there groups or individual panicles here that are similar to varieties you have grown? [Entry point for discussion]

Panicle Group Specific Data – page 1

11. Okay, so let's talk about this specific group. Can you tell me more it? [Each group in turn]. Why did you put all the panicles in this group together? What is it about the panicles that is similar?

Group: ____

12. What are favorable qualities of this group? Less preferred? [*Go into depth and detail about traits mentioned by the farmer in each group, focusing on/getting appropriate depth of information on the focus traits. Probe about threshing and hardness if isn't mentioned*].

Group: ____

Panicle Group Specific Data – page 2

13. Which groups / individual panicles are acceptable for you (that you would be willing to sow them in your field), and which not, and why? [1) Get at threshold of acceptability for specific traits 2) Is it one trait, combination of traits?]

Group: ____

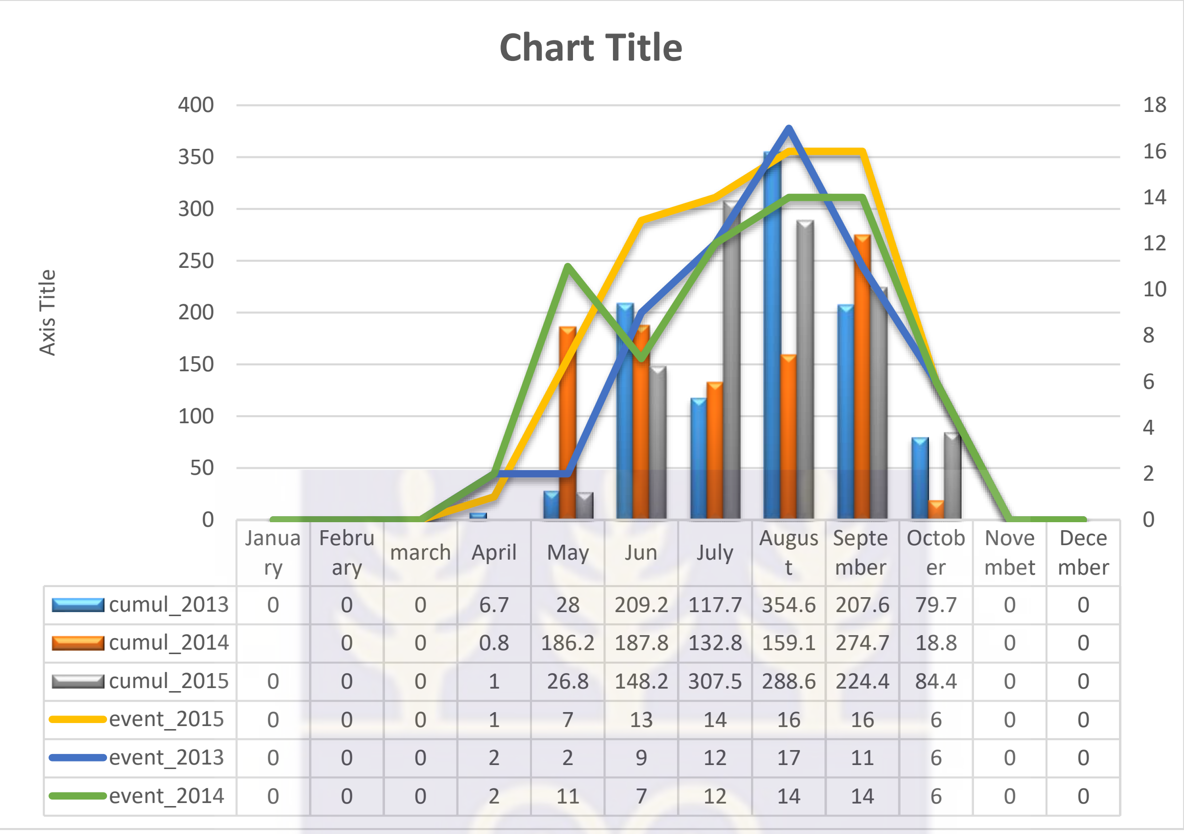
14. Are there noteworthy differences between the panicles of this same group? If so, how are they different?
Group: ____

Appendix 2. The rank, grain yield and corresponding standard error, with 298 selected progenies under LP of the 25 progenies with highest yields under HP in 2014 Samanko

ENTRY13	Pedegree	Rank 14HP	GYLD14HP	SE	Rank 14LP	GYLD14LP	SE
178	Lata//Grin-9-4-1-1	1	540.51	45.35	44	57.63	25.39
338	Lata//IS23540-9-31-1-1	2	533.35	45.74	4	200.50	20.64
5	Lata//Fram-1-8-1-1	3	489.25	45.36	42	100.82	20.69
831	Lata//Ridb-1-17-1-1	4	487.23	55.71	40	110.68	20.60
887	Lata//Ridb-8-9-1-1	5	471.86	45.93	17	173.52	20.75
667	Lata//DouaG-2-4-1-1	6	463.82	45.53	36	130.35	20.66
539	Lata//SK5912-5-3-1-1	7	456.24	45.19	41	100.88	20.71
494	Lata//SC566-7-7-1-1	8	450.63	45.33	15	177.51	20.73
611	Lata//Ngol-4-25-1-1	9	443.14	45.26	34	132.28	20.65
279	Lata//IS15401-8-10-1-1	10	437.34	45.36	27	167.34	20.82
174	Lata//Grin-8-46-1-1	11	436.68	45.51	28	156.00	20.66
35	Lata//Fram-2-8-1-1	12	435.78	45.36	35	132.11	20.95
18	Lata//Fram-1-32-1-1	13	431.75	45.47	22	171.73	21.03
18	Lata//Fram-1-32-1-1	14	431.75	45.47	23	171.73	21.03
748	Lata//DouaG-7-9-1-1	15	429.72	45.48	2	208.95	25.38
145	Lata//Grin-8-42-1-1	16	428.95	45.43	37	128.34	20.87
172	Lata//Grin-8-39-1-1	17	421.25	45.42	33	133.65	20.67
305	Lata//IS23540-1-27-1-1	18	415.51	45.42	16	174.83	20.69
136	Lata//Grin-8-24-1-1	19	413.48	45.38	29	155.35	20.69
245	Lata//IS15401-6-45-1-1	20	413.22	45.49	43	88.83	25.34
59	Lata//Fram-5-26-1-1	21	413.12	45.36	30	151.65	20.93
631	Lata//Ngol-4-15-1-1	22	412.47	45.58	39	115.07	20.72
24	Lata//Fram-1-47-1-1	23	403.94	45.22	38	127.80	20.68
231	Lata//IS15401-6-30-1-1	24	402.97	45.37	31	149.35	20.81
965	Lata//Samb-5-3-1-1	25	400.64	45.23	32	138.65	20.68
353	Lata//IS23540-4-16-1-1	26	387.45	55.56	8	188.81	20.76
844	Lata//Ridb-2-11-1-1	27	372.24	45.35	13	179.06	21.06
160	Lata//Grin-8-1-1-1	28	357.94	45.36	1	223.79	20.67
161	Lata//Grin-8-2-1-1	29	343.47	45.78	19	172.79	20.67
1023	Lata//IS24887-13-1-1-1	30	334.18	55.73	21	172.17	25.46
650	Lata//Ngol-7-12-1-1	31	326.40	45.52	10	183.59	20.72
233	Lata//IS15401-6-32-1-1	32	326.36	46.90	25	168.54	20.82
618	Lata//Ngol-7-17-1-1	33	321.12	45.17	11	182.87	25.35
277	Lata//IS15401-8-8-1-1	35	316.10	45.81	12	179.29	20.79
90	Lata//Grin-1-21-1-1	36	307.78	45.33	7	189.52	21.07
260	Lata//IS15401-7-2-1-1	37	306.39	45.22	3	201.92	20.70
126	Lata//Grin-8-12-1-1	38	300.59	45.55	24	171.03	20.79
228	Lata//IS15401-6-27-1-1	39	299.11	45.41	6	190.00	20.95
677	Lata//DouaG-4-25-1-1	40	297.13	45.47	14	178.71	20.70
610	Lata//Ngol-4-24-1-1	41	289.02	45.38	5	190.38	25.36
943	Lata//Samb-3-17-1-1	42	285.25	45.33	20	172.52	25.40
91	Lata//Grin-1-22-1-1	43	227.71	45.37	9	185.15	21.00
237	Lata//IS15401-6-36-1-1	44	189.35	45.48	18	172.89	20.64

Appendix 3. The rank, grain yield and corresponding standard error, with 298 selected progenies under LP of the 25 progenies with highest yields under HP in 2015 Samanko

ENTRY13	Pedegree	Rank HP15	GYLD15HP	SE	Rank LP15	GYLD_15LP	SE
91	Lata//Grin-1-22-1-1	1	388.77	36.85	12	180.66	26.07
160	Lata//Grin-8-1-1-1	2	359.32	35.66	6	200.48	29.37
185	Lata//IS15401-1-6-1-1	3	348.60	35.99	26	162.66	33.44
852	Lata//Ridb-3-9-1-1	4	343.11	36.33			
960	Lata//Samb-4-13-1-1	5	326.96	44.51			
314	Lata//IS23540-2-8-1-1	6	320.35	43.49	46	36.63	33.75
284	Lata//IS23540-1-4-1-1	7	318.71	44.51	37	109.05	26.27
846	Lata//Ridb-2-13-1-1	8	306.53	35.53	39	97.32	26.94
843	Lata//Ridb-2-10-1-1	9	301.16	35.67	34	126.12	38.46
887	Lata//Ridb-8-9-1-1	10	301.09	36.31	31	149.61	62.83
965	Lata//Samb-5-3-1-1	11	298.65	35.58	29	154.74	33.35
93	Lata//Grin-1-25-1-1	12	298.21	35.51	36	110.90	26.43
196	Lata//IS15401-1-19-1-1	13	294.11	35.53	45	44.99	43.62
811	Lata//Gnos-7-13-1-1	14	293.56	36.22	44	62.24	32.22
244	Lata//IS15401-6-44-1-1	15	292.08	36.07	28	157.45	27.22
998	Lata//Samb-7-1-1-1	16	291.39	43.49	43	69.03	49.10
730	Lata//DouaG-6-9-1-1	17	288.53	37.00	40	79.04	28.11
245	Lata//IS15401-6-45-1-1	18	287.51	35.56	41	73.57	35.52
264	Lata//IS15401-7-8-1-1	19	287.25	37.02	18	174.01	27.71
620	Lata//Ngol-7-19-1-1	20	286.27	35.53	30	154.70	27.08
667	Lata//DouaG-2-4-1-1	21	286.14	35.45	20	171.16	30.52
253	Lata//IS15401-6-54-1-1	22	284.10	35.55	42	73.28	35.01
24	Lata//Fram-1-47-1-1	23	283.39	35.92	38	99.04	29.17
240	Lata//IS15401-6-39-1-1	24	279.70	35.54	33	130.31	36.31
618	Lata//Ngol-7-17-1-1	25	279.45	36.27	32	140.28	39.05
237	Lata//IS15401-6-36-1-1	27	276.96	35.66	24	165.60	47.14
255	Lata//IS15401-6-57-1-1	28	275.36	35.44	19	172.53	43.69
152	Lata//Grin-9-9-1-1	29	265.61	35.39	16	175.18	31.99
963	Lata//Samb-5-1-1-1	30	265.42	43.75	5	208.41	36.35
977	Lata//Samb-6-5-1-1	31	256.63	35.53	23	165.91	48.25
650	Lata//Ngol-7-12-1-1	32	254.30	38.69	8	193.92	43.94
272	Lata//IS15401-8-3-1-1	33	239.90	36.99	11	186.16	33.84
445	Lata//SC566-6-64-1-1	34	238.36	36.10	25	162.86	30.63
338	Lata//IS23540-9-31-1-1	35	238.19	35.39	22	168.57	34.96
973	Lata//Samb-6-1-1-1	36	233.63	35.68	7	196.58	32.21
125	Lata//Grin-8-11-1-1	37	231.40	35.37	21	168.84	33.79
880	Lata//Ridb-8-2-1-1	38	228.57	35.52	1	246.52	34.26
488	Lata//SC566-6-56-1-1	39	225.59	36.08	3	226.74	42.04
133	Lata//Grin-8-21-1-1	40	220.96	36.22	9	191.26	25.41
463	Lata//SC566-3-4-1-1	41	217.26	36.31	17	174.06	35.59
692	Lata//DouaG-5-18-1-1	42	212.48	36.15	15	176.47	25.31
951	Lata//Samb-4-4-1-1	43	205.13	35.42	14	177.42	32.60
727	Lata//DouaG-6-6-1-1	44	204.66	36.90	13	180.43	27.48
279	Lata//IS15401-8-10-1-1	45	202.18	35.65	4	224.07	42.22
497	Lata//SC566-7-14-1-1	46	191.65	35.44	2	229.13	58.55
1081	Lata//IS24887-23-7-1-1	47	191.00	36.14	10	188.96	45.44



	January	February	march	April	May	Jun	July	August	September	October	Novembet	December
cumul_2013	0	0	0	6.7	28	209.2	117.7	354.6	207.6	79.7	0	0
cumul_2014		0	0	0.8	186.2	187.8	132.8	159.1	274.7	18.8	0	0
cumul_2015	0	0	0	1	26.8	148.2	307.5	288.6	224.4	84.4	0	0
event_2015	0	0	0	1	7	13	14	16	16	6	0	0
event_2013	0	0	0	2	2	9	12	17	11	6	0	0
event_2014	0	0	0	2	11	7	12	14	14	6	0	0

Appendix 4. Variation of rains of total rain per month and the number of rain event from 2013 to 2015

Appendix 5: Chemical properties of soil sample in 2016.

	unit	**LP**	**HP**
pH		5.45	5.30
N	%	0.02	0.03
P_total	mg/kg	68.38	87.54
Bray-1 P	mg/kg sol	3.38	14.32
Al^{3+} -Sat.	$Cmol^{+}Kg^{-1}$sol	0.27	0.33
K_total	mg/kg sol	643.98	950.05
Ca++	$Cmol^{+}Kg^{-1}$sol	0.79	0.99
Mg++	$Cmol^{+}Kg^{-1}$sol	0.54	0.70
K+	$Cmol^{+}Kg^{-1}$sol	0.09	0.16
CEC	$Cmol^{+}Kg^{-1}$sol	2.58	3.08

pH= soil pH, Bray-1 P= soil plant P availability, P_total=soil P total, Al^{3+} -Sat.=soil aluminum saturation, Ca= calcium, N=Nitrogen, Mg=Magnesium,