

**BREEDING SORGHUM [*Sorghum bicolor* (L.) MOENCH] FOR HIGH QUALITY
STOVER FOR NIGER**

By

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DECLARATION

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

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ABSTRACT

Pasture shortage during March to July is the main constraint in cattle productivity in Niger. To alleviate this pasture shortage, farmers use dual purpose sorghum varieties where the grain is used as food and the nutritionally low quality stover as feed for their cattle during dry season. Consequently, cattle productivity is reduced. One sustainable means is to pave the way for better stover quality availability through incorporation of the *brown midrib* trait, which is known to improve quality of stover, into sorghum varieties. The main objective of this study was to develop dual purpose sorghum lines with high quality stover for sustained livestock productivity. The specific objectives were to (1) identify farmers' and stover traders' preferences on sorghum stover varieties; (2) introgress *brown midrib6* and *brown midrib12* genes in two Nigerien elite sorghum lines; (3) determine the agronomic and nutritive potential of new *brown midrib* (*bmr*) derived lines and (4) identify Single Nucleotide Polymorphism (SNP) mutations in Ethly methanesulfonate (EMS) sorghum mutants used in the national sorghum improvement programme. A Participatory Rural Appraisal (PRA) consisting of focus group discussions followed by semi-structured interviews, was conducted in three agro-ecological areas in Niger. Two *bmr* genes (*bmr6* and *bmr12*) from three donor parents were introgressed into two Nigerien recurrent parents using hand emasculatation in Sotuba, Mali. Ninety-four (94) derived BC1F3 families plus 6 checks were phenotyped using alpha lattice design in two sites (Tillabery and Konni) in Niger in 2017 rainy season. Selected derived families' chemical compositions were analyzed at Sotuba Animal Nutrition Laboratory (LNA) in 2018 in Mali. Ten (10) EMS mutated accessions out of 554 phenotyped in Niger, were sequenced for candidate SNP discovery in Purdue University in 2018. PRA results revealed that farmers cultivated sorghum for dual purpose (grain and stover) with high preference for sorghum stover compared to pearl millet for cattle feeding. Farmers complained of

feed shortage during dry season coupled with poor quality of their millet and sorghum stovers. Stover trading was a growing business despite the traditional poor management practices. Main criteria for forage quality traits were higher biomass, juiciness, stay-green for both farmers and traders. There were significant to highly significant differences among *bmr* derived families for grain and dry matter yield potential within and across sites. Grain yield ranged from 3271.2 kg/ha to 1205.4 kg/ha while dry matter yield varied from 6909.8 kg/ha to 2867.0 kg/ha. Significant variations were observed for the dry matter, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) contents between parental and derived lines. Results from 10 EMS lines revealed *bmr2*, *bmr6*, *bmr12* and stacked *bmr2/bmr6* genotypes. No candidate SNP was found for 2 EMS mutants for the known *bmr* genes suggesting that these could represent new mutations. Further investigations may identify the nature of their candidate mutations. Deeper phenotyping on field and nutritional values are essential to detect higher yielding lines to confirm the already interesting data collected during the course of this study for improved cattle production in Niger.

DEDICATION

This work is dedicated to my wife Ramatou Adamou Djibo, our adorable daughters Aïchatou and Samira, my mother Aissata Timbo and my brothers and sisters. I love you and say thank you for your exceptional and uncommon characters.

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LIST OF ABBREVIATIONS

FAO: Food and Agriculture Organization of the United Nations.

SSA: Sub-Saharan Africa.

INS : Institut National de la Statistique.

NGO: Non-Governmental Organization.

Bmr: Brown midrib.

CGIAR: Consultative Group on International Agricultural Research.

EMS: Ethyl MethaneSulfonate.

BC: Before Christ.

KG: Kilogram.

DNA: Deoxyribonucleic acid.

INTSORMIL: International Sorghum and Millet.

PRA: Participatory Rural Appraisal

GPS: Global Positioning System

INRAN: Institut National de la Recherche Agronomique du Niger

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

UNDP: United Nations Development Program

ECOWAS: Economic Community of West African States

IRAT: Institut des Recherches Agronomiques Tropicales et des Cultures Vivrières

SNP: Single Nucleotide Polymorphism

CTAB: Cetyl TrimethylAmmonium Bromide

CHAPTER ONE

1.0. GENERAL INTRODUCTION

Sorghum bicolor (L.) Moench is an important food, feed and industrial crop world-wide. Sorghum ranks fifth in global production among cereals and is the staple food crop for 500 million people in Sub-Saharan Africa (SSA) with an annual production of 60 million tons (FAO, 2014). More than 35% of sorghum is grown for human consumption, the remaining is used for animal feed, alcohol production and industrial products (Awika and Rooney, 2004).

In Niger, sorghum is the second most important cereal crop after pearl millet and the country is ranked fifth in Africa in terms of production (FAO, 2018). All five of the cultivated races of sorghum according to Harlan's classification (bicolor, caudatum, durra, guinea and kaffir) are found in Niger but, the caudatum and durra races are more common over much of Niger (Clark and Kitch, 1982). Total area under sorghum cultivation was 3,111,100 ha with a total production of 1,375,700 tons leading to an average yield of 0.442 t ha⁻¹ (INS, 2013). Sorghum's low yield is attributed to biotic and abiotic constraints that negatively affect the agricultural systems and food supply. Sorghum is indeed grown in the marginal areas of Sahel / Sudan zones with an average annual rainfall of 350 mm, and up to 600 - 800 mm in Sudan savannah zone and more than 800 mm in the small area covered by the Guinea savannah zone in Niger (JAICAF, 2009).

Agricultural activities in Niger face serious climatic risks with soils providing low nutrients to sorghum plant which is grown from June to September. During this cropping season, rains are erratic and not well distributed. This situation is worsened by a low use of chemical fertilizers. Nevertheless, sorghum shows a good adaptation under these difficult growing conditions

conferring its strategic role in the search for food and feed security. Sorghum stover is used to feed livestock (cattle and small ruminants) and its stems as building materials by farmers.

In rural areas of Niger, sorghum is a staple food where a wide range of foods and beverages are prepared from the grain (thin = fura in local language) and thick (tuwo = in local language) porridge or couscous. Furthermore, sorghum residues constitute an important source of forage for the important Niger livestock. In fact, Niger ranks as the eighth largest livestock holder in Africa (Yameogo *et al.*, 2014). In the Sahel region, Niger with 10.5 million tropical livestock units (TLUs) has the largest herd population (World Bank, 2013). There is thus an important potential for beef production and exportation. Spore (2015) claimed that pastures and crop residues represent about 90% of feed for large ruminant animals in Niger. However, crop residues as dry roughages from cultivated grain are rich in fiber and low in nitrogen, minerals and vitamins, furthermore with forage maturity, there is decreased nitrogen, digestibility and increased fiber and lignin contents (Sultan *et al.*, 2011).

Consequently, a large part of sorghum stover is thus not efficiently used by animals. Suad *et al.* (2015) highlighted that in view of the pressing demand for fodder coupled with the importance of the grain of sorghum for people in SSA, it is imperative to reconsider the present mono-commodity breeding strategy of sorghum. Furthermore, Spore (2015) forecasted that between 2005 and 2030, meat and milk consumption in Africa will grow by 2.8 and 2.3% per year respectively and according to Aschalew (2014) many scientists can agree with rapid population growth, urbanization and rise in per capita income are opportunities for livestock revolution that is expected to be happening in the 2020s. However, Africa's livestock producers are currently not able to meet the rising demand. In particular, providing Africa's livestock with sufficient quantity

and quality feed which is a critical issue that researchers, NGOs (Non-Governmental Organizations) and policymakers, amongst others have to address (Spore, 2015).

The use of sorghum as a forage crop is in fact growing in many regions of the world due to its high biomass productivity and ability to utilize efficiently water even under drought conditions (Sanchez *et al.*, 2002). Forage sorghums constitute a particular group that was classified by Reed *et al.* (1988) into (i) greenchop-haylage-pasture grazing types that generally include sudangrass (subrace *sudanense*), sudangrass x sudangrass hybrids, or sorghum (*bicolor* grain type) x sudangrass hybrids, (ii) dual-purpose sorghums, usually *bicolor* grain types, tall 3 dwarf or normal 2 dwarf in height, that are characterized by high grain:stover ratios, (iii) sweet sorghum cultivars or hybrids involving grain x sweet hybrids fed as greenchop, silage, or hay, (iv) silage types, usually 0, 1, or 2 dwarf types, that are ensiled, or (v) stubble residue after grain harvest that is supplemented with high-protein legume forages and/or grain concentrates as a good temporary roughage source.

The genus *Sorghum* covers various species with high forage potential. Among them, the brown midrib types are well known for forage quality improvement. Incorporating the *bmr* trait into these crops could result in increased feed efficiency due to the reduction in lignin content with no repercussions on plant physiology. *Bmr* genotypes utilization in practical plant breeding programmes would not be difficult. Indeed, *bmr* genes inheritance is simple recessive and the *bmr* genes exhibit a useful morphological marker because it produces an easily identifiable phenotype (Bittinger *et al.*, 1981). Another advantage of *bmr* sorghum lines is that they tend to be less susceptible to insects and pest damage compared to their wild types (Dowd *et al.*, 2016), tolerant to fungal diseases at equal or possibly greater level than their wild-type (Funnell and Pedersen, 2006) and the resistance to lodging compared to their wild types (Oliver *et al.*, 2005). In addition,

Hisano *et al.* (2009) revealed that plants with low lignin had high total carbohydrate levels in biomass reflecting a compensation for the reduction in lignin level on a mass balance basis. The compensation mechanism suggest the existence of a cell wall sensing system which relays information from the cell wall to the interior of the cell, so it can react with regard to the synthesis of new cell wall components (Hannes *et al.*, 2015).

Several studies have been reported on the digestibility of stover. Chemical and genetic approaches have been used to improve forage fiber digestibility by reducing the amount of lignin or the extent of lignin cross linking with cell wall carbohydrates. Gerhardt *et al.* (1994) and Oliver *et al.* (2004) reported that forage sorghum digestibility can be significantly improved with the use of *bmr* genes.

In Niger, as well as in the other countries of West Africa, technology for treating cereals straw with urea and supplementation with multi-nutritional blocks have been popularized but poorly adopted by Farmers (Abdou, 2010). On the other hand, enhancing the production quality with farmers and stovers traders' implication of this abundant and available stover through genetic improvement may have a significant impact on livestock productivity. Indeed, Kelley *et al.* (1996) concluded that farmers' new variety adoption is highly linked to its stover productivity. In this view, Tunde and Augustine, (2014) reported that farmers empirically estimate the value of a given feed from observations of animal responses. Crop residues play a strategic role for livestock feeding at small scale farmers level because they are harvested from crops that are primarily cultivated for grain (CGIAR, 2011).

To date no studies have been reported on the nutritive value and the impact of sorghum containing the *bmr* trait on livestock feeding in Niger. *Bmr6* and *bmr12* genes are currently use to develop new dual purpose sorghum lines with high stover quality by introgressing *bmr* traits in local elite

varieties. In addition to *bmr6* and *bmr12*, the sorghum breeding programme hypothesized that the EMS mutations induction in BTx623 (*Sorghum bicolor* reference genome) already cultivated in Niger will lead to new *bmr* variants with better nutritive quality. Indeed, according to Yusuff *et al.* (2016) mutagenesis is now a pillar of modern plant breeding. In fact, in plants, spontaneous mutations are very rare and random in terms of time of occurrence and that makes them more difficult to use in plant breeding programmes (Lönnig, 2005).

The main objective of this thesis was to develop dual purpose sorghum lines with high quality stover to sustain livestock productivity in Niger.

The specific objectives were to:

- i. identify farmers' and stover traders' preferences on sorghum stover varieties,
- ii. introgress *bmr6* and *bmr12* genes in two Niger elite sorghum lines,
- iii. determine the agronomic and nutritive potential of new *bmr* lines, and
- iv. identify SNP mutations in EMS-induced sorghum mutants used in the national sorghum improvement programme.

CHAPTER TWO

2.0. LITERATURE REVIEW

Sorghum bicolor (L.) Moench is a tropical grass. It is considered to be predominantly a self-pollinated diploid species ($2n = 20$) and belongs to the *Poaceae* family (House, 1985). It is treated as an annual in temperate or subtropical climates where cold temperature limits growth and development (Duncan, 1996). Sorghum has a great genetic diversity composed mainly of *bicolor*, *guinea*, *caudatum*, *durra*, and *kafir* races with ten intermediate races recognizable by spikelet or the panicle morphology (Harlan and de Wet, 1972). It is also unique in its ability to thrive under a wide array of harsh environmental conditions and in some areas like Sub-Saharan Africa (SSA) and India, it is arguably the most important cereal crop and it is used as source of food and feed (Suad *et al.*, 2015). Xin *et al.* (2009) reported the high water efficiency use of sorghum and its tolerance to low soil fertility in comparison with the major cereal crops. These traits make sorghum particularly advantageous as feedstock crop. Getachew *et al.* (2016) highlighted the potential of sorghum as forage for livestock in areas with limited water availability.

History on forage crops can be traced back to about 1300 BC when alfalfa was cultivated in areas of modern Turkey (Iqbal *et al.*, 2015). Forage was defined as a succulent green plant form and as crops harvested and cured as hay or silage to feed animals during lean periods when there was scarcity of green forage. During feed scarcity, livestock rely mostly on crop residues for feeding which can be up to 45% of their total annual feed intake, and up to 80% during critical periods (Sandford, 1989). In countries like Niger natural pasture and crop residues represent about 90% of feed for large ruminant animals (Spore, 2015). In the case of Sudan, where the second largest animal wealth in the African continent exists, sorghum straw has a great contribution in maintaining the national herd (Maarouf *et al.*, 2014). In this view, Buso *et al.* (2011) emphasized

that the advantage of using sorghum is the possibility of producing greater biomass volume, associated with better quality silage, with digestible fibers for high animal consumption and performance in areas with cultivation restrictions for maize and other forages. Another advantage of sorghum is its great genetic resource variability including brown midrib types.

2.1. Nature of the Brown midrib character

The brown midrib (*bmr*) is a phenotypic marker. Sattler *et al.* (2010) reported that this phenotype has been observed in maize (*Zea mays*), sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*) and was associated with reduced lignin levels and altered lignin composition compared to wild-type. In sorghum, Ayyangar and Ponnaiya (1941) reported that the abbreviation *bmr* was adopted to distinguish it from *bm*, already in use for the sorghum bloomless mutants (lack of waxy coating, which gives the typical colour to the stem and leaves). The *bmr* plants display a useful brown morphological marker on leaves and stems (Rao *et al.*, 2012). The mutation responsible of the *bmr* phenotype occurs spontaneously or is induced by chemical or physical mutagenesis agents. *Bmr* mutants can also be created by genetic engineering.

Bout and Vermerris (2003), stated that the *bmr* phenotype is controlled by a single recessive gene and it is the result of a recessive mutation in the lignin biosynthesis pathway. Hanna (1982) reporting Porter *et al.* (1978) treated seeds with 0.1 and 0.2% diethyl sulphate (DES) to induce *bmr* mutants with reduced lignin which were identified in segregating M₃ head rows. The same conclusion was drawn by Nagaraja *et al.* (2008). Furthermore, Casler *et al.* (2003) claimed that when the mutation is present in the homozygous recessive state, it results in reduction of lignification, cell-wall concentration, increases digestibility and voluntary intake of feed by ruminants. In addition, Batista de Aguilar *et al.* (2014) reported likewise that this mutation

increases forage digestibility, dry matter intake and productivity per animal. Consequently, plants with the *bmr* genes contain less lignin and are highly digestible. Cherney *et al.* (1991), by illustration asserted that lignin concentration of *bmr* lines has been reduced by 5 to 50%; a 10 g kg⁻¹ decrease in lignin generally resulted in a 40 g kg⁻¹ increase in digestibility thus animal performance may increase by up to 30%.

In terms of physiological properties, Jung and Fahey (1983) proposed that the lignin content in *bmr* plants is poorly polymerized and contain fewer phenolic monomers that can affect digestion. It is well known that modifying lignin content or composition is important to enhance stover digestibility. In fact, plant lignin, after cellulose, is the second most important polymer in nature and while being beneficial to plants, the complex linkages between lignin and structural carbohydrates are detrimental to the digestibility of plant residues by livestock (Humphreys and Chapple, 2002). Moreover, according to Aydin *et al.* (1999) lignin is the primary indigestible component of plant cell walls, it inhibits digestion of cell wall carbohydrates in the rumen.

2.2. Types of genes controlling brown midrib trait in sorghum

Bmr mutants were first discovered in maize in 1924 in a self-pollinated line of a northwestern dent maize, resulting finally in the description of a total of four genes with each gene segregating as a simple Mendelian recessive character (Barriere *et al.*, 2004). Following the finding of these genes, a series of nineteen *bmr* mutants were isolated from diethyl sulfate (DES) mutagenized populations at Purdue University in the 1970s (Porter *et al.*, 1978). Those *bmr* mutants were designated *bmr-1* through *bmr-19*. However, six mutants were deleted due to sterility and poor phenotypic expression and three were selected as most agronomically acceptable (Fritz *et al.*, 1988). Later additional spontaneous *bmr* mutants of sorghum were identified in breeding populations (Vogler

et al., 1994). Porter *et al.* (1978) suggested that in mature *bmr* sorghum plant the lignin content is half lesser than the wild type, whereas its leaf lignin content was just one quarter. These results were also confirmed by Grant *et al.* (1995). Indeed, those authors showed that the *bmr* mutants of sorghum have significantly lower levels of lignin content; they estimated around 51% less in their stems and 25% less in their leaves and furthermore *bmr* sorghum silage had 17% less lignin than regular sorghum silage making it more digestible. Allelism tests on the sorghum *bmr* mutants showed that several of the mutations are allelic (Bittinger *et al.*, 1981). Saballos *et al.* (2008) proved that up to date four allelic classes of sorghum *bmr* mutants have been identified which are *bmr2*, *bmr6*, *bmr12* and *bmr19*, with the first three showing the greatest potential for increasing biomass conversion. Sattler *et al.* (2009) confirmed that the *bmr6* and *bmr12* genes have been cloned; later Saballos *et al.* (2012) stated that mutations in 4-coumarate coenzyme a ligase (4CL), cinnamyl alcohol dehydrogenase (CAD2) and caffeic O-methyltransferase (COMT) genes contribute to the phenotype of *bmr2*, *bmr6* and *bmr12* groups respectively.

2.2.1. Sorghum *bmr6*

In sorghum, *bmr6* encodes the major cinnamyl alcohol dehydrogenase (CAD) protein involved in lignin synthesis (Sattler *et al.*, 2009). Reduced CAD activity altered cell wall architecture, reduced lignin level, and the incorporation of phenolic aldehydes into lignin in sorghum and maize (Palmer *et al.*, 2008). *Bmr6* plays an important role in sorghum lignin biosynthesis. Sattler *et al.* (2009) affirmed that *bmr6* affects phenylpropanoid metabolism, resulting in reduced lignin concentrations and altered lignin composition in sorghum. Further, Sattler *et al.* (2010) confirmed that *bmr6* plants were shown to have limited cinnamyl alcohol dehydrogenase (CAD which is a precursor for polymerization to lignin) activity, the enzyme that catalyzes the conversion of hydroxycinnamoyl

aldehydes (monolignals) to monolignols. Humphreys and Chapple (2002) demonstrated that the last step in monolignol biosynthesis is the reduction of cinnamyl aldehyde precursors, catalyzed by the enzyme cinnamyl alcohol dehydrogenase (CAD). Reduced CAD activity results in increased digestibility on dry weight basis, altered cell wall architecture, reduced lignin level, and the incorporation of phenolic aldehydes into lignin in sorghum and maize (Pillonel *et al.*, 1991). Sattler *et al.* (2009) affirmed that genomic and cDNA sequences of Sb04g005950, the gene whose predicted amino acid sequences shared significant similarity to the Arabidopsis proteins AtCAD4 (67.7% and 83.4%) indicated that a C-to-T transition mutation is present in the *bmr6* gene and the mutation changed amino acid 132 of the protein from Glutamine (CAG) to a stop codon.

2.2.2. Sorghum *bmr12*

Bout and Vermerris (2003) claimed that among a number of the sorghum *bmr* mutants having a significantly lower lignin content in stems and leaves compared to their wild-type counterparts, the most severe reduction in lignin was found in the *bmr12* and *bmr18* mutants. Allelism tests by Bittinger *et al.* (1981) indicated that *bmr12* and *bmr18* are allelic and different from *bmr6*. However, Bout and Vermerris (2003) demonstrated that regarding their flowering time, a difference exists with *bmr12* mutant flowering later than its wild-type whereas no significant difference was observed between the *bmr18* and its wild-type. In addition, the cell wall composition of leaf blade tissue from *bmr12* mutant contained less *p*-coumaric acid which is primarily esterified to syringyl residues (Ralph *et al.*, 1994), therefore a reduction in amounts of syringyl residues is observed.

Sattler *et al.* (2010) indicated that *bmr12* encodes a caffeic *O*-methyltransferase (COMT) which catalyzes the penultimate step in monolignol biosynthesis, the transfer of a methyl group from *S*-adenosyl-methionine (SAM) to the 5-hydroxyl group of 5-hydroxy-coniferyl substrates to form

sinapyl products in conformity with Li *et al.* (2000) whose study showed that caffeic acid O-methyltransferase (COMT) controls the synthesis of syringyl lignin units.

2.3. Lignin biosynthesis and composition

Lignin is a complex, amorphous biopolymer matrix; it serves critical functions such as the structural integrity of cell wall tissues for vascular plants. Indeed, lignin is required for vascular elements to transport water under negative pressure, an important function for adaptability (Boyce *et al.*, 2004). Zheng-Hua *et al.* (2000) added that lignin offers rigidity to the walls of conducting tracheary elements (TEs) for enduring the negative pressure generated from transpiration, it also renders the walls of maturing TEs undigestible by hydrolytic enzymes released during autolysis of xylogenesis. In addition, Zheng-Hua *et al.* (2000) affirmed that the deposition of lignin in the walls of sclerenchyma cells adds physical toughness and chemical durability to the walls but may deter feeding by herbivores. Lignin action may also block the liberation of sugars from the cell wall polysaccharide moieties, release compounds that can inhibit microbes used for fermenting sugars to fuels, and adhere to hydrolytic enzymes (Sattlet *et al.*, 2009). However, lignin can also offer a physical barrier for protection of adjacent tissues from damage after deposition at the sites of wounding or pathogen invasion. Therefore, lignin content reduction in plant cells has become an area of interest for bioenergy feedstock improvement (Chen and Dixon, 2007). Lignin content of crop plants has been reduced by traditional plant breeding, natural and induced mutations, and insertion of transgenes (Pedersen *et al.*, 2005). Lignin, the third cell wall component, is an ubiquitous polymer whose abundance in the world is exceeded only by cellulose (Jung and Ni, 1998). It accounts for approximately 30% of organic carbon in the biosphere (Boerjan *et al.*, 2003). Conversely, severe reduction of lignin content may affect plant fitness (Sattler *et al.*, 2014). Indeed,

many studies such Jones *et al.* (2001); Piquemal *et al.* (1998); Ruel *et al.* (2009) reported the collapse of vascular elements in mutants extremely impaired in lignin synthesis. In the same idea, Mir Derikvand *et al.* (2008) showed that at very low levels of lignin, plant growth and development are affected.

The biogenesis of the lignified plant cell wall is a complex metabolic process in which the formation and modification of several cell wall polymers need to be coordinated (Saballos *et al.*, 2008). In the same way, lignin biosynthesis is a complex metabolic process and Dauwe *et al.* (2007) define lignin as an aromatic heteropolymer and an important component of secondarily thickened cell walls contributing to make them rigid. They have attracted significant research attention because they represent a major obstacle in chemical pulping, forage digestibility and processing of plant biomass to biofuels (Vanholme *et al.*, 2008). In addition, understanding lignin biosynthesis and properties were important targets for feed improvement (Mackay *et al.*, 1997). The deposition of lignin in plant cell walls was part of the mechanisms which allowed the development of upright plants of large size adapted to a terrestrial habitat (Alain, 2000). However, the exact mechanism of lignin biosynthesis is still unclear, and many researchers such as Saballos *et al.* (2008) affirmed that lignin is synthesized via the oxidative coupling of several phenolic monomers and the traditional view holds that lignin is derived from the monolignols *p*-coumaryl, coniferyl and sinapyl alcohol, which give rise to *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) residues respectively. Bonawitz and Chapple (2010) affirmed that the deposition of each lignin type in plant tissues is spatially and temporally controlled: first being deposited H units, followed by G units, finally S units. It is important to notice that Chloe *et al.* (2002) affirmed that in angiosperms, lignin is composed of guaiacyl (G) and syringyl (S) units, the G unit is singly methylated on the 3-hydroxyl group, whereas the S subunit is methylated on both the 3- and 5-

hydroxyl moieties. The ratio of S-to-G subunits dictates the degree of lignin condensation by allowing for different types of polymeric linkages, in fact increased G content leads to more highly condensed lignin composed of a greater proportion of biphenyl and other carbon-carbon linkages, whereas S subunits are commonly linked through more labile ether bonds at the 4-hydroxyl position (Guo *et al.*, 2001). For Pond *et al.* (1987), in forage crops harvested at maturity, lignin content increases, particularly the S subunits which may negatively affect the digestibility and ultimately the nutrient availability of alfalfa and other forage grasses. Rabinovitch *et al.* (2001) reported that the conventional nomenclature for the monomers are called monolignols. According to Sattler *et al.* (2012), lignin subunits are derived from the amino acid phenylalanine, hence both phenylalanine metabolism and lignin biosynthesis occupy central roles in plant metabolism. Boerjan *et al.* (2003) detailed that Phenylalanine, the primary substrate in the lignin biosynthetic pathway, is derived from the shikimate pathway and then modified by the enzyme phenylalanine ammonia lyase (PAL) to form cinnamic acid. Subsequently, a series of enzymes such as hydroxylases [*p*-coumarate-3-hydroxylase (C3H), cinnamate-4-hydroxylase (C4H), ferulate-5-hydroxylase (F5H)], methyl transferases [caffeic acid-O-methyltransferase (COMT), caffeoyl-CoA-O-methyltransferase (CCoAOMT)], reductases [cinnamoyl-CoA-reductase (CCR)], and dehydrogenases [cinnamyl alcohol dehydrogenase (CAD), sinapyl alcohol dehydrogenase (SAD)], produce lignin biosynthetic pathway components such as alcohols, acids and aldehydes. Alain (2000), added that lignin synthesis requires significant energy and the final product is highly heterogeneous in nature but always exhibits a higher C/H and C/O ratios than the other polymers of the cell wall resulting in a higher calorific value. Recently, Konovalov *et al.* (2015) asserted that the biosynthesis of lignin takes place on the external side of the cell, on a polysaccharide matrix at

certain positions which contain hydroxycinnamic (n-coumaric and ferulic) acids bound to the cell wall; their phenolic oxy-groups serve as “anchors” for the process of lignification.

The composition of lignin contains aromatic metabolites: aldehydes and acids (Dalimova *et al.*, 1994). Aldehydes (monolignans) are included in the lignin in the same manner as monolignols, via peroxidase and laccase (Ros Barceló *et al.*, 2001). Konovalov *et al.* (2015) believe that the lignin, having aldehydes in its composition, becomes colored red or reddish-brown.

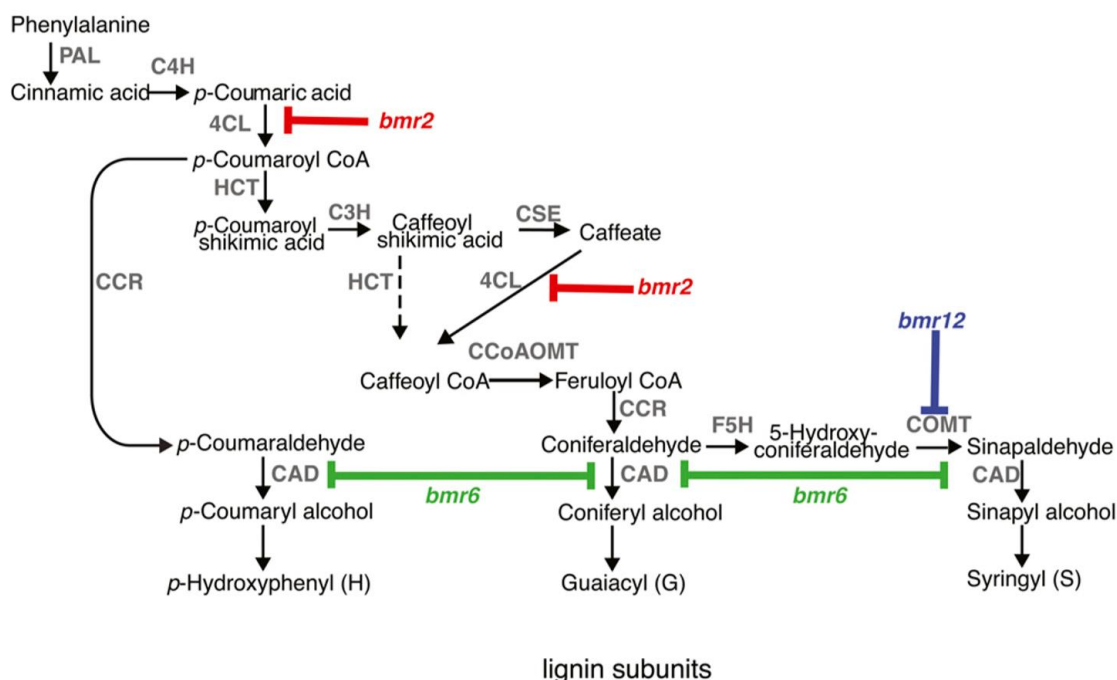


Figure 2. 1. The monolignol biosynthetic pathway in sorghum. Sattler *et al.* (2014)

The enzymatic steps (gray) are as follows: phenylalanine ammonia lyase (PAL); cinnamate 4-hydroxycinnamoyl CoA:shikimate transferase (HCT); p-coumarate 3-hydroxylase (C3H); caffeoyl shikimate esterase (CSE); caffeoyl CoA O-methyltransferase (CCoAOMT); cinnamyl CoA reductase (CCR); ferulate 5-hydroxylase (F5H); caffeic acid O-methyltransferase (COMT); and cinnamyl alcohol dehydrogenase (CAD). The *bmr2*, *bmr6* and *bmr12* mutants are impaired in 4CL, CAD and COMT enzymatic activities, respectively. Source: Sattler *et al.* (2014)

2.4. Lignin mutation degradation

Plants depend on their cell walls for rigidity and fitness (Santiago *et al.*, 2013). The cell wall of plant biomass is composed of cellulose, hemicellulose, lignin, and minor components (Cassie *et al.*, 2015). In many regions, grasses are generally used in livestock feeding however for an efficient nutrition, the degradation of fiber in animal stomach is an important factor limiting forages utilization. Carpita and Gibeaut (1993) reported that cell walls of Gramineae differ from the cell walls of many other species in terms of both their carbohydrate and lignin composition while Jung and Fahey, (1983); and Grisebach (1981) confirmed that lignin is a factor limiting the extent of digestibility of cell wall polysaccharides by animals. In addition, Jung and Allen (1995) asserted that it may highly influence rumen microbes' action on plants cellulose and hemicellulose therefore in breeding for improving the feeding value of a crop, cell wall digestibility is the target. Barrière *et al.* (2003) reported that this is obvious when the harvested plant has no grain. Most research efforts to control lignin have focused on biosynthesis of the monolignols, most of the enzymes involved in the monolignol synthesis have been cloned and characterized in *Arabidopsis thaliana* (At) and others dicot species (Anterola and Lewis, 2002).

The most rapid method of improving forage sorghum quality is to improve In Vitro Dry Matter Digestibility (IVDMD). Pedersen *et al.* (1982) and Boerjan *et al.* (2003) reported that the lignin polymer cross-links the polysaccharides, rigidifying and reinforcing the secondary cell wall structure. Sattler *et al.* (2012) reported that this resulted in a barrier that is chemically and microbially resistant. Furthermore, Sattler *et al.* (2014) showed that this resulting matrix is refractory to both chemical degradation and biological digestion, which impairs hydrolysis of the polysaccharides into their monomeric sugars in ruminant livestock or cellulosic bioenergy systems.

Chemical mutagenesis by soaking sorghum seeds in diethyl sulfate or ethyl methane sulphonate (EMS) induces mainly point mutations, and are thus ideal for producing missense and nonsense mutations that are known to produce phenotypic changes which could be desirable in crop improvement. Among the chemical agents, the alkylating agent (EMS) is the most commonly used in plants because of its potency and ease to use. Another advantage of EMS is the low level of chromosomal breaks and lethal effects (Greene *et al.*, 2003). EMS alkylates guanine bases and leads to mispairing-alkylated G pairs with T instead of C, resulting in primarily G/C- to-A/T transition mutations (Ali *et al.*, 2012). Sorghum *bmr* genotypes have been shown to contain less lignin, therefore they are more digestible than normal forage sorghum. Oliver *et al.* (2005) found that digestibility is due to two different modifications in biochemical pathways by which lignin is produced in the plant. The *bmr* phenotype is characterized by the presence of a brown coloured midrib in sorghum, maize and pearl millet (Rao *et al.*, 2012). Halpin *et al.* (1998) stated that the brownish pigments are strongly associated with the lignin as they persist in the cell wall after the removal of hemicellulose and cellulose. The *bmr* phenotype has been widely used mutants' identification (Sattler *et al.*, 2014). For the phenotype evaluation, it is important to notice that, the *bmr* phenotype is visible at seedling stage but tends to fade at physiological maturity. Although the intensity cannot be taken as a measure of reduction in lignin, it is a clear indicator that the *bmr* genes are present.

2.5. Feeding cattle with *bmr* mutants

Lima *et al.* (2005) considered that sorghum has the potential to be used as ruminants feed, especially in semi-arid regions where it is a major source of fodder and for being resistant to drought and high temperatures, high yield, and high nutritional value. In fact sorghum has been shown to be an excellent silage in areas characterized by unfavourable growing conditions for

maize. In many milk producing regions sorghum constitutes an important forage crop for grazing dairy cows. Some studies have demonstrated that the *bmr* silage with or without protein supplements increases significantly the milk production of lactating cows (Cherney *et al.*, 1991; Oba and Allen, 1999) and animal performance. These properties make the use of *bmr* sorghum for cattle feeding very attractive in many countries. According to a paper from INTSORMIL (International Sorghum and Millet), University of Nebraska-Lincoln (2013) a trial conducted in some countries in central America (Costa Rica, El Salvador, Nicaragua, Honduras, Guatemala, Panama and Haiti) using *bmr* sorghum for feeding dairy cows, dairy farmers had an average increase of 20% in milk production and weight gain of the cattle was 900 g per animal per day which represents an increase of 10% over conventional sorghum. The *bmr* sorghum lines were very rapidly spread throughout Central America and had a significant impact on increasing rural income and promoting food security.

2.6. Sorghum crossings techniques

Sorghum inflorescence is a panicle. Indeed, Brown *et al.* (2006) reported that the inflorescences show primary, secondary and tertiary branching. Morphologically, the panicle (or head) varies from compact to open. Sorghum is thus a perfect-flowered plant and is a normally self-pollinated crop with however a certain level of outcrossing. This level of outcrossing is contingent on the specific genotype being grown and the environmental conditions encountered prior to and during anthesis (Schertz and Dalton 1980). House (1985) estimated the outcrossing from 1 to 10% and can also reach to 30-60% depending to the panicle compactness. In general, the knowledge of plant reproduction mode is important for a successful improvement programme. For sorghum improvement, the discovery of genetic inheritance and the development of plant breeding principles in the early 20th century were the levers rapidly adopted by sorghum breeders and used

to address critical issues (Rooney, 2004). Sorghum flowering starts a few days after panicle emergence from the top toward the bottom of the panicle. Based on the flowering habits, sorghum breeders have developed several methods of hybridization and outcrossing elimination (genetic male sterility; cytoplasmic male sterility; hot-water emasculation; anther dehiscence control by use of humidity; chemical control of anther dehiscence). Indeed, fertile line panicles' early selfing ensure pure line maintenance whereas several crossing methods were established for segregating populations' development. Below, the principle crossing technics are described and the table 2.1. summarizes the advantages versus disadvantages of each technic.

2.6.1. Hand emasculation

The chosen panicle for emasculation is prepared 1-2 days before. Flowers are emasculated the day before anthesis. Such florets occur below and within about 3 cm of opened florets in a sorghum panicle. All open spikelets are removed with scissors. In addition, all florets except those that are to be emasculated are removed, leaving only the florets that are expected to open the next day. The three anthers are coaxed out of the enclosing lemma and palea by inserting a sharpened pencil or similar pointed instrument. Care must be taken not to break the anthers, and if the anther is breached, that flower should be removed and instruments rinsed to avoid contaminating the next floret. Every anther must be removed before the set of florets is completely emasculated. (Rooney, 2004).

Table 2.1 Advantages and disadvantages of sorghum different crossing technics

Method	Advantages	Disadvantages
Hand emasculation	Require simple tools (House, 1985)	Varieties differ in ease of emasculation (House, 1985) Produce small quantities of seeds (Schertz and Dalton 1980) Takes special skill and time consuming (Schertz and Dalton 1980)
Genetic male sterility	Simple and breeders can make a large number of crosses in a short amount of time (Rooney, 2004)	Can not be use for commercial hybrids seeds production
Cytoplasmic male sterility	Simple and breeders can make a large number of crosses in a short amount of time (Rooney, 2004)	Makes possible the commercial hybrid seeds production (House, 1985)
Hot-water emasculation	Simple to use in green house (Rooney, 2004)	High level of seedlings from self-fertilization in the nurseries. Varieties respond differently to this technic (House, 1985)
Anther dehiscence control with humidity	Simple and breeders can make a large number of crosses in a short amount of time (Rooney, 2004)	A certain level of self-pollination can occur in seed from a poured cross. (Rooney, 2004) In most cases, the proportion of progeny that are F1 hybrids will vary based on the specific genotype used as a female parent, the fecundity of the pollen parent, and the environmental conditions during the process. (Rooney, 2004)
Chemical control of anther dehiscence		Require a good control of the technic

Source: House (1985); Rooney (2004); Schertz and Dalton (1980)

2.7. Sorghum improvement: The backcross breeding method

According to Reddy *et al.* (2012), sorghum, improvement research has come a long way from using simple methods like mass selection to advanced level of selection using molecular markers for trait improvement. The backcross breeding method was first proposed by Harlan and Pope (1922). The method is widely used by sorghum breeders to transfer a gene from one source (donor parent) to another line (recurrent parent). Backcrossing is useful for several breeding objectives such as insects, diseases, drought tolerance, high lysine or proteins content (House, 1985). This author added that the relationship between the donor and recurrent parents is an important aspect in a backcross breeding method. In other words, if a desired character is found in a kafir and a durra type, and it is desired to transfer this character to a kafir, then it would be better to use the kafir rather than the durra source as the donor parent (House, 1985). In plant breeding, the backcrossing method is used to improve a well-adapted line that lacks a particular gene. In the crossing program, the well adapted desirable parent is called recurrent parent and the source of the desirable gene lacked in the recurrent parent background is called donor parent. Even though the chief role of the donor parent is to provide the missing gene, it should not be significantly deficient in other desirable traits. In fact, the rationale of backcross breeding is to replace a specific undesirable gene with a desirable alternative while preserving all other qualities (adaptation, productivity, etc.) of an adapted line (Acquaah, 2007). Therefore, this breeding method enables breeders to transfer desirable traits in a specific genetic background. Instead of inbreeding the F1 as normally done, the F1 is crossed with the desirable parent. In successive generations, progenies are selected for the characteristic of interest and then backcrossed to the recurrent parent in order to recover the maximum genome of the RP carrying the trait of interest (Hospital, 2005). Acquaah (2007) mentioned that a selection pressure is used to identify and eliminate undesirable

individuals. For recessive trait, a progeny test is necessary to determine the genotype of a backcross progeny before continuing with the next cross. This authors emphasized that backcross method of breeding is best suited to improving established cultivars that are later found to be deficient in one or two specific traits. However, Fehr (1987) reported that the success of backcrossing is influenced by the number of genes controlling a character and the role of the environment on the phenotypic expression of a gene(s). For Acquah (2007) the backcross method is most effective and easy to conduct when the missing trait is qualitatively (simply) inherited, dominant, and produces a phenotype that is readily observed in a hybrid plant. If the trait is simply inherited, the RP can be effectively covered with the trait incorporated (House, 1985). The procedure for transferring a recessive trait is similar to that for dominant traits, but entails an additional step.

2.7.1. Genetic issues of backcross method

In the breeding process to improve the recurrent parent, with each backcross step the progeny becomes more like the recurrent parent. Hospital (2005) affirmed that if selection is applied for the desired characteristic only, then the proportion of donor genome is expected to be reduced by one-half (50%) at each generation, except on the chromosome holding the characteristic. On the chromosome carrying the trait of interest, the rate of decrease is slower due to linkage drag (Stam and Zeven 1981). On the overall genome of the RP; House (1985) reported that with each generation of backcrossing, the genetic composition of the donor parent is reduced by a factor $(1/2)^n$ where 'n' is the number of generation of backcrossing. It is important to consider the status of gene to be transfer. Indeed, the process of dominant gene transfer is easy and fast whereas for a recessive gene it is slightly different because it is not expressed in the heterozygote (House, 1985). In this case, self-pollination is required at each generation in the segregation populations in order to identify the genotypes carrying the gene of interest. Moreover, Acquah (2007) reported that an

additional step is needed after each backcross to produce an F₂ generation in order to identify the recessive trait. If many genes control the character to be transfer, it is probably not possible to backcross for more than one or two generations before a new line must be selected by a pedigree breeding method (House, 1985). Moreover, continuous backcrossing becomes not possible because the expression of the trait becomes less or not measurable.

The genetic advance in backcross breeding depends on several factors. The table 2.2 below describe the advantages and disadvantages of the backcross breeding method.

Table 2. 2. Advantages and disadvantages of the backcross breeding method.

Advantages	Disadvantages
Does not have to be conducted in the environment in which the RP is adapted because all that is needed is to be able to identify and select the target trait	Not suitable for transferring quantitative traits. The trait should be highly heritable and readily identifiable in each generation
Does not require extensive testing: the new and the adapted cultivars are similar except for the newly incorporated trait.	Presence of undesirable linkages may prevent the cultivar being improved from attaining the performance of the original recurrent parent
It is possible to transfer two or more genes by simultaneous selection among the progeny. This undertaking requires a larger population that would be necessary if two genes are transferred independently	Recessive traits are more time consuming to transfer.
It is a conservative method, not permitting new recombination to occur	
Backcross breeding is repeatable. If the same parents are used, the same backcrossed cultivar can be recovered.	
It is useful for introgressing specific genes from wide crosses.	
It is applicable to both self-pollinated and cross pollinated species	

Source: Acquah (2007)

2.8. *Bmr* genes introgression

Rooney (2000) reported that there are three basic colours pigmentation that occur throughout the leaves and stems of sorghum plants: purple, red and tan. These colours are developed on the leaves or culm where the plant has been damaged by insects, diseases or mechanical means. The colours are conditioned by two genes P and Q. Tan plants are genetically ppqq and ppQQ, purple plants are genetically PPqq and PPQQ (Ayyangar *et al.*, 1933).

Morphologically inherited traits such as the *bmr* phenotype observed in maize, sorghum and pearl millet are of a great importance in plant breeding. Roy *et al.* (2004) affirmed that such traits are important from the point of identification of a cultivar. In maize, Barriere and Argillier (1993) reported that the *bmr* genes segregate in a simple Mendelian recessive character (3:1). Gupta (1995) also concluded that the *bmr* gene is controlled by a recessive allele and the segregation in fits 3:1 ratio in pearl millet. In sorghum, the same conclusions were drawn by Porter *et al.* (1978).

2.9. SNP markers for *bmr* genes

In sorghum, Porter *et al.* (1978) informed that there are at least four independent *bmr* loci exhibiting the *bmr* phenotype (*bmr2*, *bmr6*, *bmr12* and *bmr19*). For the *bmr6* phenotype, Saballos *et al.* (2009) revealed that the SNP mutation C-to-T transition mutation at position 2800 of the SbCAD2 genomic sequence. This particular nucleotide change creates a premature stop codon; the resulting protein contains 131 amino acids and lacks the nucleotide binding site (Saballos *et al.*, 2009). For the *bmr12* phenotype, Sattler *et al.* (2012) identified from an EMS-mutagenized TILLING population, four missense mutations in the coding region, which changed evolutionarily conserved amino acids Ala71Val, Pro150Leu, Gly225Asp, and Gly325Ser while for the previously characterized *bmr12* mutants all contain premature stop codons. The missense mutations found had all impact on enzyme activity, Klason lignin content, lignin subunit composition and saccharification yield (Sattler *et al.*, 2012). For the *bmr2* phenotype, Saballos *et al.* (2012) reported that a missense mutation (G to A within the genomic coding sequence at position +468) leads to an amino acid substitutions Gly111Asp and proved that the *bmr2* gene encoded a 4-coumarate coenzyme A ligase (4CL), which catalyzes an early step in monolignol biosynthesis. A single allele represents the *bmr19* locus at this time despite the large numbers of brown midrib mutants

originally isolated from sorghum (Saballos *et al.*, 2008). For Sattler *et al.* (2010) this may indicate that either genetic saturation has not been achieved or alternatively, *bmr19* is not a simple loss of function mutation. Furthermore, Sattler *et al.* (2014) reported that the *bmr19* mutant is not publicly available (effectively reducing the available sorghum *brown midrib* mutants to a set of three independent loci: *bmr2*, *bmr6*, and *bmr12*). Moreover, for Saballos *et al.* (2008), the *bmr19* appears to be of limited value for forage and bioenergy applications, because it did not significantly reduce lignin concentration and did not markedly alter lignin subunit composition.

2.10. Dual purpose cereals: Farmers and traders' preferences in SSA

According to Jacob *et al.* (1997) many countries in semi-arid regions need dual purpose crops to help alleviate feed and food shortage. In Niger, farmers struggle to feed the animals during the long dry season with the poorly nutritional stored crop residues (Abdou, 2010). According to Reddy *et al.* (2004), dual purpose sorghum development requires effort on traits that affect yield and quality of forage and grain. In fact, several studies reported on the importance of residues yield in new varieties adoption for small scale farmers (Magnan *et al.*, 2012; Kelly and Parthasarathy, 1994). Indeed, for the majority of smallholders, crop residues from dual-purpose crops constitute 40-60% of total dry matter intake (Rao et Hall, 2003). In semi-arid regions, crop residues from cereal and leguminous crops, are by far the most important feed source available to farmers (Rao et Hall, 2003). For Miller and Stroup (2004), the potential of sorghum as dual purpose crop is enormous while Tunde and Augustine (2014) reported that farmers in two villages of Niger appreciate stovers qualities based on physical aspects. Chikuta *et al.*, (2014) reported that farmers for dual purpose preferred a variety with high grain yield potential combined with high biomass and high sugar content in Zambia.

CHAPTER THREE

3.0. SURVEY ON CROP RESIDUES FOR CATTLES FEEDING IN NIGER

3.1. Introduction

In Niger agro-pastoralism is the major food and feed production system. In this system, crop residues represent an important resource used for soil fertility management and livestock feeding. Valbuena *et al.* (2014) reported that in areas with low cereal intensity in SSA, crop residues constitute a vital dry season feed resource for livestock particularly when other resources are scarce. Witcombe (1996), reported that in marginal areas, most farmers have not adopted new cultivars because of limited access to improved seeds and perhaps recommended varieties did not have attributes such as high stover yield needed or did not appear as productive as expected. Furthermore, the CGIAR (2011) concluded that crop residues, especially stover and straw are increasingly important commodities that increase the overall value of dryland cereals. In Niger, the important role of cereal stovers in livestock feeding during dry season for small scale farmers is obvious particularly in the context of limited availability of natural pasture. However, the correct feeding of livestock for more productivity in terms of fattening, milk or field work is a challenge that most farmers face. This situation combined to the recurrent feed deficit generated another recent aspect in crop residues utilization. Indeed, Valbuena *et al.* (2012) informed that the increasing use of crop residues for purposes other than mulching implies that these have become a private good with an explicit economic value. Currently in Niger's large cities, appearance of small shops specialized in forage trading is obvious everywhere on the edges of the roads and in the markets in an informal system. The main objective of this chapter was to appreciate well this growing market.

The specific objectives were to:

- determine livestock feeding constraints, stover production and utilization in Niger during the dry season,
- identify farmers' and stover traders' preferences for sorghum stover varieties.

3.2. Material and methods

The current survey was conducted on sorghum farmers and stover traders based on focus group discussion (FGD) and semi structured interviews (SSI).

3.2.1. Farmers

In collaboration with livestock extension service, three villages well known for their livestock rearing and sorghum cultivation in the country were randomly sampled. Extension service agents assisted in the sampling and gathering sorghum farmers with intensive experience beneficial to PRA survey. A FGD with an average of a group of twelve farmers was firstly conducted to have an overview on sorghum production, cattle rearing and feeding constraints. During the FGD, males and female groups were met separately to avoid men's negative influence on women's free expression of their opinions. The different topics discussed during the FGD were evaluated by ranking, rating, listing or sorting by the group of farmers. After the FGD, a list of sorghum farmers rearing cattle in each village was recorded and 30 farmers per village were randomly sampled. The selected farmers were interviewed separately in a semi-structured interview based on a questionnaire structured on four major' points: farmer identification, main activities, sorghum production and livestock rearing (constraints, opportunities, contribution in income, stover quality traits).

The following material was used during FGD:

- Discussion guide line to address keys topics
- Posters on sorghum life cycle for a better understanding
- *bmr* sorghum pictures for illustrations
- Forage sorghum pictures for illustrations
- Exotic improved cow pictures
- Tape recorder to collect oral information
- GPS to reference geographical position of each village
- Camera for pictures
- Excel software for data analyses

FGDs team was composed of: 1 facilitator and moderator: local extension agent; 1 Livestock scientist; 1 PhD candidate for FGD topic introduction and data collection.

Table 3. 1 FGD: Topics and evaluation methods.

Topics	Evaluation methods	
Importance of sorghum crop in the area compared to millet and maize	Ranking	Sorghum Millet Maize
Sorghum production system	Ranking: association vs pure	
Main sorghum production constraints (grain/stover)	Rating	Diseases Drought Fertilizer Soil Others
Domestic sorghum stem/forage utilization	Sorting	Commercialization Animal feeding Domestic utilization Other
Importance in animals feeding compared to millet and maize	Rating	Sorghum Millet Maize
Livestock feeding constraints	Listing	
Farmers' empirical preferences for specific traits and attributes of forage sorghum	Listing	
Which cow is fed during the dry season?	Sorting	Milking cow Pregnant cow Draught cow Bullock
How is cow fed during the dry season?	Rating	Stem chopped and put container Chopped stems put on ground Without chopping put on ground

3.2.2. Stover traders

A census of all the stover salesmen in each village and its surrounding was conducted. This census facilitated the collection of personal information (name, and cellphone number) and the place of every stover owner shop. A questionnaire was then conceived based on the identification, supply, methods of storage and the profitability of the activity to collect information using a semi structured interviews.

3.3. Study areas

3.3.1. Description

- **Soumarana:** is a village in the South-East Niger next to Maradi located at the geographical position of 13°26'38'' North and 6°54'11'' East. The average rainfall is 400-500 mm per year.
- **Dolli:** is a village located in the central Niger next to Tahoua region at the geographical position of 13°47'30'' North and 5°15'00'' East. The average rainfall is 400-500 mm per year.
- **Lossa:** is a village in located in the southwestern Niger next to Tillabery region at the geographical position: 13°56'2'' North and 1°34'29'' East. The average rainfall is 500-600 mm per year.

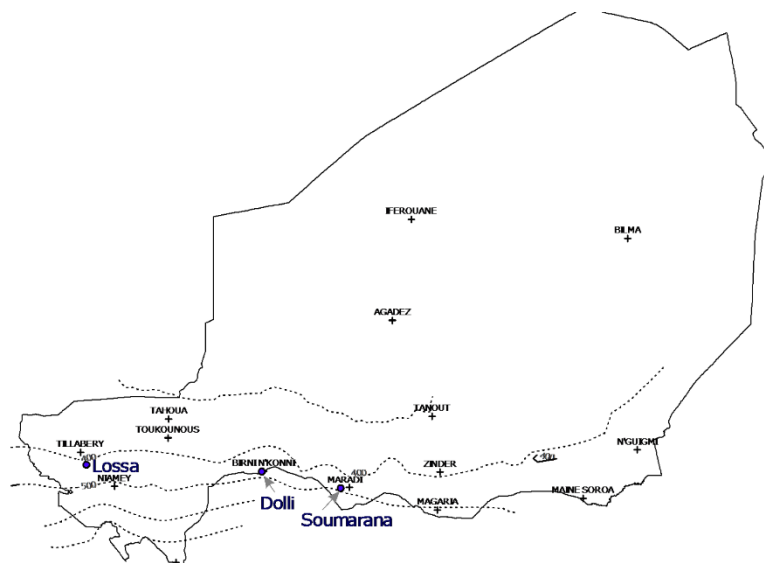


Figure 3. 1. Locations of PRA villages.

3.4. Results

3.4.1. FGD Results

Sorghum was the second cereal crop after pearl millet in all three villages. Livestock rearing was well practiced and integrated in crop farming. Farmers were well aware of livestock feeding constraints during a particular period (March-July) of the year. Indeed, they revealed feed shortage, stover poor quality, pasture unavailability which impact seriously animal and milk productivity, transport and farm management particularly for draughts (land preparation, sowing, and weed management) animals. Drought was reported as the main constraint for sorghum production in all three localities while, sorghum midge was cited as source of low grain production in Dolli and Soumarana. However, farmers were not aware of the real cause of sorghum midge causing low grain yield. One major information collected was that sorghum stover was important in livestock feeding. Indeed, at Dolli and Soumarana, it was the main feed whereas at Lossa millet stover came first. For stover, biomass was the most important attribute of preference for farmers.

Farmers had never experienced growing *bmr* sorghum and its attributes. This was confirmed through the exposition of *brown midrib* varieties' posters. However, they noticed the existence of white and yellow midribs.

Table 3. 2. Summary results of the FGDs

Topics	Results
Importance of sorghum crop in the area compared to millet and maize	2 nd cereal crop after pearl millet in all the localities
Sorghum production system	Mostly sorghum was cultivated in pure
Main sorghum production constraints (grain/stover)	Drought was the 1 st at Lossa however in Dolli and Soumarana drought and disease (sorghum midge) were the major constraints
Domestic sorghum stem/forage utilization	Livestock feeding was the main utilization of the stover
Importance in animals feeding compared to millet and maize	Sorghum was 2 nd behind millet stover at Lossa, however sorghum stover was at the 1 st position at Dolli and Soumarana
Livestock feeding constraints	-Shortage of forage -Poor quality of forage -Cereal bran price -Pasture unavailability
Farmers' empirical preferences for specific traits and attributes of forage sorghum	1 st Biomass 2 nd palatability 3 th stay-green
Which cow is fed during the dry season?	1 st Milking cow 2 nd Bullock
How is cow fed during the dry season?	Whole stem without chopping put on ground

3.4.2. Results Semi Structured Interview (SSI)

3.4.2.1. Farmers

➤ Identification

Ninety-one farmers were interviewed of which 93% were males and 7% were females. The traditional land tenure system (land belongs to extended family) was the most wide spread across the three villages (72%). (figure 3.2)

There were 4 females at Soumarana and 2 females at Dolli. Their farm land tenure was dominated by the rent followed by the legally owned (obtained from a legal structure). No female farmer was met at Lossa in the western part of the country.

On average each famer had 4.59 ha of land.

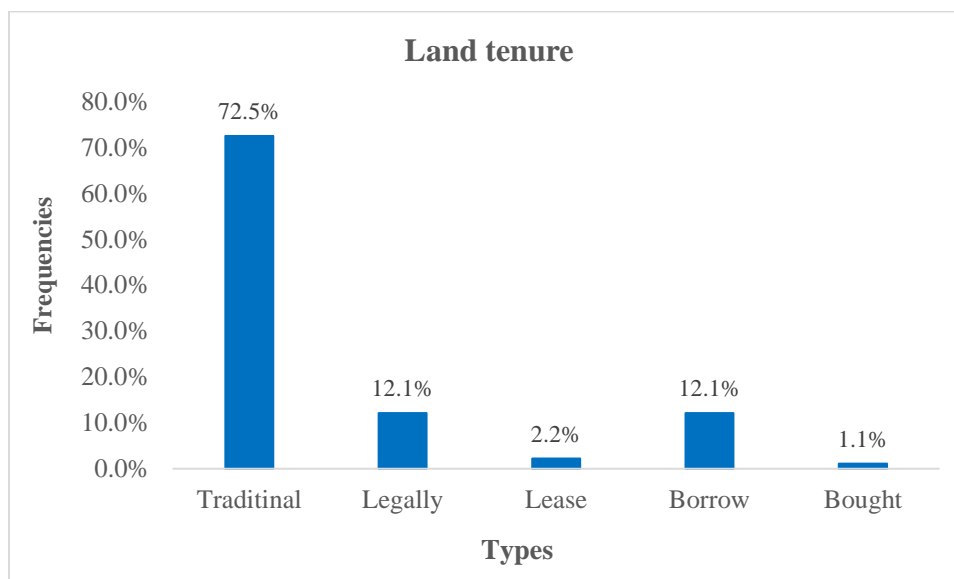


Figure 3. 2. Land tenure in the three villages.

Traditional = land belongs to extended family; Legally owned (Obtained from a legal structure (administrative); Lease /Rent; Borrow = Land owned by a parent.

➤ **Main activities**

Sixty-eight per cent of the investigated farmers were involved in other activities such as trading, or fattening livestock throughout the year to improve their income and 32% were just farmers. Trading was more developed at Dolli (80% of farmers) than the other two villages, Soumarana (71%) and Lossa (53%).

➤ **Sorghum production:**

All investigated farmers produced sorghum and reared livestock. In fact, Sorghum production and rearing livestock was a criterion of their selection. However, the survey revealed that they all produced sorghum for both grain and stover. Eighty-six per cent of interviewed farmers were favorable for adopting improved varieties while 46% bought improved seeds frequently.

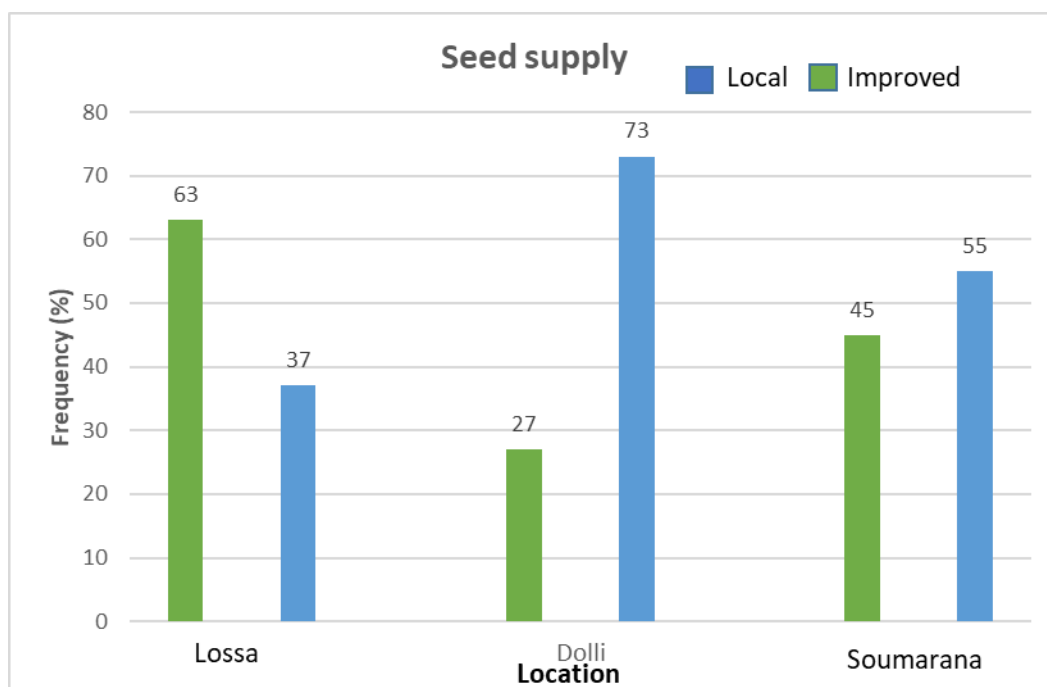


Figure 3. 3. Farmers' sorghum seed supply sources at three locations surveyed

➤ **Livestock rearing:** A profitable livestock rearing in semi-arid regions involves the control of key strategies to overcome numerous constraints.

- **Stover quality trait:** Three types of answers were given by farmers on stover quality: biomass (59.3%) was ranked first followed by juiciness (20.9%) and the stay-green (19.8%) comes which were ranked about the same.



Figure 3. 4. Farmers' perception on stover quality trait at Lossa

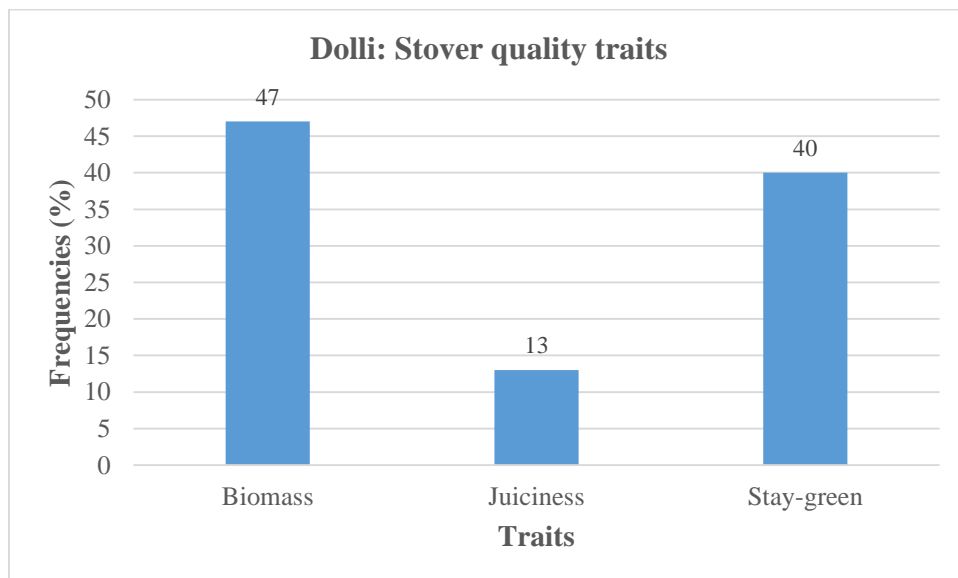


Figure 3. 5. Farmers' perception on stover quality trait at Dolli.

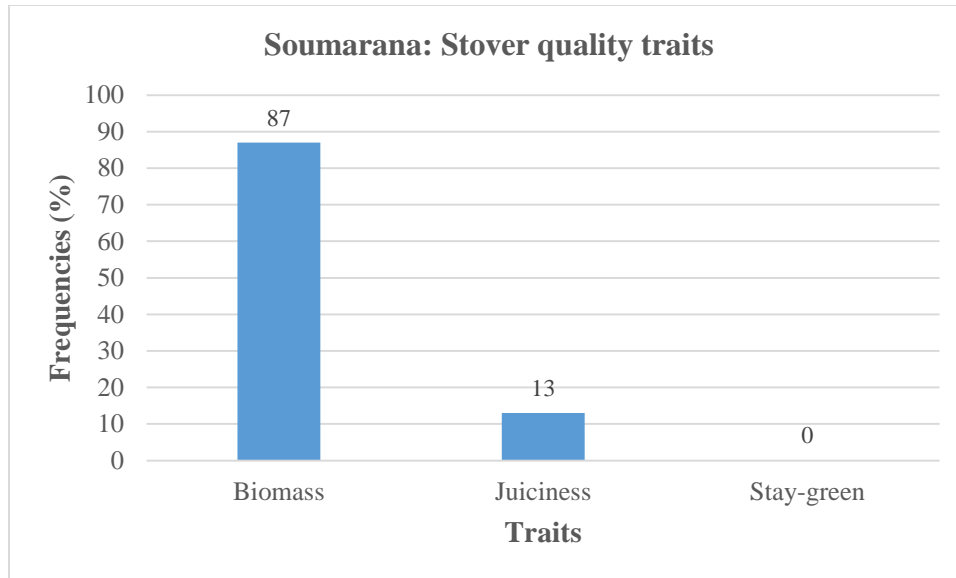


Figure 3. 6. Farmers' perception on stover quality trait at Soumarana.

- **Milk production:** Majority of farmers (59.3%) mentioned stover shortage as the main constraint for milk production, followed by malnourished cows (15.4%) and expensive cereal bran price (12.1%). However, seven per cent (7.7%) of farmers did not have constraints in milk production. Additional information showed that these farmers (7.7%) kept small size of herd (2 to 5 cattle) which were kept in the village throughout the year and fed mostly industrial feed as complement in addition to the cereal bran for their diet.

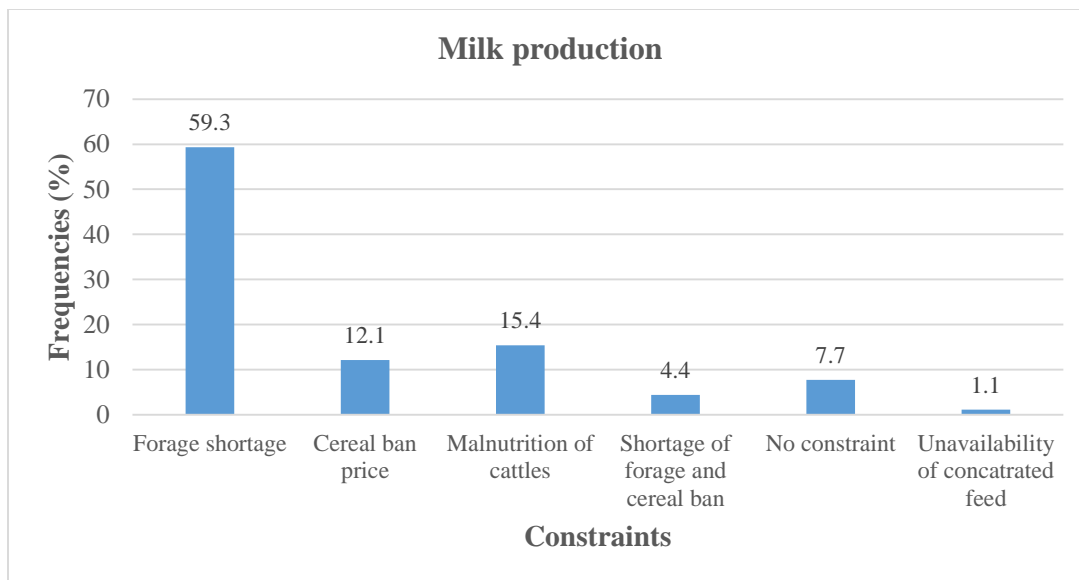


Figure 3. 7. The major constraints of milk production in Lossa, Dolli and Soumarana.

- Fattening:** Sixty-nine per cent of the investigated farmers carried out this activity and 68% responded that it was an important source of income. Thirty-one per cent did not have a particular view and for 1% fattening is a thrift. It is important to notice that those 31% with no view correspond to those who are not doing this activity.

3.4.2.2. Stover traders

Twenty-four (24) stover salesmen were identified. Among them, 21 were from Konni the principal city close to Dolli (14 miles separate the 2 localities) and 3 were from Soumarana. No stover salesman was found at Lossa and its surroundings. All traders ran their business individually and have never received a particular training. Main types of stover marketed forage were from sorghum and pearl millet crops, cowpea hay all in bundles, groundnut haulms, cowpea pod shells and cereals brans in bags.

- Supply:** Farmers are the main suppliers for stover traders. Indeed 87.5% of traders purchased from farmers principally at Konni while at Soumanarana stover salesmen are producers.

A bundle of 18 kg to 20 kg each purchased price was comprised between 250F-300FCFA (\$0.40-0.48) at the harvest time (sorghum) which was their main period of acquisition. Standard qualities requested when purchasing were good biomass (42%), stay-green+biomass (21%), stay-green (17%) and stover with enough leaves (21%).



Figure 3. 8. Purchased stover quality requirement.

- Storage:** Except two salesmen at Soumarana who stored on shade their stover, the remaining piled up their stover outside at their business place stover bundles. The storage duration was not determined since selling can happen any time, but in case of slow selling it can reach 12 months. In such situation, sellers complained about rodents, mites, thieves, fire or rains. Unfortunately, 50% of the salesmen do not have any solution for those constraints management whereas 29% utilized chemicals to control mites and rodents' attacks. The remaining 21% affirmed that vigilant and regular inspection of stock constitute their solution.

- **Sales:** In majority, salesmen affirmed peak selling time runs from April to July. During this particular period, the stover bundle price was comprised between 750-1000F CFA (\$1.20-1.61) and is mostly established according to its demand and availability. The standard qualities wished were the presence of the leaves, good biomass and the general quality of the stover. For 75% of the salesmen, stover business is a source of income and 25% were conducting to ensure their family daily expenditure. In fact, 75% save money for a continuous acquisition of stover.

3.5. Discussion

Farmer identification provided a useful starting point and served also to locate farmer establishment (experience in sorghum cultivation, farm equipment ...). The results on land tenure in the three villages in Niger showed that land tenure was mainly traditional (72.5%); in the other hand, land belonged to extended family, distributed between members or not under the responsibility of the head of the family. Land tenure revealed therefore a capital importance as it determines access to resources such as crop residues (FAO, 2014). In fact, Yazon and Peter (2003) reported that out of 1.267.000 km² in Niger, arable land occupies only 3.94% and agriculture is mainly practiced in the southern part of the country where rainfall rarely exceeds 800 mm. Further, concerning land distribution and exploitation, Ngaido (1996) reported that in a village of the country the following types of lands: individual lands, family lands and village common lands also known as chieftaincy lands. The results of this survey are consistent with Terraciano (1998) who explained that the land inheritance is through a patrilineal system. However, in the particular case of women land tenure, it was observed that 83% of their farm land was owned by a parent and only 17% belongs to them. In addition, women farm size was between 2 to 3 ha with an acceptable fertility. The overall results indicated that the average farm size was 4.59 ha. Waha *et al.* (2016) revealed that defining the farm size in Africa is country-specific and depends on the self-assessment of the households and

affirmed that the average farmed land area of small farms in Niger is 5.5 ha or less. With regards to land fertility, 85% of farmers consider their farms to be acceptable to good for sorghum production. This can be explained by the fact that sorghum is generally cultivated on clay soil opposed to millet which is cultivation on sandy soil.

The majority of interviewed farmers (69.2%) were practicing fattening to raise their income and for 75.8% of farmers, livestock contribution into their income was important, which is in harmony with Kamuanga *et al.* (2008) and Tunde *et al.* (2015). In addition, Tunde and Augustine (2014) confirmed that livestock are kept by most farmers to complement crop activities, because ruminants provide fertilizer for crop production and are also valued as assets that can be readily liquidated to meet household and farm financial obligations. According to Kamuanga *et al.* (2008), livestock rearing is one of the main economic activities on which the poorest population depends for food and income. The profitability of fattening depends however on a good control of investments in animal feeding. For this purpose, in addition to crop residues, cereal brans and other industrial concentrated feed played an important role. Findings reports that only 41.8% of farmers utilized cereals brans and concentrate as supplement due to their high cost which were also mentioned as constraints for milk production. Tunde *et al.* (2015) found that many rural households frequently used crop residues from their farm and brans from processed grains to reduce feed cost. In this view, the Food and Agriculture Organization (2014) highlighted the excessive price of wheat bran in ECOWAS countries and Niger seems to be the biggest for maize bran imports. At a single village level, the study revealed that fattening was more practiced at Soumarana (84%) than at Lossa (63%) and Dolli (60%), the proximity of Nigeria republic can explain this situation. Indeed, Soumarana is closer to Nigeria border which is one of the main destinations for livestock exportation from Niger. Guilbert *et al.* (2009) estimated that from the

Eastern network, 20% of livestock at international market in Maiduguri is thought to come from Niger and from the Western network livestock are collected from Mali, Niger, Burkina Faso and Benin to the international market in Kano although it is difficult to assess import flows of live animals from Niger, Chad or Mali due to the porosity of borders.

In general, the principal sources of household income in Niger are still the sale of crop, other income generating activities from migrants' remittances, livestock and byproducts sales (Rep. of Niger, 2002). Farming system is oriented on subsistence based characterized by low inputs utilization and not very productive material (equipment) leading thus to low productivity. Livestock holding, well integrated in farmer activities, contributes to income improvement and diversification. Among the production constraints, farmers explained that drought was the major limitation followed by sorghum midge at Soumarana and Dolli. The major biotic stresses in Niger include sorghum midge (*Stenodiplosis sorghicola*), Striga and various diseases and drought for abiotic stress. Hamidou *et al.* (2018) reported that sorghum midge is one of the most limiting factors of sorghum production and most farmers in major sorghum growing areas of Niger still rely on the low yielding, midge-susceptible local sorghum varieties. In the PRA study area, farmers considered a night wind coming from East as the cause of sorghum midge. On this subject, Kadi Kadi (1993) reported that farmers in Maradi and Konni thought a night wind caused empty glumes and reduced sorghum production. The World Bank (2013) also reported that drought was the principal risk for farmers in Niger and the country has experienced seven droughts between 1980-2010 with adverse impact on national agriculture production in agreement with investigated farmers' views included in this study.

Concerning improved seeds, 46% of investigated farmers affirmed frequent utilization, which is in disagreement with Olembo *et al.* (2010) who reported in their previous improved sorghum

varieties utilization in Niger was about 17.10%. This high level of improved seed utilization can be explained by the proximity of research farms, NGOs activities in the areas of the study but also the released varieties are well adapted with farmer preference traits. Indeed, the INRAN research farms are about at: less than three km for Lossa, fourteen km for Dolli and less than twenty km for Soumarana.

Investigated farmers produced sorghum for dual purpose. Dual purpose lines are very important for the farmers in Niger because they care as much about stover yield as feed as they are for the grain for food. Current PRA findings are in harmony with those of Thornton *et al.* (2003) who reported that mixed crop-livestock systems is well practiced and continues to be a substantial area of interest because farmers can produce both for human and livestock consumption. Singh *et al.* (2004) reported that in West Africa, farmers do not grow food and fodder separately and therefore crop residues are the major source of feed for livestock. Furthermore, Kelly and Parthasarathy (1994) reported that yield and stover quality of sorghum and millet are important choice standards in new variety adoption by farmers in semi-arid India. Despite harsh environmental conditions in Niger, sorghum shows a good adaptation and plays a strategic role in the search for food and feed security. According to JAICAF (2009), cultivars of sorghum in Niger are mostly traditional varieties and the production quantity accounts for one quarter of that for principal cereals. In certain areas however it surpasses pearl millet in importance as staple food.

During the study, farmers used biomass, the juiciness and stay-green as main parameters to evaluate the quality of cereal crop residues. Tunde and Augustine (2014) reported that in a previous study in two villages (Milli and Gourdjia) of Maradi region that farmers described 5 different physical parameters (coloration, texture, odor, age at harvest and animal behavior) to determine feed quality. In general, concerning the quality of the purchased stover, Blummel *et al.* (2013)

considered that crop residues, fodders from different crops are not considered the same by farmers and traders. Further, surveys of crop residues, fodder trading in SSA and India showed that fodder traders and customers were well aware of differing fodder quality from crop residues of different crops. Findings from this survey support the conclusions by the above authors.

During the survey in the 3 localities, the average of milk production per day was: 48 liters at Soumarana, 138 liters at Dolli and 216.5 liters at Lossa. The low level of milk production at Soumarana can be explain by the severe feed shortage (94% of famers' complained about feed shortage). Abdou (2010) reported feed deficiency and crop residues cannot satisfy the animal maintenance in Niger between April and June. At farmer exploitation level, the overall average of milk production was 4.42 liters, the maximum was 60 liters and the minimum was 0.5 liter. It is important to notice that only milk cow production was considered and 56% of the investigated farmers did not sell milk unlike the remaining 44%. The main reason was that the quantity of milk produced was not enough (42.9%) and the milk production was on seasonal basis. Indeed, milk is more produced when cows calving correspond to the rainy season thus the availability of feed. The major constraints for milk production was forage shortage (59.3%) and malnutrition of cattle (15.4%) according to farmers, in addition most of their milking cows were not improved breeds. This is consistent with Singh *et al.* (2004); Diester and Hassane (2006) who reported in a previous study that Nigerien improved cattle such as the Azawak or the Kouri rank among the best milk cows in West Africa; however the actual production is limited by malnutrition. Another reason for low milk production was the poor animal health maintenance. Health and economic constraints were mentioned by Diester and Hassane (2006) as part of low performance in term of milk production in Niger. Indeed, at Lossa and Dolli animal veterinary service was lacking. However, in case of emergency, farmers have to contact the closest livestock service and pay for the cost of

drugs and transport. The same report was observed by Tunde and Augustine (2014) in Gourdjia (Maradi region). At Dolli, the average milk production per day was 4.6 liters and 90% of farmers mentioned stover shortage as a constraint whereas the average milk production per day was 7.21 liters with lack of forage for 73% of farmers at Lossa. Tunde and Augustine (2014) found a high feed scarcity from April to June in two villages in Maradi region, in general. UNDP (2006) reported that cattle breeding activity is seriously affected by the decrease of fodder production and the reduction of pastoral areas as a result of climatic events. In addition, the availability of rice straw at Lossa may favor farmers to feed their livestock. Beside the fact that livestock products is bringing important nutritional benefits to large segments of population from developing countries (Muehlhoff *et al.*, 2013), milk production offers an important source of cash income to many of the over 200 million poor livestock keepers estimated in developing regions (Pica-Ciamarra *et al.*, 2011). However, Zezza *et al.* (2014) affirmed that at the household level data and studies on the role of milk production for human nutrition and livelihoods are severely hampered by the difficulty of producing reliable estimates of milk production in small-scale livestock production system.

In addition to this, farmers mentioned several uses of draught animal in their farming activities (farm work: ploughing, fertilizer, transport...) as advantage gains from their livestock, this result provide confirmatory evidence from previous Mohamed-Saleem and Von Kaufmann (1988) survey on farmers in Nigeria.

The results of the survey revealed also that farmers are stover market suppliers. Berazneva and Dyson (2013) supposed that in the context of African smallholder agriculture, crop residues have the most valuable application among on-farm resources. Socio economic factors such as the short term gain might influence farmers' decisions concerning this resource. In a previous study on crop residues trade in SSA and South Asia, Valbuena *et al.* (2011) reported that the need to cover their

own food requirements and household expenses pushes smallholder farmers to favor practices with positive returns in the short term. In the context of growing demand of crop residues in Niger, cereal stover represents a source of emergency revenue and can play an important role for short-term benefits for farmers. According to CGIAR (2011), the increasing value of stover has been a prominent trend in Asia and stover markets are emerging in drier, more densely populated areas of West Africa. Further, as the demand for livestock and livestock products increases, so too will the importance of fodder and feed, therefore dryland cereals will thus focus on increasing the quantity and quality of stover and straw as well as grain.

During the dry season, crop residues were important sources of diets used to feed livestock in all three villages. However, in terms of ranking, sorghum residues constituted the first choice in Dolli and Soumarana and second at Lossa. This is in harmony with Tesfaye (1998) who reported that sorghum stover was the major source of dry fodder for urban and peri-urban dairy production in Hyderabad (India). For 87.5% of traders, farmers are the suppliers for stover particularly at the harvest time, confirming Mohammad (2008) findings. Indeed, he affirmed that un-chopped stover purchased by traders from small cities are brought to Hyderabad and sold in informal fodder market. Improving storage methods can maintain the quality of the stover from harvest to feeding time during dry season. Unfortunately, 92% of the local traders piled up their stover outside with no respect to conservation and storage techniques. Mohammad (2008) confirmed that for different feed resources, trade and marketing, conservation, storage and processing technologies to mitigate spatial and temporal imbalances in feed supply and demand is not well understood in East Africa (Ethiopia and Tanzania) and South Asia (India and Bangladesh). The same constraint is reported from the current survey.

Stover trading is a source of livelihood for many farmers and traders in Konni and Soumarana. This is in agreement with Tesfaye (1998) who affirmed that stover selling, transporting, trading and its use in dairy production support the livelihood of many people from producers (farmers) to the end users in Hyderabad (India). Grunert (2005), reported that in Hyderabad buyers judge quality based on experience then use that knowledge in purchase decisions and traders infer quality differences based on intensity of demand for different types of stover. For Mohammad (2008) roughage feeds are traded in a variety of forms and generally there is no formally defined quality or standard though local buyers and sellers use informal indicators to differentiate quality to some extent. Further in Bangladesh, traders make varying sizes of small bundles of rice straw for sale but are not weighed to determine actual weight and quality is mainly differentiated based on visual observations.

3.6. Conclusion

Small scale farmers with mixed crop-livestock dominate the production system in all the 3 localities. Throughout the survey farmers' preference for dual purpose sorghum variety was evident. They highlighted the significant position of sorghum stover in their livestock feeding. Farmers also explained the importance of livestock contribution in their household income and commodities. In this context, the long dry season (November to May) constitutes an opportunity for practicing additional activities such as fattening and trading. However, those activities were seriously impacted by the shortage and mere quality of feed from their sorghum landraces stover. Drought is one of the major cause of low stover production that affect animal productivity like milk production. Indeed, in all the localities the low milk production reduces the opportunity of milk marketing. This restriction was therefore a limit of a sustainable long term revenue gain for farmers.

Farmers and stover traders appreciate essentially sorghum stover quality at harvest time based on physical aspect. High biomass and stay-green were important choice standards. The integration of those key information in breeding program might favor the rate of adoption of new sorghum dual purpose based on the *bmr* attributes.

Farmers rely on crop residues to feed their livestock during the dry season. During this particular moment, shortage of feed constitutes one of the main constraints for livestock rearing. As a result, livestock face harsh feeding conditions. This situation leads to a growing demand for stover. However, this demand is an opportunity that increases the value to crop residues particularly sorghum stover due to its high preference for livestock feeding (cattle and draught feeding) in Niger. Indeed, this survey showed that crop residues trading is a growing and profitable business

in the Southern part of the country. Un-chopped fodder was the main handling method throughout the cereal stover chain from the farm to the end user. Moreover, with the locally growing stover market, the economic value of crop residues as feed and/or source of income might have implications on farmers' variety adoption as they are the main suppliers of stover to traders. Therefore, the improvement could lead to a substantial increase of farmers' income since cereal residues are the major feed for their livestock and also farmers are the main sources of supply to traders in Niger.

CHAPTER FOUR

4.0. INTROGRESSION OF BROWN MIDRIB GENES INTO TWO NIGERIEN SORGHUM VARIETIES

4.1. Introduction

The first sorghum breeding efforts in Niger began with the creation of IRAT (Institut des Recherches Agronomiques Tropicales et des Cultures Vivrières) in 1964. The varieties Bagoba (Durra), Jan Jare (Caudatum), Gourma (Guinea caudatum), El-Mota (Guinea-caudatum) and Babbadiyah (Durra) were improved by mass selection and released to farmers (Clark and Kitch, 1982). INRAN (the National Agricultural Research Institute for Niger) was created in 1975 with the mandate of research on crops and animals in Niger. According to Adamou *et al.* (1985), the objectives of sorghum improvement is the development of varieties with high and stable yield, good grain quality, and resistance to drought, insects and diseases. In fact, in rural areas of Niger sorghum is a staple food. In addition, sorghum residues also constitute an important source of forage for livestock feeding. However, sorghum residues like other cereal residues are known to have poor feeding value due to their high lignin content. Indeed, Wilson and Kennedy (1996) revealed that the presence of lignin reduces the forage quality by hindering access to digestible energy and nutrients (i.e. proteins and minerals). Lignin is cross-linked to the cell wall matrix whereas an important aspect of a forage crop is its feeding value which involves a number of criteria affecting both plants and animals. However, Akinola *et al.* (2015) affirmed that the cereal crop residues' potential as livestock feed is enormous if particularly if appropriate methods of improving their nutritive value are used. Jung and Fahey (1983) reported that *bmr* plants have lignin which is less polymerized and contain less phenolic monomers that can affect digestion.

Ayyangar *et al.* (1936) explained that in sorghum, the D gene which affect the leaf midribs also controls stem juiciness, with pithy (D) being dominant to juicy (d) stems. According to Kumari *et al.* (2017) green color of leaf midrib in sorghum is an indicator of sweetness whereas brown colour indicates reduced level of enzyme resistant polymer 'lignin' in plants. According to Rooney (2000) a white (and dry) midrib is conditioned by a dominant allele at the D locus, while recessive genotypes have green and juicy midribs.

Porter *et al.* (1978) produced 19 *bmr* sorghum mutants by chemically treating seed from two grain sorghum lines. These mutants were numbered *bmr* 1 to *bmr* 19. Porter *et al.* (1978) suggested that the *bmr6*, *bmr12*, and *bmr18* should be selected for further evaluation. However, according to McCollum *et al.* (2005) the *bmr6* and *bmr12* genotypes are more prevalent than the *bmr18*. Adding *bmr* trait to local cultivars and newly developed lines will be more sustainable and will bring a significant impact on livestock productivity in countries like Niger. However, exotics *bmr6* and *bmr12* sources were less adapted into the Nigerien typical sahelian environment (Diakit  *et al.*, 2018). Two well adapted sorghum varieties (Sepon82, and El mota) were selected as suitable candidates for their stover quality improvement. In the Nigerien national plants catalogue Sepon82 is an elite variety for grain yield. In addition, it has a good stover potential and aptitude to stay green at the physiological maturity. El mota is a local variety widely cropped by farmers for its earliness, adaptation to erratic rainfall, poor soil fertility and its high yield potential (Kapran *et al.*, 2007).

The main objective of this chapter was develop *bmr* breeding population with Sepon82, and El mota as recurrent parents using the backcross breeding method.

The specific objectives were to:

- i. introgress *bmr6* and *bmr12* genes to local sorghum varieties,
- ii. identify and advance superior genotypes with *bmr* trait in the breeding populations.

4.2. Material and methods

4.2.1. Plant materials

The plant materials were composed of Sepon82, and El mota as recurrent parents (RP). The *bmr* donor parents were Redlan*bmr6*, Wheatland*bmr12* and Tx630*bmr12* were used as donor parents (DP). Those donor parents were from Purdue University Indianapolis (USA). Table 4.1 summarizes the agronomic characteristics of the different plant materials.

Table 4. 1. Summary of agronomic characteristics of the different parental lines.

	Plant type	Grain Yield (t/ha)	Biomass Yield	Lodging	Midrib color
Recurrent Parents					
Sepon82	Tan	2-2.5	Good	Resistant	Dull-green
El mota	Anthocyanin	1-1.5	Acceptable	Less resistant	White
Donor Parents					
Redlan <i>bmr6</i>	Anthocyanin	1.1-1.4	Low	Resistant	Brown
Wheatland <i>bmr12</i>	Anthocyanin	1-1.5	Low	Resistant	Brown
Tx630 <i>bmr12</i>	Tan	1-1.5	Low	Resistant	Brown

Source: République du Niger (2012)



Figure 4. 1. The different types of sorghum midribs involved in this study
White (El mota: left), brown (Redlan***bmr6***: centre) and dull green (Sepon82: right).

4.2.2. Development of *bmr* population

bmr genes introgression for the breeding population development activities took place in Mali from January 2016 until May 2017 at Sotuba Research Station. The geographic coordinates of Sotuba: 12° 39' N; 07° 56' O at 320 m altitude.

The crossing blocks nurseries were prepared and managed for a good growth of seedlings. The Standard agronomic practices were implemented for the growth of the plants.

Crossing blocks were established in early January 2016 off season under drip irrigation system. As sorghum is a short-day plant, its cultivation in January, when photoperiod is short, causes early flowering thus allowing crossing synchronization between the two parents. Nevertheless, two planting dates (27/01/2016 and 9/02/2016) for the donor parents (DP) and one date of planting for the recurrent parents (RP) (27/01/2016) were used to ensure flowering synchronization required

for crossings. Furthermore, too early parents were pruned during vegetative period to allow new shoots emergence for flowering synchronization between parents. There were 10 hills planted for expected 10 plants per DP or RP line.

At flowering stage (March 2016: period of the high temperature at Sotuba), RPs were hand emasculated using tweezers/forceps. The hand emasculatation technique using tweezers was chosen to minimize the amount of seedlings from self-fertilization in F1 plants. Fifty to sixty flowers of each RP per cross were chosen for each crossing. Flowers from the top of each panicle were emasculated. Anthers were removed in each female and male flower. Two to three days after emasculatation the recurrent parents were dusted with donor parent pollen. Flowers from the bottom of the same female panicle were bagged in order to produce identical recurrent parents' seeds. Other flowers on the same female plant were completely discarded to avoid seed contamination. Likewise, each donor parent was crossed to each recurrent parent to produce F1 seed for each population. Mature crosses were harvested and kept separately. Donor parent plant seed was also self-pollinated. RP and F1 seeds were threshed separately to avoid any seed mixtures and stored at 4°C.

F1s seeds from all successful crosses and their respective parental line seeds were treated with a Caïman Rouge P (Permethrine 25 g/kg + Thirame 250 g/kg) an insecticide fungicide, pre-germinated in petri-dishes, and then were transferred in plastic pots containing 1 kg of compost for BC1F1 seed production. After 20 days period, F1 were transplanted in one row of 3 m. Parental seedlings were planted next to F1 for conformity control. Heterosis and presence of anthocyanin were used to differentiate F1 from their parental phenotypes. Hybrid vigour from crosses is easy to distinguish from parental lines. Anthocyanin (purple plant) is dominant over tan plant. If male parent is purple and the female parent tan, then the F1 derived from these two parents must be

purple. Conversely, if F1 is tan plants, this is considered a self, therefore discarded. These two techniques allowed the identification of few F1 plants. From F1 deriving from two purple or tan plants, segregation of *bmr* plants in F2 or BC1F2 was a sure mean of verification of true F1 cross. The identified F1s were then used as pollen donor on the emasculated top of RP panicles while the bottom of the RP panicles were self-pollinated. This method allowed BC1F1s seeds production simultaneously with pure RP seeds while by self-pollinating the F1s' panicles F2 seeds were produced.

BC1F1 seeds obtained during 2016 cropping season crosses between F1s and recurrent parents were planted for segregation study and generation advancement to obtain BC1F2. F2 seeds were also planted in other nurseries for *bmr* trait segregation study and F3 seeds production. Each BC1F1 and F2 plant seeds were germinated in petri dishes (21-31 October), transplanted in plastic pots containing 1 kg of compost when primary root appeared, then transplanted in nursery field for BC1F2 plant identification. As the RP (El mota) population heights were heterogeneous, each crossing within this population crosses was kept separate for population identity. Harvest was done in early January.

At harvest, each *bmr* BC1F2 panicle and F3 panicles were threshed separately. Seeds were pre-germinated in petri-dish first, then transferred in pots after the appearance of their primary root and finally transplanted in the field in late January to early February. Photoperiod sensitive sorghum cultivation in long days (starting March) delays flowering until September avoiding harvest in May or early June. To speed up panicle initiation, seedlings after 21 days of transplantation in open field, were covered with cages every day from 5 pm to 8 am, during 30 days. This artificial shortening of day length induces reproductive phase and seed for day length sensitive plants. The cages comprised of black plastic layer under each white cotton tissue for

complete shade to cover each sorghum plot. All panicles were self-pollinated using appropriate paper bags during all generation advancement. Figure 4.3. below shows the general breeding scheme for the *bmr* genes introgression.

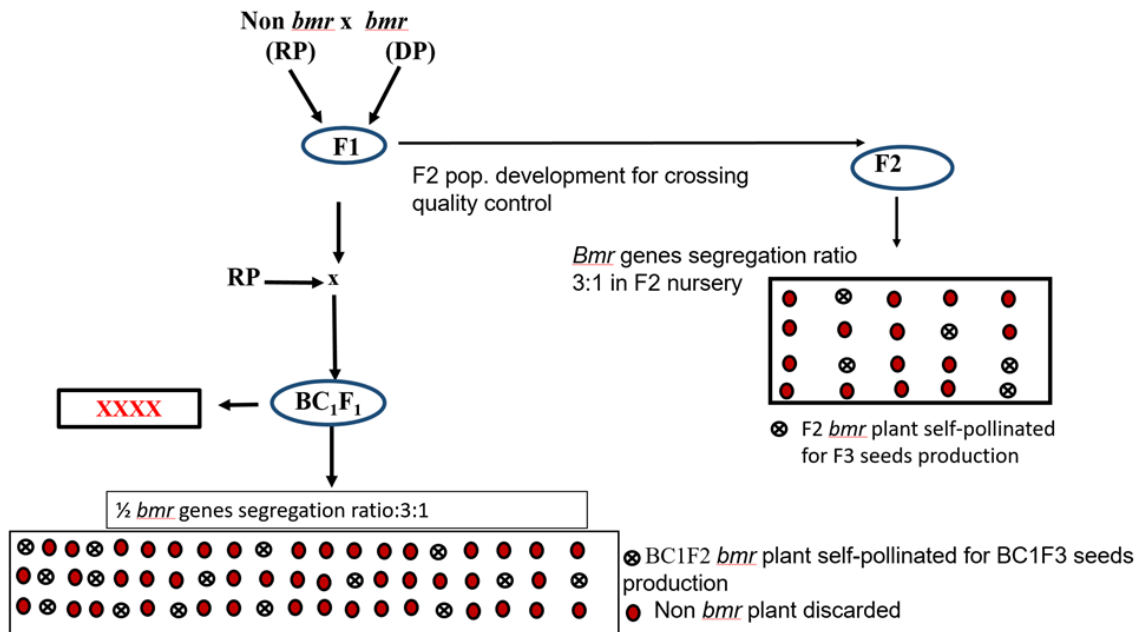


Figure 4. 2. The general scheme for *bmr* genes introgression. (RP=Recurrent parent; DP=Donor parent).

All BC₁F₁ were planted and non *bmr* segregating BC₁F₂ populations were discarded.

4.3. Data analysis

Data for *bmr* segregating phenotypes were collected in the field at BC1F2 and F2 generation for Chi-square (X^2) test on every breeding population for the 3:1 segregation ratio in order to select the genotypes with the *bmr* genes incorporated.

The following formula was used for the Chi-square test.

$$X^2 = \sum \left(\frac{d^2}{e} \right)$$

Where X^2 = Chi-square value; \sum for sum; d=observed value-expected value and e=expected value.

4.4. Results

A total of thirty-four (34) seeds were obtained as possible F1s (Table 4.2) in April 2016. All progenies were examined as follows and out of twenty-six seedlings, eight (8) F1s were identified.

- Sepon82 (*tan plant*) x Redlan *bmr6* (*purple, anthocyanin*): among the nine (9) F1s plants, only 1 plant (F1-1-3) was anthocyanin, therefore it was considered true F1 plant. The remaining seedlings were tan plants and were discarded from the breeding populations' development.
- Sepon82 (*tan plant*) x Wheatland *bmr12* (*purple, anthocyanin plant*): two (2) F1 plants were anthocyanin, thus were considered as F1s.
- El mota (*purple, anthocyanin*) x Tx630 *bmr12* (*tan plant*): twelve (12) F1 seedlings were generated and among them 3 were selected as F1s because of the heterosis observed.
- El mota (*purple anthocyanin*) x Redlan *bmr6* (*purple, anthocyanin*): both parental lines were anthocyanin. 3 F1 seedlings were produced but 2 were selected as F1s based on their visible heterosis effect.

Table 4. 2. Quantities of F1 seeds and seedlings generated per cross.

Crosses	F1 seeds harvested	Seedlings generated	True F1
El mota x Tx630 <i>bmr12</i>	14	12	3
El mota x Wheatland <i>bmr12</i>	0	0	0
El mota x Redlan <i>bmr6</i>	4	3	2
Sepon82 x Wheatland <i>bmr12</i>	2	2	2
Sepon82 x Redlan <i>bmr6</i>	14	9	1
Sepon82 x Tx630 <i>bmr12</i>	0	0	0
Total	34	26	8

- **BC1F1 populations development**

Table 4.3. summarizes the quantity of seeds harvested in the crosses between the selected F1 (*bmr* donor) and the recurrent parents.

Table 4. 3. Quantities of BC1F1 seeds produced per population.

Population BC1F1	Seeds
El mota//El mota/Tx630 <i>bmr12</i> 1-1	15
El mota//El mota/Tx630 <i>bmr12</i> 1-2	2
El mota//El mota/Tx630 <i>bmr12</i> 1-3	31
El mota//El mota/Tx630 <i>bmr12</i> 1-4	2
Sepon82//Sepon82/Wheatland <i>bmr12</i> 2-1	36
Sepon82//Sepon82/Wheatland <i>bmr12</i> 2-2	12
Sepon82//Sepon82/Redlan <i>bmr6</i> 3-1	3
Sepon82//Sepon82/Redlan <i>bmr6</i> 3-2	15
Total	116

In the F2 populations, the *bmr* genes segregation gives the following results summarized in Table 4.4.

Table 4. 4. Segregation in the F2 families.

Population	Phenotype	Observed	Expected	Cal. X ²	X ² Critical value
Sepon82//Sepon82/Redlan <i>bmr6</i>	Dull-green	13	14	0.271	3.84
	Brown	6	5		
Total		19	19		
Sepon82//Sepon82/Wheatland <i>bmr12</i>	Dull-green	14	15	0.266	3.84
	Brown	6	5		
Total		20	20		
El mota//El mota/Tx630 <i>bmr12</i>	White	15	15	0	3.84
	Brown	5	5		
Total		20	20		

For a test with 1 degree of freedom, the "critical" value of the chi-square statistic is 3.84: when $X^2 >$ to the critical value, then the data did not fit the X^2 model or deviated from the expected value. Observed: number of *bmr* plants counted in the field out of a total of 20 plants. Expected: number of *bmr* plants derived from the single dominant gene segregation.

- ***Bmr* trait segregation study in BC1F2 populations**

In the BC1F2 populations, the *bmr* genes segregation gave the following results summarized in Table 4.5.

Table 4. 5. Segregation in the BC1F2 families.

Population	Phenotype	Observed	Expected	Cal. X ²	X ² Critical value
Sepon82//Sepon82/Redlan <i>bmr6</i>	Dull-green	42	45	0.8	3.84
	Brown	18	15		
Total		60	60		
Sepon82//Sepon82/Wheatland <i>bmr12</i>	Dull-green	47	45	0.35	3.84
	Brown	13	15		
Total		60	60		
El mota//El mota/Tx630 <i>bmr12</i>	White	43	45	0.35	3.84
	Brown	17	15		
Total		60	60		

For a test with 1 degree of freedom, the "critical" value of the chi-square statistic is 3.84.

4.5. Discussion

Tan and anthocyanin sorghum plants types were used for the genetic transmission of the *bmr* trait in order to produce F1 plants. The morphological traits associated with distinct phenotype are of great importance in plant selection. In this view, Rooney (2001) stated that F1 hybrid plants must be identified by the breeder on some specific phenotypic or genetic basis. Moreover, the author concluded that this is typically accomplished by using a simply inherited phenotypic trait or heterosis that occurs between parents of different origin. In the F1 progenies derived from crosses involving tan and anthocyanin plants in this study anthocyanin marker was observed. This result showed the dominance of anthocyanin over tan character. This finding is in agreement with Nagaraja *et al.* (2008). In addition, anthocyanin marker is of great importance for sorghum biomass utilization in livestock feeding. To this end, Porter *et al.* (1978) stated that the anthocyanin pigments of sorghum leaf sheath have been found to be associated with economically important traits such as increased fodder quality. After crossing the parental lines (recurrent and donor parents), thirty-four (34) F1 seeds were harvested from six (6) crosses. This low level of success of the crosses can be due to the high temperatures occurring at this period of the year (almost 45°C). In this view, House (1985) demonstrated that crossing at a temperature of 40°C (with low humidity) usually results in failure of seed set. Regarding the low success of crosses, only eight (8) true F1s over twenty-six (26) seedlings, breakage of anthers and pollen contamination on tweezers tips during the emasculation process can be responsible. This hypothesis was supported by House (1985) who described prevention of anther rupture and pollen sticking to the emasculation tool. In addition, Schertz and Dalton (1980) reported that the success of hybridization varies with personal skill, and the amount of injuries sustained by the floret part during the emasculation.

The IBPGR and ICRISAT (1993) produced a useful descriptor of many morphological traits for sorghum. For the leaf midrib colour; the white, dull green, yellow, brown and purple were considered. Further Acquah (2007) showed that sorghum midrib colour was controlled by a single dominant (D) gene. For the particular case of the *bmr* type, Bout and Vermerriss (2003) revealed that sorghum brown midrib colour is controlled by single recessive gene. Following this, Nagaraja *et al.* (2008) reported the dominance of white midrib over brown midrib colour in F1 population. Further those authors showed in the following F2 segregating population, a good fit of the ratio 3:1 revealing *bmr* trait as single recessive gene. The same report was observed in nurseries at BC1F1 and F1 levels in this study. Indeed, all the individual plants in every breeding population exhibited the parental midrib colour. Those results are also consistent with the basic 3:1 Mendelian dominant and recessive phenotypic traits inheritance ratio for a single gene. House (1985) too stated that a recessive trait is not expressed in F1s. Later with the advancement to BC1F2 and F2 breeding populations, segregation for the *bmr* trait was observed. Results on segregation patterns in this study confirmed the recessive inheritance of the *bmr* genes as concluded by earlier authors (Akira *et al.*, 2012) on recessive mutation in a single locus. Based on this information, Chi square test was performed to verify the segregation ratios of *bmr* trait in all the breeding populations. The results were in harmony with the following authors Acquah (2007); Bout and Vermerriss (2003); Nagaraja *et al.* (2008). Therefore, in breeding population advancement each identified *bmr* plant in every breeding population was self-pollinated.

4.6. Conclusion

The results from Chi-square test confirmed that *bmr6* and *bmr12* genes were transmitted successfully in the local varieties El mota and Sepon82 genetic background. Three breeding populations were generated out of the six (6) breeding populations expected. In each population, every panicle was kept separately as a particular family. The genetic transfer of the *bmr6* and *bmr12* genes laid out the dual purpose sorghum varieties with high stover quality programme. Indeed, in most cases, in the breeding programmes sorghum new lines were bred for high grain yield potential. However, with the increased demand of livestock feed, breeding new dual-purpose varieties need to be developed. A total of 94 *bmr* BC₁F₃ families were selected for phenotyping in the Nigerien environment for their potential in terms of grain, fresh stover and dry matter yield.

CHAPTER FIVE

5.0. AGRONOMIC POTENTIAL OF TWO NIGERIEN SORGHUM VARIETIES WITH *BMR 6* AND *12* GENES IN TILLABERY AND KONNI

5.1. Introduction

In Niger like other South Saharan Africa countries, the mixed crop-livestock production system dominates in most small scale farmer holders. Cereal crops are mostly cultivated for dual purpose targeting human consumption and livestock feeding. Indeed, as the grain is used for human consumption, the stover part is used as feed. In this situation, farmers therefore care equally for grain and stover yields. Cereals residues are significant sources for feeding livestock. In this context, Youngquist *et al.* (1990) reported that in low rainfall environments, livestock depend mainly on crop residues for feed during dry season and drought years. Stover yield is particularly important after the years of low rainfall because its unavailability can affect animal survival. The World Bank (2013) reported that drought is the principal risk in Niger where the country experiences high frequency of drought with adverse impact on the national agricultural production. However, little research has been done to increase the quantity and the nutritive value of dryland crop residues (CGIAR, 2011). In this situation, the development of dual purpose cereal varieties constitutes a pressing need and an important goal for the SSA countries. Dual purpose sorghum lines with high stover quality can constitute a sustainable solution if agronomic potentials are acceptable. Indeed, Oliver *et al.* (2005) affirmed that it may be possible to add value to crop and animal systems by enhancing the digestibility of the stover residues by the use of *bmr* genes if grain yields can be maintained. This will make genetic improvement of dryland cereals more efficient and effective in addressing the needs of producers and consumers of grain, and crop

residues from these crops (CGIAR, 2011). To ascertain the latter two ideas (CGIAR, 2011; Oliver *et al.*, 2005), conventional breeding method was used to introgress *bmr6* and *12* genes in two adapted Nigerien sorghum varieties for grain yield, stover yield and quality improvement. The main objective was to enhance stover and grain yield potentials of *bmr6* and *bmr12* derived populations. The specific objectives were to:

- determine the agronomic performance of 94 BC₁F₃ *bmr 6* and *12* derived families and,
- identify superior *bmr 6* and *12* families for grain and stover yields.

5.2. Material and methods

5.2.1. Sites

Phenotyping trials were conducted in two Niger INRAN research farms (Tillabery and Konni). These localities are the closest research farms to PRA villages, thus in the same agro ecological environment. Tillabery is in the western part of the country (1°26'98'' North; 14°13'59'' East) and its annual average rainfall is 400 mm. Konni (13°47'23'' North and 5°14' 57'' East) and has 500 mm rainfall annually. The Figure 5.1 shows the rainfall patterns during the last ten years in the two localities.

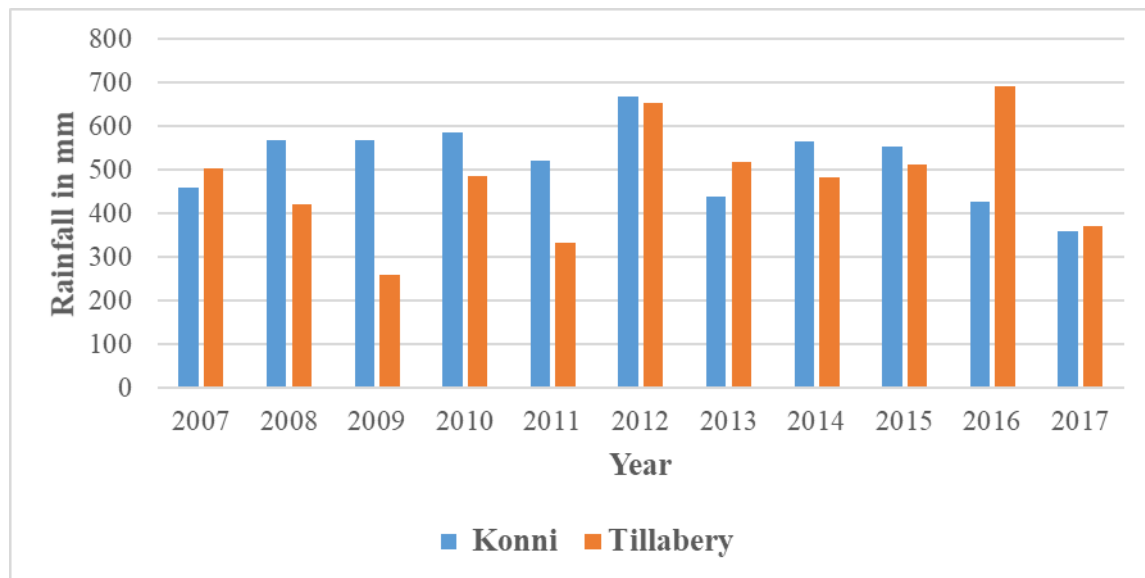


Figure 5 1. Ten years average annual rainfalls in Tillabery and Konni.

5.2.2. Plant Materials

The genetic material was composed of hundred (100) entries, of which 94 BC1F3 were *bmr6* and 12 derived families from 3 breeding populations and six checks. The *bmr* derived families were composed of three populations. Population1 (Pop1) called BCS1 resulted from El mota//El mota /Tx630*bmr12* had 60 families. Population2 (Pop2) named BCS2, derived from Sepon82//Sepon82/Redlan*bmr6*, had 27 families. Population3 (Pop3) called BCS3 was from Sepon82//Sepon82/Wheatland*bmr12* covered 7 families. There were 6 checks added to the *bmr* derived families. Checks were composed of three *bmr* donor parent (Readlan*bmr6*, Wheatland*bmr12* and Tx630*bmr12*), two recurrent parental checks (El mota, Sepon82) and a local check called Tillabery landrace.

5.2.3. Methodology

5.2.3.1. Experimental Design

The experimental design was a square lattice: (number of treatment $t = 100$; number of block per replicate $b = 10$; number of plot per block $p = 10$) with 3 replications in every location. Entries were randomized using GenStat 12th Edition. Each entry was sown on 3 rows. The row length was 3 m and the sowing density was 0.80 m between rows and 0.50 m between hills on the row (0.80 m x 0.50 m). All phenotypic data were collected on plants in the middle row. After thinning, in every planting hill, 3 plants were retained.

5.2.3.2. Cultural practices

The following standard cultural practices were performed during the phenotyping:

- Land preparation: The experimental land of each location was cleaned of debris. Seed bed was plowed and ridged.
- Sowing: Seeds were treated with Caïman Rouge P (Permethrine 25 g/kg + Thirame 250 g/kg) an insecticide fungicide. Sowing was done after the first good rainfall.
- Fertilizer applications: Diammonium phosphate (DAP) was applied before planting.

Ridge sides were ‘opened’ and DAP (18N-46P₂O₅-0K₂O) fertilizer was applied at the rate of 100 kg/ha. Urea with 46%N was applied (100 kg/ha) in two sets. The first set (50 kg/ha) was applied at tillering and the second one (50 kg/ha) at booting stage.

- Thinning: thinning was done two weeks after the emergence of seedlings and 3 plants per hill were maintained. The final density per hectare was 75000 plants.

- Field management: weed control was performed three times during plant development in every locality. The first one was done at tillering stage, the second at booting and the third at heading stage.
- The Carbodan 3% (a systemic insecticide containing cabofuran 30 g/kg) was applied at seedling stage to avoid stem borer attack.

5.3. Phenotypic data collection

Two types of data were collected: quantitative and qualitative. Phenotypic data were collected on 7 variables across the two environments for every family and recorded in Excel spread sheet.

The following data were collected:

- 50% flowering days (50%FL)
- Plant height (PH)
- Grain yield (GY)
- Fresh stover yield (FSY)
- Dry matter yield (DMY)
- Lodging (LGG)
- Foliar diseases (FDS)

5.3.1. Description of observations

- **50% flowering days:** This parameter was noted daily by using a visual observation since the appearance of the first panicle in each experimental plot. The number of days to 50% flowering

of a family was recorded as the number of days to 50% of the plants in the middle row of the experimental unit reached the anthesis at least halfway down the panicle.

- **Plant height:** this measurement was done at each experimental plot physiological maturity.

In the middle row of every family, 5 plants were randomly taken and measured from the base of the plant to the top of the panicle. Then the average height was calculated and recorded as the family height in the replication in cm.

- **Grain yield:** the middle rows were harvested. Then the panicles were dried and threshed.

For every family, the quantity of grain was weighted and finally the grain yield was calculated in kg/ha based on the sowing density in the experimental unit surface per replication (experimental unit = $(0.80 \text{ m} \times 0.50 \text{ m}) \times 6 \text{ (hills)} = 2.4 \text{ m}^2$).

- **Fresh stover yield:** this parameter was computed just after harvesting panicles. The fresh stover of every family per experimental unit was harvested and directly weighted in kg. The fresh stover yield per hectare was then estimated in kg/ha per net plot.

- **Dry matter yield:** For the dry matter estimation, a sample of 1 kg of fresh stover was taken per experimental unit after harvesting sorghum panicles. Then each sample was dried in an oven at 105°C. The samples were daily weighted until dry weight stability of each sample. This final dry weight was obtained after 5 days in general. The obtained value per sample was used to calculate the total moisture of every sample and finally the dry matter per family per plot.

The following formula was used to obtain the total humidity:

- Total Humidity (H):

$$H = \frac{FSw - DSw}{DSw} * 100$$

- FSw: Fresh Sample weight
- DSw: Dried Sample weight

The dry matter was calculated using the following formula:

$$SDM_{tot} = FSS_{tot} - \frac{H}{100} * FSS_{tot}$$

Where SDM_{tot} is the Sample Dry Matter total; FSS_{tot} is the Fresh Sample Stover and H is the moisture.

- **Foliar disease:** Sorghum foliar diseases can be due to the harmful attacks from various pathogens. Consequently, in the normal growing conditions a wide range of foliar diseases are observed on sorghum plants. Biomass free of disease is important for the quality of the stover. Therefore, throughout the experiment regular inspection was done to identify and record all the plants in every family with visible infected leaves (abnormal colour or shape of leaves) or insect damages. At the physiological maturity, data for the tolerance disease damage were collected based on (House, 1985) scale: 1=experimental unit free of disease; 2=1 to 10% plant with disease; 3=25% plant with disease; 4=40% plant with disease and 5=more than 40% plant with disease. From this scale, 1=absence of foliar disease; 2 and 3=presence of foliar diseases and finally 4 and 5=severe presence of foliar disease.

- **Lodging:** this variable was measured at each family physiological maturity. In practice, the number of stems lodged in the middle row of each family and replication was counted based on House (1985) scale: 1=0-10% plant lodged; 2=10-25% plant lodged; 3=25-50% plant lodged; 4=50-75% and 5=75-100% plant lodged.

5.4. Data analysis

Qualitative data: Descriptive analysis using Excel followed by the Kruskal-Wallis non-parametric test using Minitab Eds.14 software were performed.

Quantitative data: different analyses were carried out:

- A descriptive analysis with Excel.
- Normality of data distribution using the Anderson-Darling test.
- Homogeneity of the variances using the Hartley's Fmax test.
- Analysis of variance (ANOVA): two types of ANOVA were performed. Firstly, a single

separate ANOVA was performed for every variable in each site. These ANOVA analyses were done with R 3.4.1 environment for statistical computing using PBIB.test function (package 'agricolae') for the alpha design analysis of variance using the linear model as follow:

$$Y_{ijl} = \mu + t_i + r_j + b_{i(j)} + e_{ijl}$$

Where; Y_{ijl} = value of the observed trait; t_i = treatment effect and ($i=1 \dots 100$); r_j = replication effect and ($j=1..3$); b = block within replicate effect and ($j=1 \dots 10$); e = random error.

Secondly, a combined ANOVA was also performed for the variables showing homogeneity of variances. Indeed, data for each site were checked for homogeneity of variance using the Hartley's Fmax method. The homogeneity of variance is an assumption of ANOVA. The Hartley's Fmax method consist of a ratio of the larger variance/smaller variance and a result less than 3 indicate similarity of the variances. Therefore, the combined ANOVA was performed for the following variables: grain yield, fresh stover and dry matter yields. The combined ANOVA was performed using R 3.4.1 software of 'agricolae' package. The sites, replicates and blocks were considered as random while the entries were fixed. Statistical model was: site + replicates (sites) + site.replicates (blocks) + site*varieties. The mean separation was done with the Tukey Honestly Significant Difference (HSD).

- Genotypes by Environment (GxE) analysis: In order to check the response of the different entries across the locations, a GxE study was performed. The following statistical general linear model: site + replicates (site) + entries + entries*site was used with the Minitab Eds.14 software. The sites were considered as random factor while the entries were fixed. The different genotypes interactions and performances in the two environments (Tillabery and Konni) were performed using the Breedingview software. The entries sensitivity and stability were checked using the GGE biplot and ranking. A twenty per cent (20%) selection pressure was applied to detect the best and stable lines across the two sites. For this purpose, the Wricke's ecovalence test was used.

- Genotypic variance (σ^2_g) and Phenotypic variance (σ^2_p) Phenotypic coefficient of variation (PCV) and Genotypic coefficient of variation (GCV) Broad sense heritability (H^2), Genetic Advance (GA), Genetic Advance of the Mean (GAM) were computed.

- The genetic and phenotypic variances were computed based on the mean square genotype, the residual and the number of the replication of the trial in each location according to Wricke and Weber (1986) and Prasad *et al.* (1981) respectively.

- The Phenotypic and genotypic coefficients of variation were calculated using the phenotypic, genotypic variances and the grand mean of the trait under consideration according to Burton (1952) and Johnson *et al.* (1955). The results were then classified from low (0-10%), moderate (10-20%) to high (>20%) according to Sivasubramanian and Menon (1973).

- The broad sense heritability was also calculated and hierarchized as follow 0-30%=low, 30-60%=moderate and >60%=high as described by Allard *et al.* (1960) for every trait.

- The genetic advance was calculated using the data from the broad sense heritability, the selection intensity using K value (k is a constant; at 20% selection pressure $k=1.40$) and the

phenotypic variance then it was classified based on the following scale: 0-10%=low, 10-20%=moderate, >20=high with Fehr *et al.* (1987) method.

- The genetic advance of the mean was calculated in percentage using the genetic advance and the mean value of the trait according to Johnson *et al.* (1955) and categorized as: low=0-10%; moderate=10-20% and high=>20%.

Table 5. 1. Formula for phenotypic, genetic and heritability components estimation.

Parameters	Formulas	Significance
Genetic variance	$GV = (MSG-MSE)/r$	MSG = mean square genotype, MSE = mean square error, r = number of replications
Phenotypic variance	$PV = GV+MSE$	GV=genetic variance, MSE = mean square error
Phenotypic coefficient of variation	$PCV = \sqrt{PV} * (100/\bar{X})$	PV=phenotypic variance, \bar{x} = the grand mean of the trait
Genotypic coefficient of variation	$GCV = \sqrt{GV} * (100/\bar{X})$	GV = Genotypic variance, \bar{x} the grand mean of the trait
Broad sense Heritability	$H^2_B = (GV/PV)$	GV = Genotypic variance, PV = Phenotypic variance
Genetic advance	$GA = H^2_B * K * \sqrt{PV}$	H^2_B = Broad sense heritability, K is a constant (1.40 at 20% selection pressure), PV = Phenotypic variance
Genetic advance of mean	$GA (\%) = \left(\frac{GA}{\bar{X}}\right) * 100$	GA=Genetic advance, \bar{x} = the grand mean of the trait

5.5. Results

5.5.1. Traits analysis of variances

The single ANOVA (Table 5.3) showed in both locations very highly significant statistical differences between the entries for maturity, plant height, grain yield, stover fresh and dry matter yields. Replications were not homogenous in Tillabery while; they were in Konni. The blocks were homogenous within replication in both sites. The table 5.3 below describes the statistical expression of the different traits in the two locations.

Table 5. 2. Mean squares of *bmr* derived families.

Site	Source of variance	Df	50%FL	PH	GY	FSY	DM
Tillabery	Replication	2	147.343***	5670.9***	6168268***	431619342***	31231918***
	Treatment	99	249.869***	5918.1***	1161950***	44170996***	4623682***
	block/replication	27	10.903ns	385.6ns	276610ns	15021836ns	1959056ns
	Residual	171	11.07	425.1	286471	14385282	1909355
Konni	Replication	2	22.773ns	499.5ns	1447635**	5299815ns	466812ns
	Treatment	99	124.567***	3765.1***	503894***	24382567***	1753026***
	block/replication	27	31.886ns	1541.2ns	174572ns	8034003ns	676789ns
	Residual	171	34.5	1658.9	242280	13015877	932540

0.05=*; **=0.01; ***=0.001: significant at probability level; ns=not significant; Df =Degree of freedom; 50%FL=Days to 50% flowering; PH=Plant height; GY=Grain yield; FSY=Fresh stover yield; DM=Dry matter yield.

5.1.1. Time of flowering (50% FL)

- Tillabery,

there were highly significant differences observed among families. BCS1-33 with 51 days was the earliest family and BCS2-150 with 88 days was the latest. Families from population1 (BCS1) were earlier while their two parents were different in flowering by 21 days (52 days for El mota and 73 for Tx630*bmr12*).

In population1, all families flowered between 51 to 67 days: BCS1-33 was the earliest followed by BCS1-9 while BCS1-66 the latest.

In population2, days to 50% flowering varied from 60 to 88 days whereas their RP (Sepon82) and DP (Redlan*bmr6*) flowered at 76 and 68 days, respectively. Families BCS2-165 (60 days), BCS2-149 (64 days), BCS2-155 (65 days), BCS2-140 (65 days), BCS2-167 (67 days), BCS2-144 (67 days) were the earliest while BCS2-150 (88 days) was the latest.

In population3, days to 50% flowering ranged from 70 days (BCS3-192, earliest) to 80 days (BCS3-179, latest). The RP (Sepon82) and the DP (Wheatland*bmr12*) flowered at 76 and 65 days respectively.

Among local checks, El mota (52 days) flowered earlier than Sepon82 (76 days) and Tillabery landrace was the latest at 77 days.

- Konni

Highly significant differences were observed among families. Days to 50% flowering distribution ranged from 49 (BCS2-160) to 85 (BCS2-203). Families from population1 (BCS1) flowered earlier than the families from the other two populations.

In population1, days to 50% flowering ranged from 52 days (BCS1-13) to 73 days (BCS1-106). The earliest families were BCS1-13, BCS1-40, BCS1-15 (53 days) while the latest were BCS1-20 (70 days), BCS1-106 (73 days). The RP (El mota) and DP (Tx630 *bmr12*) flowered in 62 and 65 days respectively.

In population2, days to 50% flowering ranged from 49 days (BCS2-160) the earliest to BCS2-203 (85 days) the latest in flowering. Their RP (Sepon82) and DP (Redlan*bmr6*) flowered at 74 and 66 days, respectively.

In population3, days to 50% flowering ranged from 55 days (BCS3-183, the earlier) to 69 days (BCS3-210 the latest). Their RP (Sepon82) and DP (Wheatland*bmr12*) flowered at 74 and 62 days, respectively.

El mota flowered (62 days) earlier than Sepon82 (74 days) and the Tillabery (76 days) later.

5.1.2. Plant height (PH)

- Tillabery

Very highly significant differences were observed among the entries. Plant height varied from 3.18 m to 0.88 m. The Tillabery landrace was the tallest (3.18 m) followed by BCS1-102 (2.86 m) and El mota (2.54 m). Sepon82 had 2.01 m.

In population1, BCS1-102 (2.86 m) was the tallest followed by BCS1-82 (2.47 m), BCS1-95 (2.45 m) while BCS1-45 (1.53 m) was the shortest. RP (El mota) reached 2.54 m and the donor parent Tx630 *bmr12* had 1.09 m.

In population2, plant height varied from 2.22 m (BCS2-142), followed by BCS2-138 (1.73 m) to 1.10 m (BCS2-157). The RP (Sepon82) had 2.01 m and the donor parent (Redlan*bmr6*) had 1.32m.

In population3, BCS3-181 with 2.37 m was the tallest followed by BCS3-210 (2.32 m) and BCS3-179 (1.31 m) was the shortest. The RP (Sepon82) and DP (Wheatland*bmr12*) reached 2.01 m and 0.87 m, respectively.

- Konni

Very highly significant differences were observed among families. Plant height varied from 3.01 m to 0.82 m. The local Tillabery landrace was the tallest (3.01m) followed by BCS1-102 (2.57m).

In population1, BCS1-102 (2.57 m) was the tallest followed by BCS1-92 (2.47 m) and the shortest families were BCS1-20 (1.15 m) and BCS1-44 (1.12 m). Parental lines reached 2.43 m (El mota) and 1.14 m (Tx630*bmr12*) respectively.

In population2, BCS2-160 (2.17 m), BCS2-148 (2.13 m), BCS2-133 (2.09 m), BCS2-166 (2.07 m) and BCS2-158 (2.01m) were the tallest while BCS2-203 (1.15 m), BCS2-144 (1.22 m), BCS2-159 (1.36 m) were the shortest. The RP (Sepon82) had 1.82 m whereas the DP (Redlan*bmr6*) had 1.19 m.

In population3, BCS3-183 (2.28 m), BCS3-179 (2.02 m), BCS3-197 (1.87 m), BCS3-192 (1.83m), and BCS3-210 (1.83 m) were the tallest, while BCS3-181 (1.43 m) and BCS3-188 (1.71 m) were the shortest families. RP (Sepon82) had 1.82 m and the DP (Wheatland*bmr12*) had 0.82 m

5.1.3. Grain yield (GY)

- Tillabery

Very highly significant differences were observed among families. GY varied from 3504.2 kg/ha (BCS1-88) to 700.2 kg/ha (BCS3-181). Among local lines, Sepon82 (2631.1 kg/ha; 33.6% harvest index) produced better, followed by El mota (2415.0 kg/ha; 27.7% harvest index) and the Tillabery landrace (2402.06 kg/ha; 21.5% harvest index). The *bmr* donors produced less than the recurrent parents.

In population1, several families yielded better than their two parents (El mota and Tx630*bmr12*). BCS1-88 (3504.2 kg/ha and 46.5% harvest index) produced the highest grain yield followed by BCS1-102 (3428.5 kg/ha; 35.2% harvest index) and BCS1-17 (1083.6 kg/ha) had the lowest grain yield. The parental lines of this population yielded respectively 2415.0 kg/ha (El mota) and 1512.5 kg/ha (Tx630*bmr12*).

In population2, five families yielded better than their recurrent parents (Sepon82 and Redlan*bmr6*). BCS2-203 (2851.1 kg/ha; 48.8% harvest index) was the best followed by BCS2-155 (2731.2 kg/ha; 34.5% harvest index) and BCS2-148 (1155.8 kg/ha) was the least yielding family. Their parental lines yielded respectively 2631.1 kg/ha (Sepon82) and 1131.0 kg/ha (Redlan*bmr6*).

In population3, no derived lines yielded more than the recurrent parent Sepon82 (2631.1 kg/ha). BCS3-192 (2240.8 kg/ha; 27.5% harvest index) was the highest yielding family whereas BCS3-181 was the least yielding family (700.2 kg/ha). The DP yielded 1363.2 kg/ha less than the best family.

- Konni

There were very highly significant differences among entries. Families from population1 were better yielding. Sepon82 (2123.55 kg/ha; 38.7% harvest index) yielded better than El mota (1974.10 kg/ha; 31.7% harvest index) and the Tillabery landrace (2014.01kg/ha). The *bmr* donor lines produced lesser than the local sorghum lines.

In population1, grain yields varied from 3226.6 kg/ha (BCS1-60) to BCS1-17 (1285.8 kg/ha). The most productive families were: BCS1-60 (3226.6 kg/ha; 37.6% harvest index); BCS1-62 (3104.44 kg/ha; 48.5% harvest index); BCS1-95 (2945.93 kg/ha; 40.3% harvest index); BCS1-102 (2919.35 kg/ha; 35.3% harvest index); BCS1-127 (2828.23 kg/ha; 44.6% harvest index); BCS1-71 (2751.45

kg/ha; 35.4% harvest index). Their parental lines yielding respectively 1974.10 kg/ha (El mota) and 1472.6 (Tx630*bmr12*).

In population2, BCS2-203 with 2706.5 kg/ha yielded more while BCS2-157 yielded less. The highest yielding families were: BCS2-203 (2706.5 kg/ha; 35.3% harvest index); BCS2-138 (2355.69 kg/ha; 29.8% harvest index); BCS2-155 (2346.49 kg/ha; 38.8% harvest index). The parental lines produced 2123.55 kg/ha and 1414.0 kg/ha respectively for Sepon82 and Redlan*bmr6*.

In population3, BCS3-181 (2250.4 kg/ha; 41.3% harvest index) and BCS3-192 (2239.5 kg/ha; 28.7% harvest index) were highest grain yielding. BCS3-188 with 1859.8 kg/ha was the lowest. The recurrent parent (Sepon82) yielded 2123.55 kg/ha and Wheatland*bmr12* had 1119.8 kg/ha.

Grain yield performance was more variable in Tillabery (StDev=781.1) than in Konni (StDev=575.2).

5.1.4. Fresh Stover Yield (FS)

- Tillabery

There were very highly significant differences observed among families. The Tillabery landrace yielded 26053.54 kg/ha followed by BCS2-138 and BCS2-201 with respectively 23372.33 kg/ha and 19843.02 kg/ha. Wheatland*bmr12* had the lowest yield (3206.22kg/ha). Recurrent parent El mota and Sepon82 yielded on average 16658.29 kg/ha and 15776.26 kg/ha, respectively.

Population1: Fresh stover yields varied from 18945.42 kg/ha (BCS1-102) to 5568.40 kg/ha (BCS1-49). The RP (El mota) yielded 16658.29 kg/ha while the donor parent (Tx630*bmr12*) had 8161.53 kg/ha. In this population, superior families were BCS1-102 (18945.42 kg/ha), BCS1-66

(18288.44 kg/ha), BCS1-78 (17680.11 kg/ha), BCS1-71 (17141.93 kg/ha), BCS1-20 (17131.79 kg/ha), BCS1-127 (16972.37 kg/ha).

Population2: fresh stover yields varied from 23372.33 kg/ha (BCS2-138) to 8073.09 kg/ha (BCS2-150). The recurrent parent had 15776.26 kg/ha and the donor parent yielded 6841.42 kg/ha. The families with superior fresh stover yield were respectively: BCS2-138 (23372.33 kg/ha); BCS2-201 (19843.02 kg/ha); BCS2-199 (18090.62 kg/ha) and BCS2-145 (16969.51 kg/ha).

Population3: BCS3-201 with 16965.95 kg/ha was the highest yielding followed by BCS3-197 (16470.96 kg/ha) and BCS3-192 (16039.83 kg/ha). Parental lines yielded respectively 15776.26 kg/ha (Sepon82) and 3206.22 kg/ha (Wheatland*bmr12*).

- Konni

Very highly statistical significant differences were observed among families. BCS1-102 (20623.18kg/ha), BCS2-138 (20537.39kg/ha) and BCS2-166 (20481.78kg/ha) had arithmetically better yield than all families. In contrast, Wheatland*bmr12* (2783.81 kg/ha), Tx630*bmr12* (6780.08 kg/ha) and Redlan*bmr6* (9488.77 kg/ha) were respectively less yielding compared to their derived families. The Tillabery farmer landrace (18839.01kg/ha) yielded more fresh stover than El mota (14761.07 kg/ha) and Sepon82 (11869.29 kg/ha).

Population1: fresh stover yield ranged from 20623.18 kg/ha (BCS1-102) to 10499.142 kg/ha (BCS1-44). The RP and DP yielded respectively 14761.07 kg/ha and 6780.08 kg/ha. Better yielding families in this population were BCS1-102 (20623.18 kg/ha), BCS1-27 (20356.56 kg/ha), BCS1-60 (18150.42 kg/ha), BCS1-71 (18100.66 kg/ha).

Population2: BCS2-138 and BCS2-166 with respectively 20537.39 kg/ha and 20481.78 kg/ha were the elite families followed by BCS2-201 (20082.99 kg/ha) and BCS2-203 (18486.30 kg/ha).

Their parental lines produced 11869.29 kg/ha (RP) and 2783.81 kg/ha (Wheatland*bmr12*).

Population3: BCS3-192 with 20272.22 kg/ha yielded more followed by BCS3-183 (19471.22 kg/ha). The RP yielded 11869.29 kg/ha while the DP produced 2783.81 kg/ha.

5.1.5. Dry matter Yield (DM)

- Tillabery

Families exhibited very highly significant differences among them. The farmer landrace was higher yielding (8802.63 kg/ha) followed by BCS2-138 (8221.073 kg/ha), BCS1-102 (6336.27 kg/ha) and BCS3-210 (6197.56 kg/ha). Among the two RP, El mota (6297.64 kg/ha) yielded better than Sepon82 (5195.44 kg/ha).

Population1: In this population the dry matter yield ranged from 6336.27 kg/ha (BCS1-102) to 2116.65 kg/ha (BCS1-49). The parental lines gave respectively 6297.64 kg/ha (RP) and 2710.27 kg/ha (DP). The highest yielding families were respectively: BCS1-102 (6336.27 kg/ha), BCS1-66 (5849.70 kg/ha), BCS1-20 (5792.33 kg/ha), BCS1-78 (5640.15 kg/ha), BCS1-68 (5598.24 kg/ha).

Population2: In this population, the dry matter yields varied from 8221.07 kg/ha (BCS2-138) to 2596.5 kg/ha (BCS2-150). Sepon82 (RP) yielded 5195.44 kg/ha whereas Redlanbmr6 (DP) yielded 2729.92 kg/ha. Among the different families of this population, BCS2-138 (8221.07 kg/ha), BCS2-167 (6070.52 kg/ha), BCS2-199 (5990.39 kg/ha), BCS2-201 (5623.36 kg/ha), BCS2-145 (5592.97 kg/ha) were higher yielding.

Population3: In population3, BCS3-210 (6197.56 kg/ha) had better dry matter yield followed by BCS3-192 (5909.12 kg/ha), BCS3-197 (5202.07 kg/ha) while BCS3-179 (3880.63 kg/ha) had the lowest dry matter yield. The RP (Sepon82) yielded 5195.44 kg/ha and the DP (Wheatlandbmr12) had 1261.84 kg/ha.

- Konni

Very highly significant differences were observed among families. The Tillabery landrace yielded higher dry matter yield (5639.55kg/ha). This landrace was followed respectively by BCS3-192 (5566.28 kg/ha), BCS2-138 (5536.52kg/ha), BCS1-27 (5510.09kg/ha) and BCS2-166 (5507.19kg/ha). El mota (4258.90 kg/ha) yielded better than Sepon82 (3367.06 kg/ha).

Population1: BCS1-27 with 5510.09 kg/ha had the highest dry matter yield while BCS1-103 (2766.28 kg/ha) had the lowest dry matter yield. The RP (El mota) yielded 4258.90 kg/ha and the DP (Tx630*bmr12*) had 2091.69 kg/ha. Superior families of this population were: BCS1-27 (5510.09 kg/ha), BCS1-102 (5359.95 kg/ha), BCS1-60 (5345.31 kg/ha), BCS1-118 (5052.28 kg/ha), BCS1-71 (5016.62 kg/ha).

Population2: dry matter yields varied from 5536.52 kg/ha (BCS2-138) to 3095.56 kg/ha (BCS2-140). Parental line performances were respectively, Sepon82 (3367.06 kg/ha) and DP, Redlan *bmr6* (2387.87 kg/ha), while BCS2-138 (5536.52 kg/ha), BCS2-166 (5507.19 kg/ha), BCS2-201 (5385.87 kg/ha) and BCS2-203 (4958.55 kg/ha) were superior families.

Population3: dry matter yields ranged from 5566.28 kg/ha (BCS3-192) to 3205.00 kg/ha (BCS3-181). The RP (Sepon82) yielded 3367.06 kg/ha while the DP (Wheatland*bmr12*) yielded 1074.56 kg/ha. Superior families were BCS3-192 (5566.28kg/ha), BCS3-183 (5034.00 kg/ha), BCS3-179 (4646.96 kg/ha).

Table 5. 3. Summary of the single ANOVA and homogeneity of variances.

Traits	Site	Mean Sq	Df	F value	Fmax
50% Flowering-(50%FD)	TL	249.869***	99	22.5722	6.251
	KN	124.567***	99	3.6106	
Plant Height (PH-cm)	TL	5918.1***	99	13.922	6.133
	KN	3765.1***	99	2.2697	
Grain Yield (GY-kg/ha)	TL	1161950***	99	4.0561	1.95
	KN	503894***	99	2.0798	
Fresh Stover Yield (kg/ha)	TL	44170996***	99	3.0706	1.639
	KN	24382567***	99	1.8733	
Dry matter Yield (kg/ha)	TL	4623682***	99	2.4216	1.288
	KN	1753026***	99	1.8798	

0.05=*; **=0.01; ***=0.001: significant at probability level; Df =Degree of freedom;

TL=Tillabery and KN=Konni.

5.6. Combined ANOVA results

The Hartley's Fmax results indicate that variances are homogeneous for Grain yield, fresh stover yield and dry matter yield because the ratio of maxi variance divided by mini variance is less than 3. A combined ANOVA was therefore performed on those variables to selected *bmr* families exhibiting stable and acceptable performance across the two sites (table 5.5). Highly statistical differences were observed between the sites. Entrees performed diversely with high interaction between sites.

The data normality test was checked (Figure 5.2.). The normality test showed that, phenotypic data follow normal distributions for traits under study. However, a tendency of shift was observed at the tails for the fresh stover and dry matter yields.

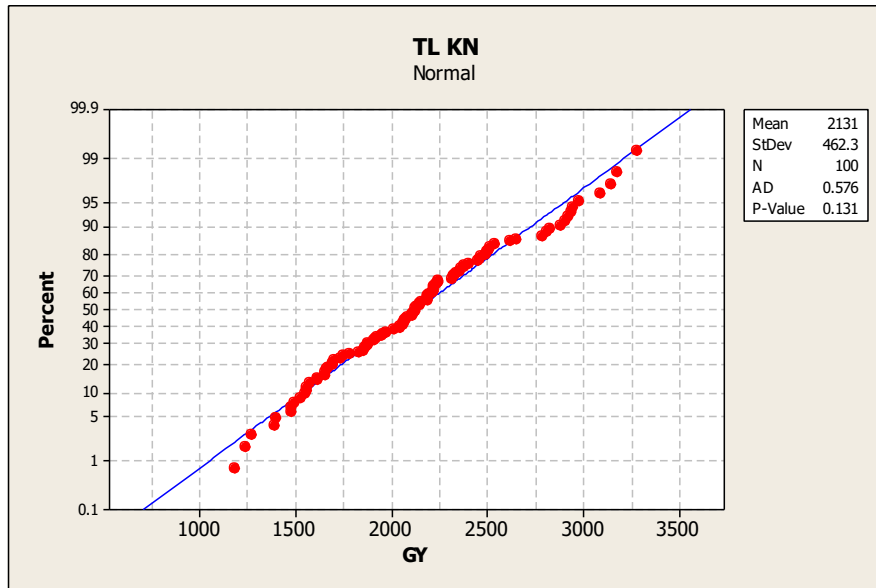


Figure 5 2. Grain yield data distribution

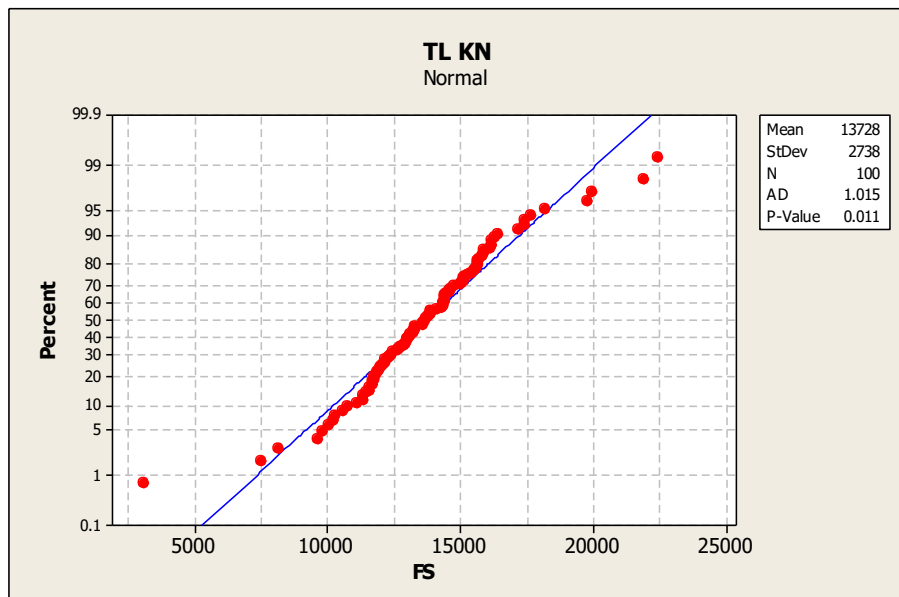


Figure 5 3. Fresh stover yield data distribution

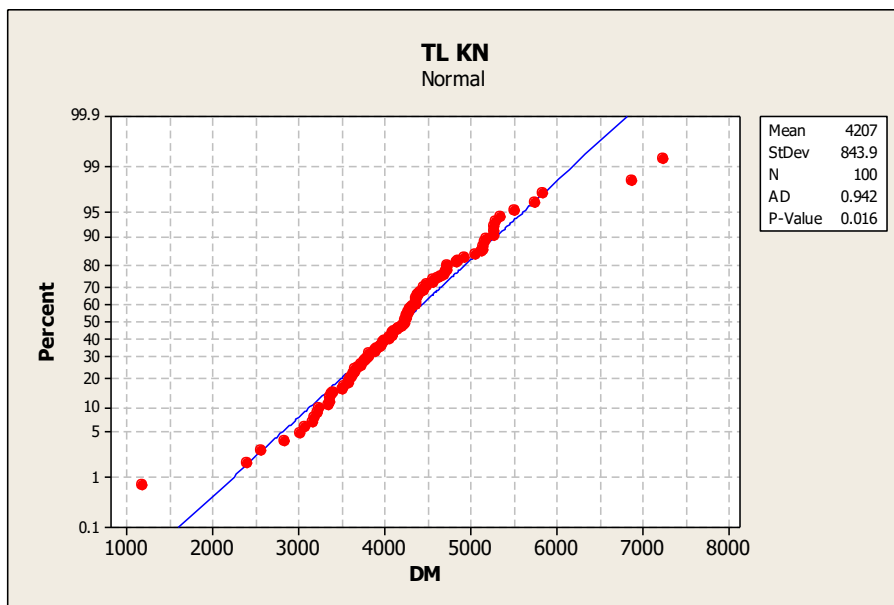


Figure 5 4. Dry matter yield data distribution

Table 5. 4. Results Combined ANOVA.

Variables	Source of variation	Df	Mean Sq
Grain yield	VAR	99	1281462***
	Site	1	7286547***
	Site*Replicates	4	3807953***
	VAR*Site	99	384383**
	Site*Replicates*Blocks	54	236568ns
	Residuals	342	262642
	Source of variation	Df	Mean Sq
Fresh stover yield	VAR	99	44580000.00***
	Site	1	1288000000.00***
	Site*Replicates	4	218500000.00***
	VAR*Site	99	23970000.00***
	Site*Replicates*Blocks	54	11310000.00ns
	Residuals	342	13740000
	Source of variation	Df	Mean Sq
Dry matter yield	VAR	99	4251992***
	Site	1	5169888ns
	Site*Replicates	4	15848919***
	VAR*Site	99	2124832**
	Site*Replicates*Blocks	54	1309670ns
	Residuals	342	1422247

0.05=*; **=0.01; ***=0.001: significant at probability level; ns = not significant

5.7. Interaction genotypes by environment-GxE

Results for the grain yield, fresh stover and dry matter yields indicate variation between the sites. Varieties performed diversely across the two sites (table 5.6). Indeed, the entries stability across the two environments revealed diverse adaptation levels for the grain, fresh stover and dry matter yield. The Wricke's ecovalence for genotypes consistency response results indicated that families from population1 and population2 were more consistent across the two locations (table 5.7).

Table 5. 5. Trait means dispersion per population and site of experiment.

Traits	Populations means dispersion								
	Entries	StDev		Mean		Maxi		Mini	
		TL	KN	TL	KN	TL	KN	TL	KN
50%FL	Whole Pop	9.541	8.000	63.003	61.553	99	89	47	46
	Pop1	4.138	6.902	56.81	59.711	70	81	47	46
	Pop2	7.576	9.45	72.42	63.89	99	89	58	48
	Pop3	4.392	7.27	75.095	63.14	86	80	65	51
PH	Whole Pop	47.70	48.35	184.83	184.07	356	350	76.33	72.3
	Pop1	31.05	45.49	205.81	191.14	294.66	350	125.33	87.7
	Pop2	32.23	42.11	136.56	170.19	356	263.7	90	107.3
	Pop3	38.18	49.3	191.82	185.5	259.33	310	125.33	119
GY	Whole Pop	784.1	575.2	2241.7	2021.3	4971.7	3753.9	450	862.9
	Pop1	754.2	599.5	2524.2	2104.1	4971.7	3753.9	844.6	889.8
	Pop2	620.3	547.0	1872.7	1895.9	3525.4	3145	1026.7	862.9
	Pop3	541	338.8	1528	2058.7	2398	2576.3	450	1412.5
FS	Whole Pop	5205	4035	12263	15193	34083	29583	1667	1750
	Pop1	4564	3657	11625	15300	24583	29583	4167	8333
	Pop2	5363	3806	13301	15628	26250	22917	3333	8790
	Pop3	5004	4405	13260	16230	23750	26250	6250	8750
DM	Whole Pop	1735	1085.4	4300	4114.6	11119	7980	543	642
	Pop1	1509	989.7	4119	4128.6	8626	7980	1355	1808
	Pop2	1832	1035	4496	4231	11119	6483	1541	2455
	Pop3	1643	1138	4923	4361	8047	6509	2021	2359

50%FL=days to 50% flowering; PH=plant height; GY=grain yield; FS=fresh stover yield; DM=dry matter yield; StDev=Standard deviation; Maxi=Maximum; Mini=Minimum; TL=Tillabey; KN=Konni.

Table 5. 6. Top 20 stable entries across Tillabery and Konni in Niger in 2017.

Rank	GY		FSY		DMY	
	Genotypes	Value	Genotypes	Value	Genotypes	Value
1	BCS2-201	3	BCS3-188	53	BCS1-59	314
2	BCS2-165	3	Wheatland <i>bmr12</i>	126	BCS1-41	365
3	BCS1-26	126	BCS1-44	362	BCS2-149	755
4	BCS2-142	396	BCS1-59	787	BCS2-141	919
5	BCS1-86	618	BCS1-108	1831	BCS1-73	1279
6	BCS1-90	679	BCS1-33	1997	BCS1-128	1886
7	BCS1-65	999	BCS2-133	5409	BCS2-156	1903
8	BCS2-172	1042	BCS1-72	9381	BCS1-121	2086
9	BCS1-10	1113	BCS1-88	14120	BCS1-60	2215
10	BCS2-159	1147	BCS3-192	15361	Tx630 <i>bmr12</i>	3024
11	BCS2-203	1403	BCS2-141	30221	BCS1-44	3199
12	BCS2-199	1484	BCS2-143	34001	BCS1-88	3827
13	BCS1-80	2174	BCS1-26	34720	BCS1-80	4159
14	BCS1-9	2220	BCS1-60	86041	Redlan <i>bmr6</i>	4539
15	BCS1-105	2957	BCS1-80	88379	BCS2-133	4945
16	BCS2-138	3460	BCS2-156	116123	BCS2-159	5485
17	BCS1-112	3648	BCS3-181	117842	BCS2-201	7801
18	BCS3-192	3748	BCS1-73	121070	BCS3-188	8167
19	BCS1-72	3853	BCS1-42	174930	BCS1-46	8725
20	Local Tillabery	3985	BCS1-82	239011	BCS1-33	9170

GY=grain yield; FSY=fresh stover yield; DM=dry matter yield; Value=coefficient of consistency.

5.8. Genetic and Agronomic parameters

The PCV and GCV were moderate for days to 50% flowering but were high for other variables at Tillabery (table 5.8). At Konni, the PCV was moderate for days to 50% flowering but high for other variables whereas the GVC values were low for 50% flowering and moderate for the remaining variables (table 5.9).

At Tillabery, the broad sense heritability (H^2B) was high for days to 50% flowering and plant height but moderate for the remaining variables. In Konni, H^2B was moderate for days to 50% flowering but was low for the all other variables.

The genetic advance (GA) was high for the plant height and the fresh stover yield, moderate for days to 50% flowering and finally low for the dry matter and grain yield in Tillabery. In Konni, the GA was moderate for the fresh stover yield but low for all the traits.

The GAM was moderate for days to 50% flowering and the dry matter but high for the other traits in Tillabery while it was moderate for the plant height and grain yield but low for all the traits at Konni.

Table 5. 7. Agronomic and genetic parameters of *bmr* derived families at Tillabery.

Traits	Site	VG	VP	PCV	GCV	H ² B	GA	GAM (%)
50%FL(days)	TL	79.599	90.669	15.113	14.16	0.877	0.117	18.575
PH(cm)	TL	1831	2256.1	25.698	23.15	0.811	0.539	29.198
DM(kg/ha)	TL	904775.7	2814131	39.01	22.119	0.321	7.55	17.559
FSY(kg/ha)	TL	9928571	24313853	40.21	25.695	0.408	28.189	22.987
GY(kg/ha)	TL	291826.3	578297.3	33.923	24.098	0.504	5.372	23.966

TL=Tillabery

Table 5. 8. Agronomic and genetic parameters of *bmr* derived families at Konni.

Traits	SITE	VG	VP	PCV	GCV	H ² B	GA	GAM(%)
50%FL(days)	KN	30	64.5	13.05	8.902	0.465	0.052	8.5
PH(cm)	KN	702	2360.9	26.397	14.394	0.297	0.202	10.9
DM(kg/ha)	KN	273495.3	1206035.3	26.69	12.71	0.226	3.486	8.4
FSY(kg/ha)	KN	3788896.6	16804774	26.981	12.811	0.225	12.939	8.5
GY(kg/ha)	KN	87204.6	329484.6	28.397	14.609	0.264	2.126	10.5

KN = Konni

5.9. Qualitative data analysis: Lodging and foliar diseases

5.9.1. Lodging

Recurrent (RP) and *bmr* donor (DP) parents showed a good resistance to lodging and tolerance to foliar diseases. Indeed, among parental lines, El mota was less sensitive to lodging. El mota showed 5.55% lodging at Tillabery (3 plants over 54 in the 3 replications) and 3.70% at Konni (2

plants over 54 at Konni in the 3 replications). One plant lodged in Sepon82 and no plant lodged in the Tillabery farmer landrace and the DP. At both Tillabery and Konni, 81.4% and 82.3% of the families showed resistance to lodging effect respectively.

At Tillabery resistance to lodging varied very highly among entries ($H_0 = 223.23$, $P = 0.000$). In population1, BCS1-45 and BCS1-86 with -1.86 and 2.89 respectively had the lowest and the highest z-value (z-value corresponding statistically to the standard deviation). In population2 BCS2-150 had the lowest z-value (-2.91), while a group of families (BCS2-152, BCS2-153, BCS2-155, BCS2-156, BCS2-157, BCS2-158, BCS2-159, BCS2-160, BCS2-165, BCS2-166, BCS2-167, BCS2-172, BCS2-199, BCS2-201 and BCS2-203) with -1.34 had the highest z-value. In population3, z-value varied from -1.34 (BCS3-179, BCS3-181, BCS3-183, BCS3-188, BCS3-192) to 1.03 (BCS3-210).

At Konni ($H_0 = 218.47$ and $P = 0.000$). The z-values for resistance to lodging varied from -1.46 to 2.88 (BCS1-86) in population1. In population2, z-values were -1.46 for all the families except BCS2-203 (-0.77) whereas in population3 z-values were -1.46 for all the families.

5.9.2. Foliar diseases

Few families (6%) showed leaf disease symptoms. Indeed, at Tillabery only 2.7% of the entries showed severe leaf disease symptoms (more than 40% of the plant with infected leaves). El mota was free of diseases while Sepon82 showed 3% presence of infected leaves. Local Tillabery exhibited 7% foliar disease presence. Among *bmr* donor parents, only Tx630*bmr12* was susceptible (25% of plant with foliar disease). In population1, BCS1-41 and BCS1-68 with 8% each and BCS1-43 (3%) were susceptible families. In population2, BCS2-143 (8%) was the most susceptible family whereas in population3 BCS3-179 (17%) was the only susceptible family.

At Konni, entries showed a good level of resistance to foliar diseases. Indeed, only 3.3% showed severe leaf disease symptoms. Sepon82 was free of foliar diseases while El mota and the Tillabery farmer landrace each 6.66%. Between the *bmr* donor parents, Tx630*bmr12* with 20% was the most susceptible. Readlan*bmr6* showed 16.66% and Wheatland*bmr12* 3.33%. The repartition between populations showed that in population1, BCS1-72 (16.33%) and BCS1-127 (21.66%) were the most susceptible families. In population2 and population3 respectively BCS2-143 (6%) and BCS3-179 (4.66%) were also the most susceptible families like in Tillabery.

Results from Tillabery on foliar diseases showed highly significant differences among entries ($H_0 = 135.96$ and $P = 0.008$). The z-values were -0.28 for all the families excepted BCS1-68 and BCS1-41 which had z value of 0.74 in population1 while in population2, the values varied from -0.28 (BCS2-138; BCS2-140; BCS2-141; BCS2-142; BCS2-145; BCS2-146; BCS2-149; BCS2-158; BCS2-159; BCS2-165; BCS2-167 and BCS2-201) to 2.81 (BCS2-203). In population3, only BCS3-179 with 0.79 showed different z-values from the -0.28 of the other families (BCS3-181, BCS3-183, BCS3-188, BCS3-192, BCS3-197, BCS3-210). At Konni, ($H_0 = 172.69$ and $P = 0.000$). In population1, the z-values were -0.75 however, BCS1-61 with 2.98 showed the highest value. In population2, the z values varied from -0.75 to 2.71 (BCS2-160) whereas in population3 only BCS3-179 with 2.26 exhibited a different value from the other families (-0.75 in general).

The z-values were used to compare the average rank of each population to the overall rank of all families (entries). In both Tillabery and Konni, lodging was more spread in population1 (TL=-1.86 to 2.89; KN=-1.46 to 2.88) compared to population2 (TL=-2.91 to -1.34; KN=-1.46 to -0.77) and population3 (TL=-1.34 to 1; KN=-1.46). The recurrent parents score for lodging were negative thus lesser than the mean. Sepon82 and the local Tillabery exhibited also lesser z-values compared to the overall mean. However, El mota with 0.11 and -0.05 at respectively Tillabery and Konni

was closer to the overall entries mean for the susceptibility to lodging. This may indicate a tendency for susceptibility to lodging of the recurrent parent El mota.

With regards to foliar diseases, population2 had more varied tolerance at Tillabery (TL=-0.28 to 2.81) while in Konni the range was most widely spread in the population1 (KN=-0.75 to 2.98).

The z-value scores showed that at Konni, the parental lines, were poorly resistant to foliar diseases except Sepon82. However, at Tillabery, Tx630*bmr12* was the most susceptible line.

Table 5. 9. z-values distribution of *bmr* derived families and checks.

Population	Konni		Tillabery	
	Lodging	Foliar Disease	Lodging	Foliar Disease
Population1	-1.46 to 2.88	-0.75 to 2.98	-1.86 to 2.89	-0.28 to 0.74
Population2	-1.46 to -0.77	-0.75 to 2.71	-2.91 to -1.34	-0.28 to 2.81
Population3	-1.46	-0.75 to 2.26	-1.34 to 1.03	-0.28 to 0.79
Checks				
Redlan <i>bmr6</i>	-1.46	2.86	-1.34	-0.28
Wheatland <i>bmr12</i>	-1.46	2.2	-1.34	-0.28
Tx630 <i>bmr12</i>	-1.46	1.6	-1.34	1.81
El mota	0.11	2.41	-0.05	-0.28
Local Tillabery	-1.46	2.41	-1.34	1.63
Sepon82	-0.85	-0.75	-0.79	0.67

5.10. Discussion

The results of this study showed that the families derived from population1 exhibited earliness compared to populations2 and population3 and the Tillabery landrace. In addition to their earliness, families from population1 showed high grain yield in both locations. Shapiro *et al.* (1993) reported that earliness gives drought escape and thus have the potential to increase yields in low-rainfall years. The results of this study are thus in agreement with those authors. Earliness combined with acceptable grain and healthy dry matter stover yields are essential breeding traits for new sorghum lines for local small-scale farmers facing shortage, erratic and variability in rainfall. Camara *et al.* (2006) revealed that characteristics of the variety such as early maturity, less diseases and drought resistance incidences and productivity influenced sorghum and millet adoption by farmers. Current study findings pave the road for sustainable livestock feeding in Niger with rain fed cropping environments.

The Tillabery landrace had the highest fresh stover and dry matter yield. El mota, the farmer variety also ranked among the best for dry matter yield. High stover yield of local landraces indicates farmers' varietal preference for varieties with high stover potential and shows the high importance of stover from cereal crops in the country. Magnan *et al.* (2012) reported that the importance of crop residue as feed has an implication for farmers' cereal variety choice. On the other hand, superior varieties with low biomass yield might not be selected by farmers in areas where stover value is high. The results of the current study are in agreement with those authors. There were families performing better than their parental lines in fresh and dry matter yields. Current results are in harmony with Kotasthane *et al.* (2015) who evaluated *bmr* sorghum parental lines and their derivatives for fresh and dry biomass and found some derived lines superior to their parental lines checks.

The results of this study indicate that several elites *bmr* derived families produced better grain yield than their recurrent parents in both locations. Oliver *et al.* (2005) reported that *bmr* genes were found to have negative agronomic impact on grain and stover yields in grain sorghum lines but the yield-drag can be overcome with heterosis. Furthermore, the improvement of the stover quality due to *bmr* genes may raise the potential of *bmr* derived families and compensate grain yield-drag for other families. Indeed, with an equal to greater harvest index, their dry matter yields were higher than the recurrent parental lines. In this view, the CGIAR (2011) reported that the classical target of research has been on increasing the output of grain, while the value of the stover was of secondary interest.

The current study results revealed that across locations and for all the traits, phenotypic variances were superior to genotypic variances. The results are thus in harmony with Sawadogo *et al.* (2014) who found similar results in a genetic evaluation of sweet sorghum accessions from Burkina Faso. This shows variability in plant material used (BC1F3) and selection opportunities to identify superior families. According to Bhagasara *et al.* (2017) variability availability is a pre requisite for any improvement program. The phenotypic and genotypic coefficients of variation were moderate for the 50% flowering time in both two locations. The current results are in agreement with those of Godbharle *et al.* (2010). This indicates that different families seemed to mature within a short interval. For the plant height, grain, fresh stover and dry matter yields, the phenotypic coefficients of variation were higher than the genotypic coefficient of variations. This indicates that those traits were highly influenced by the environmental conditions, corroborating with findings from Bhagasara *et al.* (2017).

In Tillabery, high heritability was observed for days to 50% flowering and plant height, moderate heritability was observed for the fresh stover, dry matter and grain yields while the genetic advance

was high for plant height and fresh stover yield. Bhagasara *et al.* (2017) also reported high heritability for days to 50% flowering and plant height. The heritability of a trait is important in plant breeding because it is required in determining its response to selection. The traits exhibiting high heritability (days to 50% flowering and plant height) can be improved through direct selection. The traits with moderate and low heritability can be improved through pedigree selection. In contrast, low heritability was observed at Konni for all traits excepted days to 50% flowering. This reveals high environmental conditions on families in this location.

The genetic advance was low for grain and dry matter yields at both locations. This indicates that those two traits are under the control of several genes and suggest that phenotypic selection may not be effective. Govindarasu *et al.* (1990) revealed that high heritability of a trait does not signify a high genetic advance. The results are thus in agreement with these authors.

Families performed diversely across and within location on lodging and foliar diseases during agronomic performance trials. In breeding for high stover quality, lines resistant to lodging and free of foliar diseases are important assets to consider. Lodging and foliar diseases can considerably decrease productivity (grain and stover yields) and also their quality. Therefore, those two qualitative variables were visually and daily investigated from vegetative until maturity time for each family across and within location. A good resistance to lodging was observed in both locations. The score1 (very good resistance) and score2 (good resistance) accumulated 81.4% at Tillabery and 82.3% at Konni. Indeed, 59.7 families were scored 1 and 21.7 families had score 2 at Tillabery. At Konni, 62 families obtained score 1 while 20.3 families scored 2. It is important to know that only 2.3% at Tillabery and 3.67% at Konni of families were very susceptible to lodging (score5). This good level of *bmr* derived families' resistance to lodging was supported by Bean *et al.* (2013), who observed no significant differences in lodging between *bmr* and

conventional forage sorghum. Sattler *et al.* (2010) reported that increases in lodging attributable to *bmr* genes were not detected and the genetic background of sorghum affects lodging more than brown midrib. Li *et al.* (2015) also concluded that genetic characteristics are likely to play a significant role in the lodging resistance of *bmr12* plants, and lodging resistance associated with *bmr12* plants can be significantly improved by heterosis. Getachew *et al.* (2016) informed that in *bmr* hybrid variety, lodging was more influenced by the variety or delayed harvest period than the *bmr* genotype. At population levels, Population1 was more susceptible to lodging than population2 and population3 across locations. *Bmr12* gene was successfully introgressed in both population1 and population3. Thus, lodging observed in population1 can be due to the genetic background of its recurrent parents or a combination between the two parents. Indeed, the recurrent parent El mota reached 254.99 cm at Tillabery and 196.33 cm at Konni. Sepon82 was 201.88 cm at Tillabery and 124.33 cm at Konni. Saballos (2008) affirmed that tall plants are more prone to lodging. Findings from this current study are in agreement with those reported by (Cherney *et al.*, 1991 and Oliver *et al.*, 2005) who asserted that the effect of *bmr* mutations on forage quality varies depending on the genetic background of the recurrent parent. To prevent the effect of such phenomenon, Vogler *et al.* (2009) suggested the importance of identifying a suitable genetic background that allows for optimal impact of the mutation. Kruskal-Wallis non-parametric test for families' resistance to lodging showed a high range of z-value in population1 compared to population2 and population3 across locations. This result shows a great diversity among families in population1. It is important to mention that population1 was derived from a farmer variety with heterogeneous plant height. The results are in agreement with Sawadogo *et al.* (2014) who reported the high diversity in farmers' varieties due to their seed production and management systems. Indeed, farmers produce their own seeds in areas where several varieties coexist. In contrast, the

low range of z-values in population2 and population3 explains the character of improved varieties of both RP and DP.

Families showed resistance to foliar diseases across both locations. This good resistance to foliar diseases observed is in agreement with Dowd *et al.* (2016) who observed in laboratory no consistent increase in insects or pathogen damage for the low lignin sorghum lines. Later under field condition, the same authors concluded that sorghum lines *bmr6* and *bmr12* generally do not have increased susceptibility to insects and disease as compared to wild type. For the leaf diseases, the identified pathogens in Niger include: *Ramulispora sorghicola* and *R. sorghi*, *Cercospora sorghi*, *Colletotrichum graminicola*, *Ascochyta sorghina*, *Helminthosporium turcicum* and *Gloeocercospora sorghi* (Sidibe, 1978). In general, a low level of sorghum susceptibility to foliar diseases was reported in previous study (Bandyopadhyay *et al.*, 1987) who claimed that the *Acremonium strictum* disease has been observed in some African sorghum growing countries like Lesotho, Zimbabwe, Malawi, Tanzania and Mali but does not appear to be a serious disease on varieties currently grown by-farmers. The comparison between different populations showed that in population1, five different families BCS1-41, BCS1-68, BCS1-43, BCS1-72 and BCS1-127 were susceptible to foliar diseases in the two locations. This result can be due to the susceptibility or the poor adaptability of Tx630*bmr12*, the donor parent. Indeed, this line was the most susceptible line among the *bmr* donor parents and exhibited a high level of 25% susceptibility at Tillabery and 20% at Konni. In contrast, in population2 and population3, families BCS2-143 and BSC3-179 were susceptible in both locations at different degree respectively. Kruskal-Wallis non-parametric test tolerance to foliar diseases showed that families derived from population1 exhibited the lowest z-value in Tillabery, but the highest z-value in Konni. Those results show the

good adaptability of the RP in the Tillabery compared to Konni in addition to the high level of susceptibility of the *bmr* DP in Tillabery and Konni.

The following *bmr* derived families BCS1-60, BCS1-102, BCS2-138, BCS2-201, BCS3-192, BCS3-210 exhibited promising potential for dual purpose target. However, susceptibility to lodging in BCS1-60, BCS1-102 is observed and additional backcrossing may contribute for more genetic stability.

The GxE analysis was performed for three quantitative traits (grain, fresh stover and dry matter yields) in order to identify the most stable entries across the two locations. The different lines performed very diversely with various ranking stability levels. Kang (2002) found that for quantitatively inherited traits, the genotype values and their relative rankings can change from one environment to another. The results are in agreement with this author. Furthermore, Kimbeng *et al.* (2009) asserted that the rank changes confound the determination of the overall true genetic value of the prospective varieties.

5.11. Conclusion

Different *bmr* derived families performed diversely across locations for all seven variables studied. Recurrent parents had good grain and dry matter yields. The *bmr* donor parents' agronomic performances were poor to moderate in grain and dry matter productivity in the local growing conditions. However, the *bmr* breeding populations productivity was acceptable for both grain and dry matter yields. The agronomic performance of some *bmr* derived families from population1 and population2 for both grain and dry matter yields were higher than those of their recurrent parent alones. However, in population3, *bmr* derived families had grain yield reduction tendency while dry matter production was superior in all populations compared to recurrent parents' performance. Families from population1 exhibited early maturity pattern while families from population3 were later maturing.

Stover quality traits of *bmr6* and *bmr12* genes introgression did not significantly decrease the resistance to lodging and foliar disease in the breeding populations.

The recurrent parent El mota exhibited a little sensitivity to lodging.

Within each population, the following *bmr* derived families BCS1-102; BCS1-60; BCS2-138 and BCS3-192 were identified respectively in Population1, Population2 and in Population3 as superior families across locations for grain and stover yields. These families are potential candidates for stover and grain yield improvement for sustainable cattle productivity (milk and beef) and will open for the next future cattle intensive production. Opportunity to use *bmr6* and *bmr12* genes enriched sorghum lines as an alternative for sorghum stover quality improvement at small scale farmers' level is opened.

CHAPTER SIX

6.0. DETERMINATION OF NUTRIENT CONTENT OF *BROWN MIDRIB* DERIVED POPULATION

6.1. Introduction

Stover quality can be defined in various ways. Practically it can be considered as the quantity intake to which it has the potential to produce a desired animal response. According to Ball *et al.* (2001), factors that influence forage quality include the following: intake, palatability, digestibility and nutrient content. The nutrient content of a given line is therefore of great importance in the extensive livestock husbandry. In the developing countries, the FAO declared that the cereals residues are major forages and sorghum residues was qualified as a valued feed, especially if cut and dried immediately after the heads have been harvested for grain (www.fao.org). In small-scale farming systems, stover is usually harvested and dried after grain harvest. The unchopped stover is further stored and used as livestock feed. In maintaining sustainable production systems, crop residues can play a strategic role if high quality of stover is used for livestock feeding. Indeed, Elbasha *et al.* (1999) foresee a shift to greater importance of crop residues to increase livestock production. FAO (2012) reported that in tropical countries, dairy animals are primarily fed on crop residue based diets with very little green fodder/hay/silage, which if available may only be for a limited time.

A participatory rural appraisal conducted in 2016 in three localities in Niger revealed that during the dry season, which coincides with shortage of feed, milking cows, bullocks and then draught animals were the main beneficiaries of sorghum stovers (Diakit  *et al.*, 2017). However, those stovers were qualified of low nutritive values by farmers and stover traders. In this view, Suharti

et al. (2011) asserted that low cattle production may be caused by inadequate nutrient supply in a high-forage based ration in developing countries.

In countries like Niger, there is a pressing demand for efficient stover improvement for both quantity and quality to sustain livestock productivity. To date no studies have been reported on sorghum stover nutritive value. Furthermore, the value of local sorghum varieties containing the *bmr* trait need to be assessed. Ninety-four (94) BC1F3 derived *bmr* families were phenotyped for agronomic performances specially for the grain and dry matter yields. Among them, 20 elite families were selected based on their superior phenotypic performance in one location (Tillabery) to determine their nutritional contents. Families' parental lines were also analysed along with their progenies. The main objective of this study was to assess nutrient content of selected *bmr* derived families and respective parental lines. The specific objectives were to:

- determine the nutritional composition of the *bmr* derived families, and
- identify superior families with high nutritional values.

6.2. Material and methods

6.2.1. Plant material

The plant material was composed of 20 entries selected for their superior agronomic performance plus six checks composed of the recurrent and *bmr* donor parents and one farmer landrace (Table 6.1.).

The Baker's Standard Deviation for multi traits selection method (BSD) was used to select the elite *bmr* derived families. Early maturing (days to 50% flowering time), grain and dry matter yields were considered.

$$BSD = \Sigma \bar{X}_I / \sigma_{pi} :$$

Where $\Sigma \bar{X}_I$ is the sum of mean of the ith trait and

σ_{pi} is the phenotypic standard deviation of the ith mean.

The selected families were set out as follow: Population1 (El mota//El mota/Tx630bmr12) was represented by 12 families. Population2 (Sepon82//Sepon82/Redlanbmr6) contains 6 progenies families and finally population3 (Sepon82//Sepon82/Wheatlandbmr12) includes 2 families.

Table 6. 1. List of selected families and parental lines.

Populations								
Number		Code		Number		Code		
Population1	1	BCS1-18	Population2	13	BCS2-138	Population3	14	BCS2-145
	2	BCS1-60		15	BCS2-172		15	BCS3-192
	3	BCS1-66		16	BCS2-199		16	BCS3-210
	4	BCS1-68		17	BCS2-201		17	El mota
	5	BCS1-71		18	BCS2-203		18	Local Tillabery
	6	BCS1-82		19	BCS2-203		19	Redlanbmr6
	7	BCS1-88	Checks	20	BCS3-192	20	Sepon82	
	8	BCS1-92		21	BCS3-210	21	Tx630bmr12	
	9	BCS1-95		22	El mota	22	Wheatlandbmr12	
	10	BCS1-102		23	Local Tillabery	23		
	11	BCS1-118		24	Redlanbmr6	24		
	12	BCS1-127		25	Sepon82	25		
			26	Tx630bmr12	26			
				Wheatlandbmr12				

6.2.2. Methodology

Stover samples (leaves + stems) of every family was collected at after panicle harvest at maturity. Sorghum plants were cut at ground level. The collected stover samples were dried in shade on shelves during two months.

Stover chemical composition determination was conducted at the Animal Nutrition Laboratory (LNA) of IER/Sotuba in Mali in 2018. All samples were dried at 50°C for 48 hours before the chemical analysis. Drying sample was to eliminate all remaining humidity in all samples in order to work with complete dried samples.

6.2.3. Stover nutrient content data analysis

A descriptive analysis was firstly done for the mean distribution of every variable (Appendix 1). Then a one sample t-test for differences of the means was performed for all the variables. In each population, the recurrent parents' mean was tested against its derivative progenies' mean. The t-value were used to measure the size of the difference while the p-value were used for statistical differences observation.

6.2.3.1. Data collection

The stover quality analysis were based on the following elements: Dry matter, Net Energy, Crude Protein content, mineral content (calcium and phosphorus), Cellulose content, Neutral Detergent Fiber, Acid Detergent Fiber, and the Acid Detergent Lignin using the sequential filter bags method of the ankom technology for the last three elements (Ankom, 2010). For the other elements the analysis were conducted according to the description below:

- **Dry matter (DM):** The samples dry matter consisted as their final mass when they were completely dried except their water content. Its estimation was done by sampling 2 grams (P1) in each sample. After that, those 2 grams were placed in the oven at 105°C for 4 hours. Then all the samples were weighed to get their dry weight (P2). The difference P1-P2 gives the dry matter content of each sample.

- **Net Energy (NE):** The energy is a quality associated with the nutrient content of feedstuffs and mixed diets and is therefore, the most accurate and unbiased way to date of characterizing the energy content of feed (Moehn *et al.*, 2005). In each sample 0.22 g was weighted. Then the 0.22 g was put into pellets to prevent its spraying throughout the calorimeter during the influx of oxygen. Pure oxygen was used to combust the sample in the calorimeter using a bomb calorimeter.

- **Crude Proteins content (CP):** CP is widely used to determine the protein requirements in a feed. It is thus a significant indicator of feed quality. CP determination was conducted using the Kjeldahl method. In practice, this method consists of 3 main steps (digestion, distillation and the titration) leading to the determination of the amount of nitrogen (N) in each sample. In general, the term CP refers to all the nitrogenous compounds in a feed therefore the total amount of CP was finally obtained as follow: $CP = N \times 6.25$.

- **Minerals:** The major mineral requirement for cows feed include calcium and phosphorus. The calcium content in every sample was estimated using a photometer while the phosphorus content was determined using the spectrolitic²¹.

- **Cellulose content:** The Wend method was used to estimate the cellulose content in every sample. In practice, 1 g was taken from every dried sample and put in a flask. Then sulfuric acid was added and the flask was heated in an oven at 150°C for 2 hours. After that the sample was

weighted to get P1. At this point, the sample was burned at 700°C for 2 hours and weighted again to get P2. Finally, the percentage of cellulose of the sample was obtained by doing $(P1-P2) * 100$.

- **Neutral Detergent Fiber (NDF):** The NDF is basically a mixture of hemicellulose + cellulose + lignin. With a very highly sensitive balance, 0.5 g portion was taken from every sample and put in a centrifugal mill with a 2 mm screen. A heat sealer was utilized to completely seal the upper edge of the filter bag within 4 mm of the top. Two blank bags were added to the experiment to get the blank bag correction. The experiment was conducted in conformity to the ankom protocol (Ankom, 2010) and finally the percentage of NDF of every sample was determined using the following formula:

$$\%NDF = (W3 - (W1 * C1)) * 100 / W2$$

Where: W1= bag tared weight; W2= Sample weight; W3= Dry weight of bag with fiber after extraction process; C1= Blank bag correction (running average of final oven-dried weight divided by the original blank bag weight).

- **Acid Detergent Fiber (ADF):** The ADF is a mixture of cellulose + lignin. It was determined after the NDF. The same bags containing the same samples from the previous protocol were used in conformity to the ankom protocol. The percentage of ADF was calculated as follow:

$$\%ADF = (W3 - (W1 * C1)) * 100 / W2$$

Where: W1=Bag tared weight; W2 = Sample weight; W3 = Dry weight of bag with fiber after extraction process; C1 = Blank bag correction factor (running average of final oven-dry weight divided by original weight).

- **Acid Detergent Lignin (ADL):** The ADL correspond to the lignin content in a sample. It was determined on the residue from the ADF procedure. Practically, an acidified detergent solution was used to dissolve cell soluble, hemicellulose leaving a residue of cellulose, lignin, and heat

damaged protein, a portion of cell wall protein plus the minerals. ADL was determined gravimetrically as the residue remaining upon ignition after 72% sulfuric acid (H₂SO₄) treatment.

$\% \text{ Lignin} = (\text{Crucible Weight after Acid Soak} - \text{Crucible Weight after Ignition}) / (\text{Sample Weight} \times \text{DM})$

6.3. Results

The Baker's Standard Deviation (BSD) for multi traits selection method allowed a clear identification of the top 20% superior families in every population. Table 6.2. shows the agronomic characteristics and BSD score of every selected BC1F3 family. The mean distribution gave the spread tendency and the variability in every population for all the variables (Appendix 1). The one sample t-test allowed the comparison between the recurrent parental lines and their derivative *bmr* progenies for the nutrient content in each population. Table 6.3. shows the t-test results.

Table 6. 2. Agronomic characteristics and BSD values of the entries.

Families	50%FL (Day)	GY kg/ha	DMY kg/ha	BSD 50%FL	BSD GY	BSD DM	BSD TOTAL
BCS1-66	67	2732.64	5849	19	5.19	5.48	29.89
BCS1-95	63	3334.58	5014.47	18	6.33	4.69	29.29
BCS1-102	58	3429.58	6322.07	17	6.51	5.92	29.25
BCS1-127	62	3125.42	5219.5	18	5.93	4.89	28.6
BCS1-82	58	3163.33	5614.68	17	6.01	5.26	27.99
BCS1-71	57	3113.19	5516.24	17	5.91	5.16	27.61
BCS1-118	56	3294.31	5490.05	16	6.26	5.14	27.54
BCS1-18	57	3035.69	5505.19	16	5.76	5.15	27.35
BCS1-88	59	3505	4037.18	17	6.66	3.78	27.35
BCS1-92	55	3373.06	5318.14	16	6.41	4.98	27.34
BCS1-60	55	3330.97	5324.22	16	6.33	4.98	27.17
BCS1-68	58	2665.42	5600.68	17	5.06	5.24	27.13
BCS2-138	71	2536.53	8199.72	11	5.5	6.98	23.22
BCS2-172	87	2100	4780.64	13	4.55	4.07	21.67
BCS2-203	86	2854.16	2991.09	13	6.19	2.54	21.64
BCS2-199	74	2434.37	6004.29	11	5.28	5.11	21.48
BCS2-145	69	2561.81	5599.27	10	5.55	4.76	20.76
BCS2-201	76	1881.11	5628.04	11	4.08	4.79	20.36
BCS3-210	74	2114.91	6169.08	24	4.01	7.15	35.14
BCS3-192	70	2238.61	5908.43	23	4.24	6.84	33.99
Local Tillabery	77	2405	8805.6	22	4.57	8.24	34.92
Sepon82	76	2634.72	5200.26	22	5	4.87	31.88
Tx630 <i>bmr12</i>	73	1514.3	2713.1	21	2.88	2.54	26.37
Redlan <i>bmr6</i>	68	1131.66	2732.45	20	2.15	2.56	24.22
Wheatland <i>bmr12</i>	66	1356.7	1287.07	19	2.58	1.21	22.72
EL mota	52	2413.05	6296.52	15	4.58	5.89	25.57

50%FL=50% Flowering time; GY=Grain yield; DMY= Dry matter yield; BSD= Baker's Standard Deviation

- **Dry matter (DM):** In population1 the dry matter varied from 94.05% (BCS1-71) to 96.67% (BCS1-60). The dry matter content of their two parental lines was 95.32% and 95.25% respectively for the *bmr* donor and the recurrent parents. The t-test analysis revealed a t-value of 2.71 while the p-value was 0.019. Those results revealed the difference between the RP mean and the progenies mean.

In population2, the dry matter varied from 94.42% (BCS2-203) to 95.98% (BCS2-138). The dry matter content of their two parental lines was 95.00% and 95.32% respectively for the *bmr* donor and the recurrent parents. The t-value was 0.23 while the p-value was 0.826. Therefore, no statistical significant difference was observed between the RP mean and the progeny mean.

In population3 the dry matter varied from 94.48 % (BCS3-210) to 95.15% (BCS3-192). The dry matter content of their two parental lines was 95.02% and 95.32% respectively for the *bmr* donor and the recurrent parent. There were no significant differences between the means of the recurrent parental line and the progenies means (t-value=-1.31 while p-value=0.320).

Among the local sorghum lines, the Tillabery farmer landrace with 95.35% exhibited the highest dry matter content followed by Sepon82 and finally El mota. The figure 6.1. shows the performance of every family.

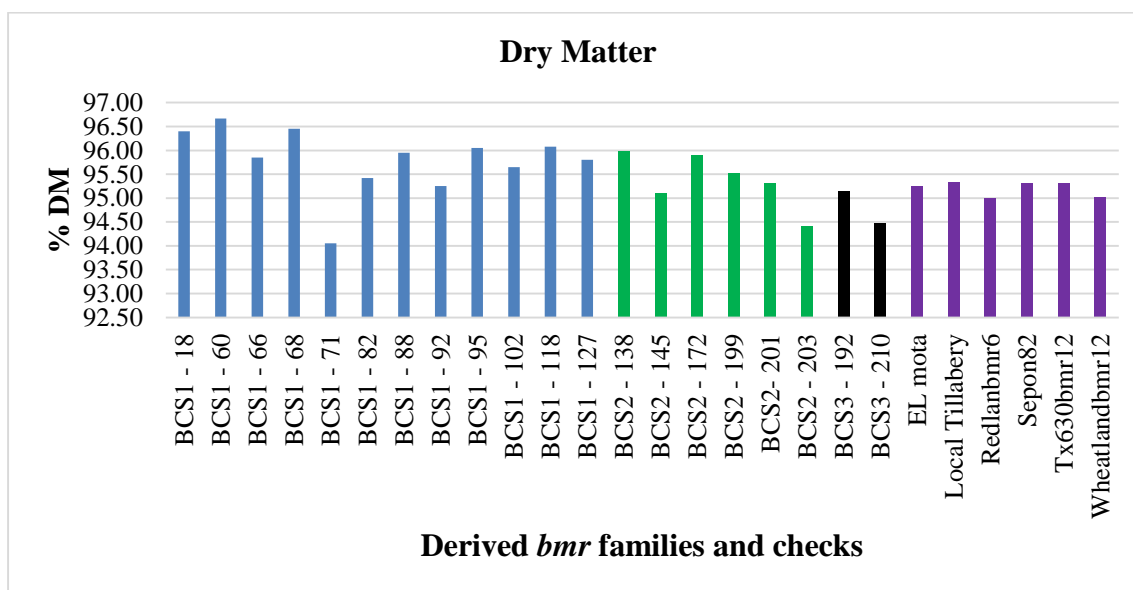


Figure 6. 1. Dry matter content of the selected families and the checks.

- **Net Energy (NE):** In population1 the net energy varied from 2893.41 kcal/kg DM (BCS1-118) to 3755.02 kcal/kg DM (BCS1-71). Their two parents produced respectively (3392.76 and

3611.37 kcal/kg DM) for the *bmr* donor and the recurrent parent. The t-value (2.44) and p-value (0.031) revealed the statistical difference between the RP and progenies means.

In population2, the net energy varied from 3506.83 kcal/kg DM (BCS2-172) to 3732.31 kcal/kg DM (BCS2-201) while their parental lines produced respectively 3664.32 for the *bmr* donor and 3525.24 for the recurrent parent. The t-test revealed a t-value of 2.17 while the p-value was 0.073. Those results indicate that there were no statistical differences between the RP mean and its' progenies mean.

In population3, the net energy varied from 3673.57 kcal/kg DM (BCS3-192) to 3692.49 kcal/kg DM (BCS3-210). The t-value was 1.99 and the p-value was 0.185. Therefore, the DP mean and the derivative progenies' mean were statistically equal.

Among the local lines, the Tillabery farmer landrace with 3714.26 kcal/kg DM and had the highest net energy followed by El mota and finally sepon82.

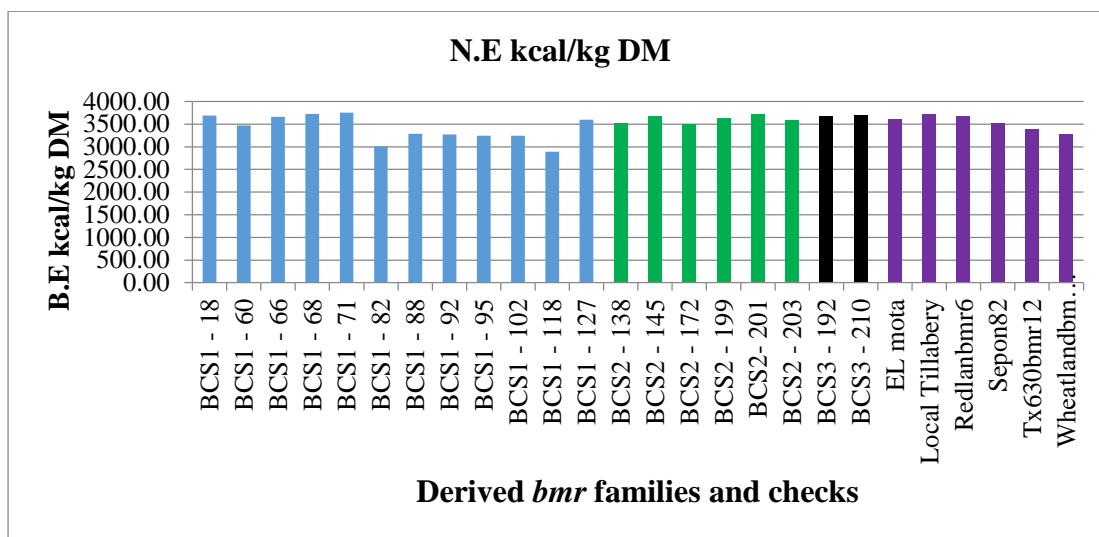


Figure 6. 2. Net energy content of the selected families and the checks.

- **Crude Proteins (CP):** In population1 the CP content varied from 2.75% (BCS1-60) to 5.63% (BCS1-82) while their two parents had respectively 3.81% for the *bmr* donor and 3.75%

for El mota. The t-test revealed a t-value and p-value of 1.51; 0.156 respectively. The results indicate no statistical difference between the RP mean and its' derivative progenies' means.

In population2, the CP content varied from 2.63% (BCS2-201) to 7.00% (BCS2-172). Results of their two parental lines were 5.31% for the *bmr* donor and 3.94% for Sepon82. The results from the t-test indicate a statistical equality between the RP mean and the derivative progenies means: (t and p-values were respectively: 1.27 and 0.251).

In population3, the CP content varied from 4.06% (BCS3-210) and 4.13% (BCS3-192). Parental lines produced respectively 5.44% for the *bmr* donor and 3.94% for Sepon82. The t-test revealed that the RP mean was not greater than the derivative progenies mean (t-value=1.86 and p-value=0.204).

The Tillabery farmer landrace with 4.25% had more CP than sepon82 which was followed by El mota. Among all the entries, the families from population2 (BCS2-172 and BCS2-138) exhibited the highest CP content (figure 6.3.)

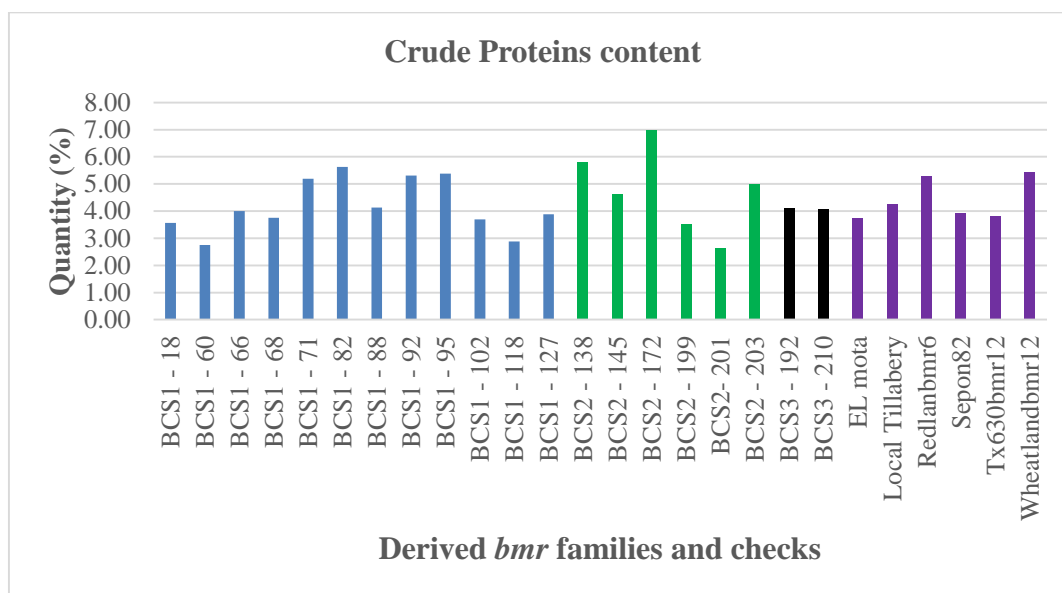


Figure 6. 3. Crude proteins content of the selected families and the checks.

- **Minerals:** Minerals, particularly the macro minerals play an important role in cattle correct nutrition. Generally, the calcium/phosphorus ratio is reported as important because an imbalance can cause infertility.

In population1 the calcium content varied from 0.10% (BCS1-127) to 0.39% (BCS1-68). Tx630*bmr12* the *bmr* donor line produced 0.05% while El mota the recurrent parent had 0.10%. The t-test results revealed a t-value of 2.99 while the p-value was 0.011.

In the population2 the calcium content varied from 0.05% (BCS2-199 and BCS2-172) to 0.15% (BCS2-203 and BCS2-138). Redlan*bmr6* the *bmr* donor parent had 0.13% while Sepon82 the recurrent parent had 0.15%. There was no statistical difference between the RP mean and the progenies mean. Indeed, t-value=1.27 while the p-value=0.251.

In the population3, the calcium content varied from 0.04% (BCS3-210) to 0.15% (BCS3-192). Their parental lines had respectively 0.21% for Wheatland*bmr12* the *bmr* donor and 0.15% for Sepon82 the recurrent parent. The t-test revealed a t-value=1.86 and p-value=0.204. The calcium content was therefore homogenous in the population3.

The Tillabery farmer landrace with 0.17% had the highest calcium content among the local lines and was followed by Sepon82 and finally by El mota.

In population1 the phosphorus content fluctuated from 0.10% (BCS1-118 and BCS1-92) to 0.32% (BCS1-127). Tx630*bmr12* the *bmr* donor line contained 0.05% while El mota the recurrent parent held 0.21%. The t-test revealed no statistical difference between the RP and the derivative progenies. Indeed, t-value=-1.01 while the p-value=0.333.

In population2, the phosphorus content ranged from 0.06% (BCS2-203) to 0.27% (BCS2-199). Redlan*bmr6* the *bmr* donor parent contained 0.13% while Sepon82 the recurrent parent held

0.17%. No statistical difference was observed between the RP and its' progenies: t-value=0.1 while p-value=0.926.

In population3, the phosphorus content varied from 0.02% (BCS3-210) to 0.23% (BCS3-192). Wheatland**bmr12** the *bmr* donor contained 0.12% while Sepon82 the recurrent parent had 0.17%. No statistical difference was observed between the RP and its' progenies: t-value=-0.48 while p-value=0.678.

Among the local lines, the Tillabery farmer landrace with 0.25% had the highest values and was followed by Sepon82 and finally El mota.

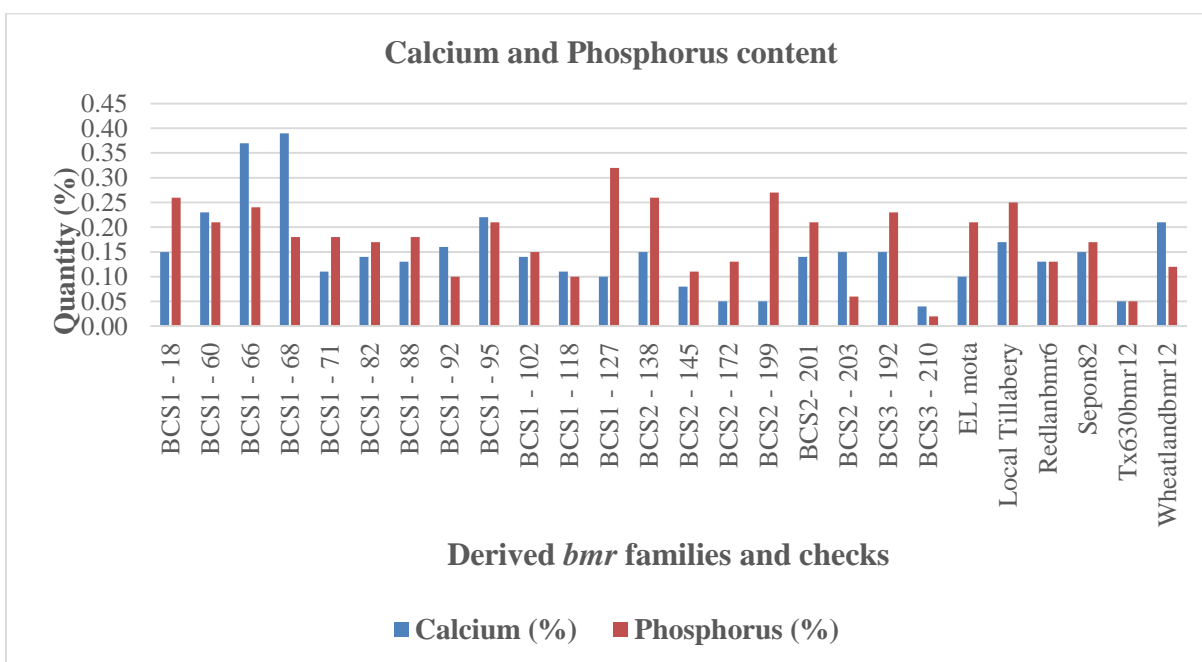


Figure 6. 4. Calcium and Phosphorus content of the selected families and the checks.

- Fibers content:** Fiber can be described as the carbohydrate fraction of the feed that cannot be digested. Indeed, fibers are resistant to digestive enzymes. Fibers are mostly composed of

cellulose, neutral detergent fiber, acid detergent fiber and acid detergent lignin. Each of the cited element was measured and the following results were obtained.

- **Cellulose content:** Cellulose is an important structural component of the plants cell walls. The cellulose content obtained fluctuated from 28.56% (BCS1-92) to 32.56% (BCS1-68) in the population1. Tx630*bmr12* the *bmr* donor parent had 30.53% while El mota the recurrent parent held 34.70%. The t-test revealed a very highly statistical difference between the RP and its' derivative progenies. Indeed, the t-value=-7.95 while p-value=0.000.

In the population2, the cellulose content ranged from 24.2% (BCS2-203) to 31.75% (BCS2-172). Redlan*bmr6* the *bmr* donor parent contained 31.58% while Sepon82 held 33.31%. The t-value was -3.5 while the p-value was 0.013. A statistical difference exists in population2 for the cellulose content.

In population3, the cellulose content varied from 26.20% (BCS3-192) to 28.31% (BCS3-210). Wheatland*bmr12* the *bmr* donor contained 33.83% while Sepon82 had 33.31%. The results from the t-test showed an uniformity of cellulose content between the RP and the progenies: t-value=-1.91 while the p-value=0.196.

Among the local lines, El mota had the highest cellulose content followed Sepon82 and finally the Tillabery farmer landrace. Several families from population1 exhibited high cellulose content.

- **Neutral Detergent Fiber (NDF):** In population1 the NDF content fluctuated from 55.66% (BCS1-71) to 65.17% (BCS1-60) while for the parental lines the NDF values were 62.77% for Tx630*bmr12* the *bmr* donor and 67.15% for El mota the recurrent parent. A highly statistical difference exists between the RP mean and the progenies: t-value=-6.34 and p-value=0.000.

In population2, BCS2-145 with 53.99% had the lowest NDF and BCS2-172 the highest value (62.15%). Redlan*bmr6* the *bmr* donor held 62.58% while Sepon82 contained 62.45%. The t-test

indicate a statistical difference between the RP mean and the progenies: t -value=-3.34 and p -value=0.016.

In population3 were 59.29% for BCS3-192 and 63.32% for BCS3-210. Wheatland*bmr12* the *bmr* donor contained 64.82%. No statistical difference was observed between the RP and the progenies in the population3: t -value=-0.62 while the p -value=0.597.

Among the local lines, El mota had the highest NDF value followed respectively by the Tillabery farmers' landrace (64.90%) and finally Sepon82.

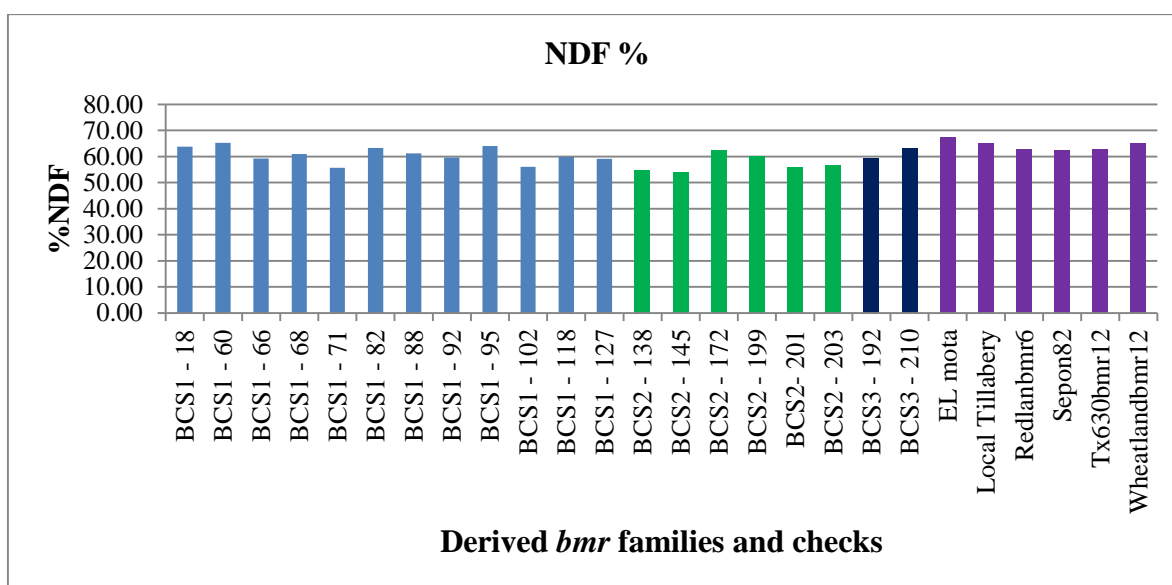


Figure 6. 5. NDF content of the selected families and checks.

- **Acid Detergent Fiber (ADF):** In the population1 the ADF values fluctuated from 31.43% (BCS1-102) to 37.60% (BCS1-60). Tx630*bmr12* the *bmr* donor contained 33.95% while El mota had 38.23%. A very highly statistical difference was observed between the RP and its' derivatives progenies: t -value=-6.62 and p -value=0.000.

In the population2, the ADF values ranged from 28.08% (BCS2-138) to 33.39% (BCS2-199) while the results of their two parental lines were respectively 33.38% for Redlan*bmr6* and 33.56% for

Sepon82. The t-test indicate a statistical difference between the RP mean and the progenies: t-value=-2.75 and p-value=0.033.

In population3, the ADF values were 31.85% (BCS3-192) and 34.04% (BCS3-210). Wheatland*bmr12* the *bmr* donor contained 37.61%. No statistical difference was observed between the RP and the progenies in the population3: t-value=-0.62 and p-value=0.600

Among the local lines, El mota had the highest ADF value followed by the Tillabery farmers landrace (34.85%) and finally Sepon82.

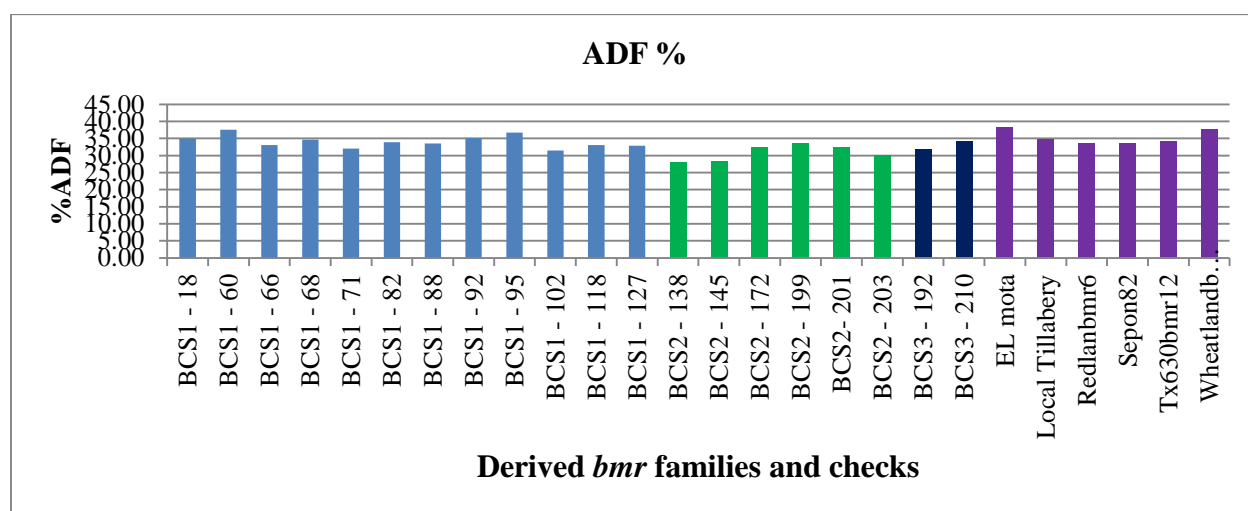


Figure 6. 6. ADF content of the selected families and the checks.

- **Acid Detergent Lignin (ADL):** In the population1, the ADL values fluctuated from 1.70% (BCS1-118) to 4.45% (BCS1-127). Tx630*bmr12* the *bmr* donor contained 2.56% while El mota the recurrent parent had 4.67%. A very highly statistical difference was observed between the RP and its' derivatives progenies: t-value=-7.34 and p-value=0.000.

In the population2, the ADL values ranged from 1.93% (BCS2-145) to 3.93% (BCS2-138) while their parental lines had respectively 2.83% for Redlan*bmr6* and 4.06% for Sepon82. The t-test

indicate a statistical difference between the RP mean and the progenies: t -value=-3.45 and p -value=0.014

In population3, the ADL values varied from 2.65% (BCS3-192) to 3.45% (BCS3-210). No statistical difference was observed between the RP and the progenies: t -value=-1.65 and p -value=0.241.

Between the local lines, the Tillabery farmers' landrace had the highest ADL content with 5.17% followed by El mota 4.67% and finally Sepon82 4.06%.

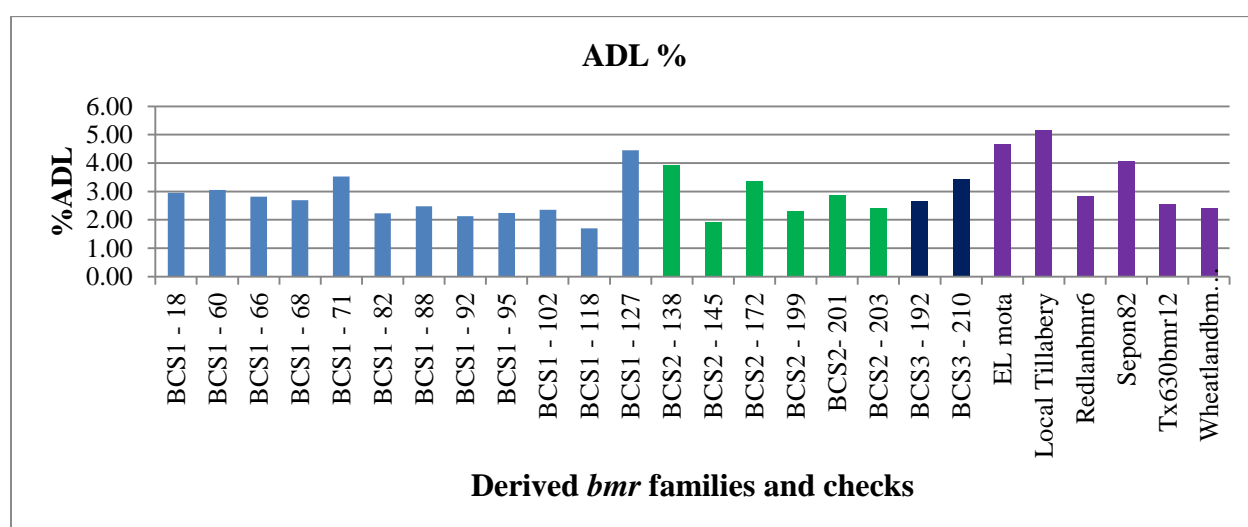


Figure 6. 7. ADL content of the selected families and the checks.

Table 6. 3. Results t-test population one.

Variable	N	Mean	95% C.I	SEM	T	P
DM	13	95.7592	(95.3494, 96.1690)	0.1881	2.71	0.019
Ash	13	7.33846	(6.45940, 8.21752)	0.40346	5.82	0
Cellulose	13	31.3415	(30.4209, 32.2621)	0.4225	-7.95	0
CP	13	4.14615	(3.57610, 4.71621)	0.26164	1.51	0.156
Lipids	13	1.34385	(1.03038, 1.65731)	0.14387	5.31	0
Ca	13	0.180769	(0.121898, 0.239641)	0.02702	2.99	0.011
P	13	0.193077	(0.156536, 0.229618)	0.016771	-1.01	0.333
NDF	13	61.1585	(59.1007, 63.2162)	0.9444	-6.34	0
ADF	13	34.3892	(33.1250, 35.6534)	0.5802	-6.62	0
ADL	13	2.86923	(2.33474, 3.40372)	0.24531	-7.34	0
E.B	13	3420.2	(3249.76, 3590.65)	78.23	-2.44	0.031

N=Sample size; C. I=Confidence interval; SEM=Standard error of the mean; p<0.05

Table 6. 4. Results t-test population two

Variable	N	Mean	95% C.I	SEM	T	P
DM	7	95.365	(94.8799, 95.8516)	0.1986	0.23	0.826
Ash	7	9.03143	(6.52659, 11.53627)	1.02367	-1.9	0.106
Cellulose	7	29.0443	(26.0631, 32.0255)	1.2183	-3.5	0.013
CP	7	4.64429	(3.28831, 6.00026)	0.55416	1.27	0.251
Lipids	7	1.64857	(1.32231, 1.97483)	0.13334	-0.24	0.821
Ca	7	0.11	(0.065646, 0.154354)	0.018127	-2.21	0.069
P	7	0.172857	(0.100287, 0.245427)	0.029658	0.1	0.926
NDF	7	57.9786	(54.7000, 61.2572)	1.3399	-3.34	0.016
ADF	7	31.1314	(28.9728, 33.2900)	0.8822	-2.75	0.033
ADL	7	2.97857	(2.21261, 3.74453)	0.31303	-3.45	0.014
E.B	7	3596.04	(3516.29, 3675.79)	32.59	2.17	0.073

N=Sample size; C. I=Confidence interval; SEM=Standard error of the mean; p<0.05.

Table 6. 5 Results t-test population three.

Variable	N	Mean	95% C.I	SEM	T	P
DM	3	94.9833	(93.8801, 96.0866)	0.2564	-1.31	0.32
Ash	3	8.17333	(1.86165, 14.48502)	1.46693	-1.91	0.196
Cellulose	3	29.2733	(20.2023, 38.3444)	2.1082	-1.91	0.196
CP	3	4.04333	(3.80463, 4.28203)	0.05548	1.86	0.204
Lipids	3	1.35	(0.63735, 2.06265)	0.16563	-1.99	0.185
Ca	3	0.113333	(-0.044431, 0.271097)	0.036667	-1	0.423
P	3	0.14	(-0.128701, 0.408701)	0.06245	-0.48	0.678
NDF	3	61.6867	(56.4186, 66.9547)	1.2244	-0.62	0.597
ADF	3	33.15	(30.2904, 36.0096)	0.6646	-0.62	0.6
ADL	3	3.38667	(1.63006, 5.14328)	0.40826	-1.65	0.241
E.B	3	3630.43	(3402.91, 3857.96)	52.88	1.99	0.185

N=number of entries; C.I=confidence interval; SEM=Standard Error of the Mean; $p < 0.05$.

6.4. Discussion

The results of the current study revealed high values of dry matter content of the selected families (94.05% to 96.67%). The results of this study are higher than those of Sultan *et al.* (2011). Indeed, those authors found 88.38% dry matter in sorghum stover dry roughage from common crops residues fed to livestock in India. In cereal crops, the dry matter content reveals an important aspect as it can be considered as the overall indicator of the total amount of nutrient available for the animal. Furthermore, in Niger farmers and stover traders preferred varieties with high biomass potential (Diakité *et al.*, 2017). With regards to the differences between the parental lines and the derived progenies, a tendency of increased dry matter yield was observed across all three populations. These findings are in contrast to those of Beck *et al.* (2014) who showed that dry matter at harvest was greater for the non *bmr* Sweet Sunny Sue (SSS) than NutriPlus (NP-BMR), and Dry Stalk *bmr* (DS-BMR). However, the authors affirmed that the superiority of the non *bmr* SSS versus the two *bmr* types was less than 3%. In this study the elite families were BCS1-60,

BCS1-68, BCS1-18, BCS1-127, BCS1-95, BSC2-138, BCS1-88, BCS2-172. Those families' performances were 1.45%, 1.22%, 1.17%, 0.8%, 0.73%, 0.7%, 0.65%, 0.54% respectively superior to the parental lines' performance. The best families belong mostly to population1. The phenotyping results showed that population1 contains the tallest families. Costa *et al.* (2016) reported that the dry matter production of forage sorghum is generally correlated with plant height and that is, the dry matter production potential increases with height. The low progress observed can be explained by the complexity of the trait. Indeed, it is generally reported that if a character is governed by non-additive gene action, it may give high heritability but low genetic advance as confirmed by the moderate heritability and low genetic advance observed for this trait during its' phenotyping. The net energy values for this study varied from 2893.41 kcal/kg DM to 3755.02 kcal/kg DM. For sorghum cultivars stovers, Singh *et al.* (2018) found 0.95-1.33 kcal/g DM less than current study findings. The energy content of cereal stovers remains of great importance in feed diet determination. According to Moehn *et al.* (2005) the net energy is the most precise and unbiased way of a feed's energy value. However, FAO (2012) asserted that crop residues are also low in energy content, which therefore has to be supplied from concentrate feeds. Families superior to their parental lines were identified: BCS1-71 (7.22%), BCS1-68 (6.33%), BCS1-18 (5.29%), BCS1-66 (4.62%); BCS2-201 (3.82%), BCS2-145 (2.24%), BCS3-210 (8.47%) and BCS3-192 (7.92%).

The results of this study showed that the recurrent parental lines El mota with 3.75% and Sepon82 with 3.94% exhibited low levels of Crude Proteins (CP) content. However, the results from different derived breeding populations varied from (2.63% to 7%) indicating a low to acceptable levels of CP in comparison to the acceptable standards and significant level of improvement. The results of this study for the CP content are in harmony with Sultan *et al.* (2011); Singh *et al.* (2018).

Indeed, these authors found that dry roughages mainly from the cereal straw and stover particularly from tropical countries are usually low in CP and high in cell-wall constituents. CP content of their tested dry roughages except one (LS), were below the ruminant maintenance requirement. They found CP content of 3.7 to 6.7% in sorghum stovers. Kubkomawa *et al.* (2015) and asserted that cereals straw or residues are inherently low in CP. They also found that cows generally require crude protein in the range of 7 to 14% of daily dry matter intake with lactating cows requiring especially more. Beck *et al.* (2014) found that concentrations of CP decreased with increasing phenological growth stage. Moreover, Elliot and Topps (1964) reported that maintenance requirements of Digestible CP (DCP) of cattle can be reduced by supplementing low quality roughages with concentrate, and the practice is normally undertaken during the long dry season. In this study, some families from population₂ had higher CP content (5.81% and 7%) compared to the other two populations. Badve *et al.* (1993) also found varietal differences for sorghum stover quality for CP content and cell wall concentrations. The superior families among the three *bmr* breeding populations with percentage of improvement compared to the average level of their parental lines were respectively: BCS2-172 (51.51%), BCS1-82 (48.94%), BCS1-95 (42.32%), BCS1-92 (40.47%), BCS1-71 (37.30%), BCS2-138 (25.75%), BCS1-88 (9.25%), BCS2-203 (8.22%) and BCS1-66 (5.82%).

The findings from this study showed differences between *bmr* breeding populations with low levels of calcium and phosphorus corroborating with those of Kubkomawa *et al.* (2015). These authors asserted that cereal hays and silages and such crop residues are relatively low in calcium. For a given diet, Kubkomawa *et al.* (2015) suggested that the total ration should provide a calcium/phosphorus ratio of 1.2 to 2:1, with cows at minimum of 1.2:1. The current results of this study are insufficient for such requirement. However, phosphorus has been described as the most

prevalent mineral deficiency for grazing cattle worldwide (Onyeonagu *et al.*, 2013). Khan *et al.* (2005) reported that levels of minerals in plants is a function of interaction between several factors which include soil type, plant species, stage of maturity, dry matter yield, grazing management and climate.

A diverse distribution pattern was observed for the fibers content in the current study. For the cellulose content, the *bmr* breeding populations ranged between 24.20% to 32.56% while El mota and Sepon82 had respectively 34.70% and 33.31%. This results are lower than Singh *et al.* (2018) who found 27.9% to 33.8%. Harper and McNeill (2015) reported that among the fiber elements, the NDF plays a considerable role in forage intake and digestibility. Schroeder (2004) concluded that NDF increases with the advancing maturity of forages and as the NDF in forages increases, animals will be able to consume less forage. The NDF content in the *bmr* breeding populations ranged from 53.99% (BCS2-145) to 65.17% (BCS1-60) whereas El mota and Sepon82 had 67.15% and 62.45%. Singh *et al.* (2018) found 55.0% to 68.2% in stover of sorghum cultivars. The NDF content in the *bmr* breeding populations are lower than 59.9% to 79.3% found by Hamed *et al.* (2015) in sorghum stover. The low NDF content can be attributable to the improvement made with the introgression of the *bmr* genes in the breeding populations. In this view, Beck *et al.* (2014) also found and asserted that at all phenological growth stages, *bmr* hybrids were predicted to contain less NDF than non*bmr*. Furthermore, in a study of comparison of the effect of the sorghum *bmr6*, *bmr12* genes in six grain sorghum lines for their grain yield, stover yield and stover quality, Oliver *et al.* (2005) found on average more NDF content in the wild-type compared to its derivative counterpart. Fritz *et al.* (1981) published similar results. The current results are therefore in harmony with those authors. The ADF content of the *bmr* breeding populations varied from 28.08% (BCS2-138) to 37.60% (BCS1-60) while for the recurrent parents it was 38.23% (El mota)

and 33.56% (Sepon82). Singh *et al.* (2018) found 35.3% to 43.1% in stover of sorghum cultivars. All *bmr* breeding families contain less ADF than their respective recurrent parents. For the same element Oliver *et al.* (2005) found similar results. Casler *et al.* (2003) also reported that *bmr* Sudangrass hybrids contained 3% less ADF than normal hybrids when harvested at heading stage. For the sorghum x Sudangrass hybrids, Fritz *et al.* (1988) found that presence of the *bmr* gene decreased ADF concentration by 8 to 10%. The results of the current study are thus in harmony with those authors. However, McCollum *et al.* (2005) reported no difference in ADF concentration between *nonbmr* and *bmr* forage sorghum varieties. The ADL content in the *bmr* breeding populations ranged from 1.70% (BCS1-118) to 4.45% (BCS1-127) while the two recurrent parents had 4.67% and 4.06% respectively for El mota and Sepon82. Singh *et al.* (2018) found 4.33% to 5.79% lignin in stover of sorghum cultivars. All the *bmr* breeding families contain less ADL than their respective recurrent parents. For the same element Oliver *et al.* (2005) found similar results. The cellulose, NDF, ADF and ADL content were very highly significant between the recurrent parent El mota and its' derivatives *bmr* progenies (population1) and significant in population2. On the contrary, in the population3, the recurrent parent Sepon82 and its' progenies were statistically equal. Current results are in harmony with (Badve *et al.*, 1993; Hamed *et al.*, 2015).

To sum up, the NDF, ADF and ADL content of this study ranged from 53.99% to 65.17%; 28.26% to 37.60% and 1.70% to 4.45% respectively. For the same elements, Singh *et al.* (2018) found 55.0% to 68.2%; 35.3% to 43.1%; and 4.33% to 5.79% respectively. The current findings are therefore inferior to those authors' results. Moreover, it is obvious that the lignin content in the *bmr* breeding populations is lower than their findings.

6.5. Conclusion

Several *bmr* derived families exhibited an acceptable to good levels of dry matter, net energy, and crude protein. A good level of ADL reduction was observed in the *bmr* derived families. However, their minerals content (calcium and phosphorus) remains low and may imply supplementation efforts. For their fiber content, a great variability was found across the different families. The relatively lower cellulose content in the *bmr* breeding populations compared to their parental lines may suggest better digestibility quality. Some families (BCS1-102, BCS1-66, BCS2-138, BCS2-145, BCS2-203, BCS2-201, BCS3-192, BCS3-210) combined well the considerable lignin reduction without impacting their fitness to an acceptable level of NDF and ADF.

CHAPTER SEVEN

7.0. EMS SORGHUM SNP MUTATIONS IDENTIFICATION

7.1. Introduction

The molecular identification of allelic variation responsible for induced mutant phenotypes is one of the most effective and convincing methods to demonstrate gene function (Addo-Quaye *et al.*, 2016a). According to Vermerris, (2008) mutant phenotypes are of great help in the identification of genes that are involved in a product of interest. These make *bmr* mutations induction a sustainable alternative breeding method for dual purpose sorghum production and stover quality improvement. Among the chemical agents, the alkylating agent (EMS) is the most commonly used in plants because of its potency and ease to use. Another advantage of EMS is the low level of chromosomal breaks and lethal effect (Greene *et al.*, 2003). Practically, the ethyl group of EMS reacts with guanine in DNA, forming the abnormal base *O*⁶-ethylguanine. During DNA replication, DNA polymerases that catalyse the process frequently place thymine, instead of cytosine, opposite *O*⁶-ethylguanine. Following subsequent rounds of replication, the original G:C base pair can become an A:T pair (a transition mutation); this changes the genetic information (Wikipedia.org). For Per Sikora *et al.* (2011), chemical mutagens tend to cause single base-pair (bp) changes or single-nucleotide polymorphism (SNPs) and provide a very high mutation frequency. In plant improvement and genetics, mapping the genes responsible for traits remains one of the most basic goals (Brauer *et al.*, 2006). A large number of EMS mutants (~12,000) derived from Tx623 were generated at Purdue University (Addo-Quaye *et al.*, 2016b). Selection for agronomic potential was further conducted and finally 554 EMS mutant lines were evaluated

in the Niger sahelian environment. Ten (10) EMS mutants were finally selected based on their clear brown midrib colour exhibition during the rainy season 2015.

In Niger, proper animal feeding is one of the major challenge for livestock productivity. The main objective of this chapter was to identify the EMS candidate SNP mutations responsible of the *bmr* trait from those ten (10) EMS mutant lines to speed up sorghum stover quality improvement.

The specific objectives were to:

- i identify the nucleotide change and the type of mutation observed,
- ii identify the amino acid change,
- iii classify the different EMS mutants' phenotype.

7.2. Material and methods

7.2.1. Plant materials

The plant materials were composed of twelve (12) sorghum entries set up as follow: ten (10) *Sorghum bicolor* EMS (SbEMS) families and two (2) *bmr6* and *bmr12* sources. The ten (10) families were crossed to Tx623 for F1 production. The obtained F1 were self-pollinated to produce F2 seeds. In the F2 segregating nurseries, selection based on the midrib colour was conducted, thus only the genotypes exhibiting the *bmr* character were self-pollinated. The two (2) *bmr* sources (Tx631 *bmr6* x N223 and Tx631 *bmr12* x N223) were developed in the same breeding conditions and used as checks. The progenies of every cross were considered as a family therefore the entire population was constituted of twelve (12) families. The Table 7.1 shows the entries pedigrees and family sizes

Table 7. 1. Pedigree and size of the different EMS families.

Number	Entries	Source	Family Size
1	SbEMS 1453 mut-1 - Niger 13 x Tx623	WL16-2700-1	26
2	SbEMS 2354 mut-1 - Niger 18 x Tx623	WL16-2704-1	13
3	SbEMS 3779 mut-1 - Niger 27 x Tx623	WL16-2708-1	27
4	SbEMS 4465 mut-1 - Niger 29 x Tx623	WL16-2712-1	21
5	SbEMS 4791 mut-2 - Niger 31 x Tx623	WL16-2720-1	9
6	SbEMS 4848 mut (#1)-1 - Niger 32 x Tx623	WL16-2724-1	21
7	SbEMS 5095 (#1)-1 - Niger 34 x Tx623	WL16-2752-1	32
8	SbEMS 5199 mut (#1)-1 - Niger 38 x Tx623	WL16-2728-1	16
9	SbEMS 5398 mut-1 - Niger 39 x Tx623	WL16-2732-1	24
10	SbEMS 932-1-1 - Niger 553 x Tx623	WL16-2736-1	18
11	Tx631 <i>bmr-6</i> x N223	WL16-2762-1	60
12	Tx631 <i>bmr-12</i> x N223	WL16-2795-1	64

7.2.2. Methodology

The seedlings were cultivated in a green house. Each family was composed of many plots (9 to 64: Table 7.1.). In each plot twelve (12) seeds were sown on a slightly alkaline soil (pH=7.8) for germination. The green house was kept at 27°C and seedlings were irrigated daily with tap water. Labels were used to distinguish each family. Two weeks after germination, miracle Growth fertilizer containing both macro and micro nutrients was applied to maintain seedlings in good growing condition.

7.2.2.1. Leaf tissue sampling

DNA extraction was conducted on three weeks old seedlings. To avoid any contamination among families, sampling was done by wearing laboratory gloves. In order to form a bulk of leaves of each family, one plant per plot was randomly selected considering that in each plot all the plants carry the same mutation. Subsequently, this bulk made up of equal size of pieces of leaves was

designed to represent every family. Twelve (12) envelopes containing each family were kept in liquid nitrogen from the green house to the storage at -80°C in the laboratory.

7.2.2.2.DNA extraction

For the DNA extraction a Cetyltrimethylammonium bromide (CTAB) protocol method was used. In practice, every family's bulk of leaves was ground separately in a cold mortar and the entire powder was used for DNA extraction. Thus DNA obtained was a pooled DNA and was representative of each family. This allows the capture of all the mutations in a family during a single run sequencing. Kyle *et al.* (2011) asserted that by analysing DNA from a mixture of phenotypically mutant progeny from the cross, it is possible to define the genetic region containing the mutation and even to identify the mutation itself. DNA quantification and quality measurements were also performed; those measurements are important to get an optimal performance during the sequencing step.

7.2.2.3. DNA quality and quantity measurements

The DNA quantities and qualities were checked twice in order to satisfy the Illumina sequencing for the whole genome sequence requirements. Firstly, with the electrophoresis technique using $1\mu\text{g}$ DNA per family on agarose gel. This method was used to determine the presence or absence of the DNA in all the samples. The agarose gel was at 0.8% concentration and the electrophoresis duration was 1 hour at 100 Volts; 400 mA. Secondly the DNA amount was estimated with a spectrophotometer (NanoDrop 1000) using $1\mu\text{l}$ of no diluted DNA sample from every family. The spectrophotometer was set to the default that is DNA-50.

The DNA bands from the agarose gel electrophoresis were appreciated by visualization under ultraviolet light. For the spectrophotometer, the ratios of absorbance at 260 nm/280 nm was used to

assess the DNA purity and 260/230 to measure the nucleic acid purity. The Spectrophotometer gives also the DNA concentration based on the absorbance at 260 nm. Indeed, DNA has a maximal absorbance near 260 nm, higher levels of absorbance are indicative of greater concentrations of DNA present within the samples (www.lgcgroup.com/genomics). For the first ratio (260/280), a value of ~1.8 is generally accepted as 'pure' DNA while nucleic acid purity was accepted in the range 1.8 to 2.2 looking at the ratio 260/230. (Thermo Scientific 2008). All the DNA samples were checked to Electropherogram for the sequencing requirements and were further sequenced with a >8.33x genome coverage. In order to identify the causative nucleotide change candidate of the *bmr* phenotype observed in the derived EMS sorghum families the Whole Genome Sequencing results were analyzed with the MutMap method.

7.2.2.4. MutMap analysis

The following bioinformatics softwares were used during the sequencing data processing: MobaXterm v10-9 for the Unix command utilization and sequencing data transfer in the WinSCP.

Akira *et al.* (2012) proposed and described the MutMap as an elegant and rapid method for the identification of nucleotides changes in plant genomes. The method involves F2 segregating populations derived from a cross between a mutant line and the wild type (the reference genome) used to generate the mutant. This MutMap is however based on the whole genome resequencing of pooled DNA from segregating population of plant that show a useful marker (Akira *et al.*, 2012).

In practice, the EMS mutants' sequencing data processing started firstly with the download of the BTx623 (Rooneys' data) parent from European BioInformatic. This BTx623 was used as the *Sorghum bicolor* reference genome and each EMS family was aligned to BTx623 for the MutMap configuration. A high quality read was selected for the genome sequencing data using the ml bwa

and ml samtools/0.1.8. The BWA (Burrows-Wheeler Aligner) is a software package for mapping low-divergent sequences against a large reference genome (Li and Durbin, 2009). The samtools/0.1.8. are genetic format for storing large nucleotide sequence alignments (Kwangsik, 2014). Then to develop a reference sequence for one of the parental lines used for crossing, the module load gcc/5.2.0 r/3.3.1 was used. Consensus map was made using QTL-seq_test/2.make_consensus/90.align_to_this_fasta with module load gcc/5.2.0 r/3.3.1. Figure 7.1. describes the MutMap principles.

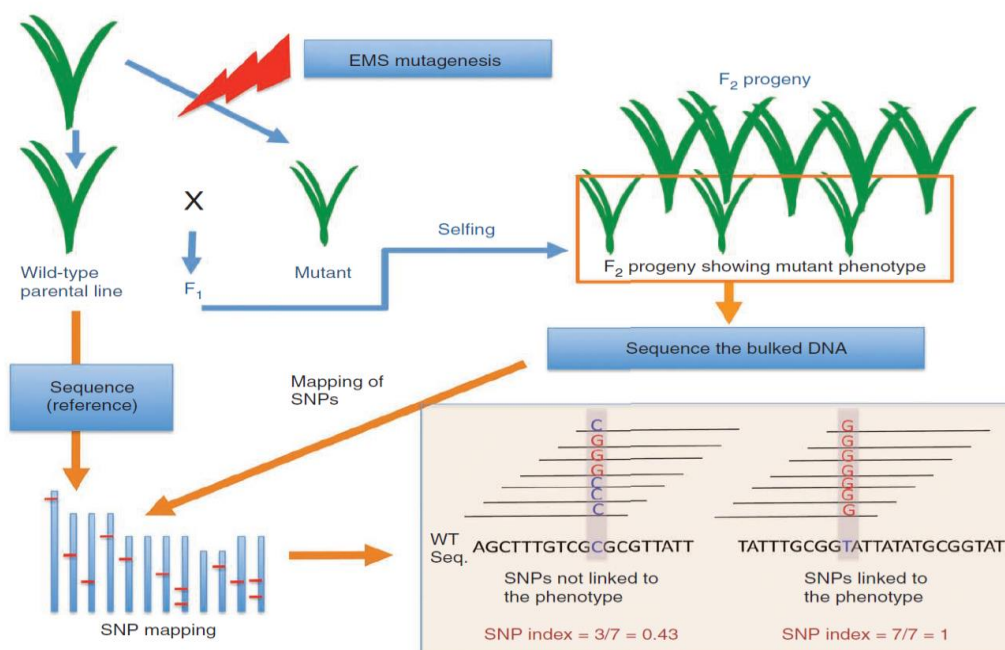


Figure 7. 1. Simplified scheme for application of MutMap to rice.

A rice cultivar with a reference genome sequence is mutagenized by ethyl methanesulfonate (EMS). The mutant generated, in this case a semi-dwarf phenotype, is crossed to the wild-type plant of the same cultivar used for the mutagenesis. The resulting F₁ is self-pollinated to obtain F₂ progeny segregating for the mutant and wild-type phenotypes. Crossing of the mutant to the wild-type parental line ensures detection of phenotypic differences at the F₂ generation between the mutant and wild type. DNA of F₂ displaying the mutant phenotype are bulked and subjected to whole-genome sequencing followed by alignment to the reference sequence. SNPs with sequence reads composed only of mutant sequences (SNP index of 1) are closely linked to the causal SNP for the mutant phenotype. Source: Akira *et al.* (2012)

7.2.2.5. SNPs identification

The Integrative Genomics Viewer (IGV) and the MultAlin (Multiple alignment) softwares were used for SNPs identification and amino acid changes across the mutants' genome. In practice the sequencing BAM file of every EMS mutant was compared to the new sorghum reference genome (*Sorghum bicolor* v3.1.1). Akira *et al.* (2011) reported that in F2 progeny, the majority of SNPs segregate in a 1:1 mutant/wild type ratio except the SNP responsible of the phenotype change which is homozygous. After alignment to the reference genome, the SNP-alleles frequencies were determined as the percentage of the reference genomes' allele versus the percentage of the alternative allele. The SNPs alleles frequencies follow a 50% mutant genotype and 50% wild type genotype for all the majority of the positions across the sequences excepted at causative mutations' position. In total, our number of reads depth varied from 1 to 16. The SNP index were calculated in percentage for all the EMS families as defined by Akira *et al.* (2011): $\text{SNP INDEX} = \text{Number of read of the mutant SNP} / \text{Total reads corresponding to the SNP} * 100$.

All the mutants were firstly screened for the known *bmr2* (Sobic.004G062500), *bmr6* (Sobic.004G071000.2) and *bmr12* (Sobic007G047300) genes positions.

7.3. Results

7.3.1. DNA quantity and quality

For the DNA quantity and quality requirements, the following results were obtained for every sample: Table 7.2 shows results obtained from the spectrophotometer readings of the different families while the Figure 7.2 shows the results from the agarose gel. The results obtained revealed the presence of DNA that all the DNA extracted from all the twelve (12) families were acceptable for sequencing.

Table 7. 2. Concentration, absorbance of bulk DNA of SbEMS families.

Number	Entries	Concentration (ng/μl)	Absorbance	
			260/280	260/230
1	SbEMS 1453 mut-1 - Niger 13 x Tx623	662.84	1.85	1.62
2	SbEMS 2354 mut-1 - Niger 18 x Tx623	590.62	1.88	2.06
3	SbEMS 3779 mut-1 - Niger 27 x Tx623	513.64	1.85	1.99
4	SbEMS 4465 mut-1 - Niger 29 x Tx623	554.14	1.82	1.92
5	SbEMS 4791 mut-2 - Niger 31 x Tx623	510.52	1.92	2.14
6	SbEMS 4848 mut (#1)-1 - Niger 32 x Tx623	441.94	1.91	1.84
7	SbEMS 5095 (#1)-1 - Niger 34 x Tx623	405.82	1.89	2.1
8	SbEMS 5199 mut (#1)-1 - Niger 38 x Tx623	532.08	1.86	2.1
9	SbEMS 5398 mut-1 - Niger 39 x Tx623	968.72	1.89	2.12
10	SbEMS 932-1-1 - Niger 553 x Tx623	1190.01	1.91	2.34
11	Tx631 bmr-6 x N223	623.15	1.93	2
12	Tx631 bmr-12 x N223	598.89	1.93	2.15

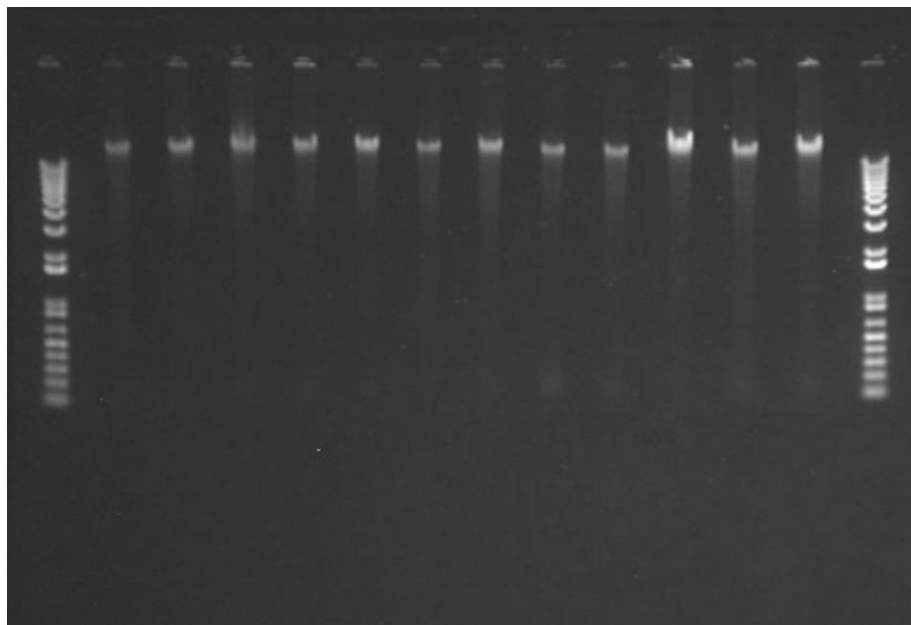


Figure 7. 2. DNA bands on the agarose gel.

Samples loaded in the order above at the indicated amounts. 6μl DNA ladder (1kb) was loaded onto the gel on the first and last wells.

7.3.2. SbEMS phenotypes identification

The following results were obtained for every SbEMS mutant: EMS 2354; 932-1-1 as *bmr2*, EMS 1456; 4848; 3779; 4465 as *bmr6* and EMS 5095 as *bmr12*. The EMS 4791 was classified as possible double mutant *bmr2/bmr6* while EMS 5199 and 5398 were suggested as new *bmr* variants. Below the detailed SNP information on every EMS mutant is described.

7.3.2.1. Family 1: SbEMS 1453 mut-1 - Niger 13 x Tx623

The sequence alignment of the SbEMS 1453 mut-1 - Niger 13 with the *Sorghum bicolor* reference genome v3.1.1 revealed a Cytosine:Thymine (C:T) nucleotide change in the Sobic.004G071000.2 gene on the chromosome 4 at the genetic position 5731439 base pair. Indeed, a Cytosine nucleotide was found at the reference genome while for the same position with four read depth a 100% Thymine nucleotide was present for the mutant. This transition mutation in the protein coding sequence (CDS) resulted in a change of the reading frame TGC/TGT indicating a candidate mutation for the *bmr6* phenotype. In fact, an amino acid change Alanine to Cysteine was observed in the amino acid chain.

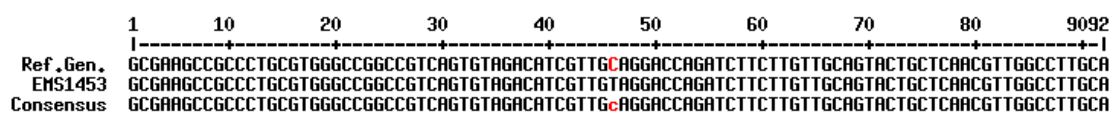


Figure 7. 3. The T:C mutation contrasting the EMS1453 mutant to the reference genome.

7.3.2.2. Family 2: SbEMS 2354 mut-1 - Niger 18 x Tx623

The sequence alignment of the SbEMS 2354 mut-1 - Niger 18 with the *Sorghum bicolor* v3.1.1 reference genome showed a Cytosine:Thymine (C:T) nucleotide change in the Sobic.004G062500 gene on the chromosome 4 at the genetic position 5042955 base pair. The reference genome carried a Cytosine nucleotide while a 100% Thymine nucleotide with three read depth was observed on

the mutant. The following transition mutation CCC/CTC in the protein coding sequence resulted in a modification from Proline to Leucine amino acid. This point mutation constitutes a candidate mutation for the *bmr2* phenotype.

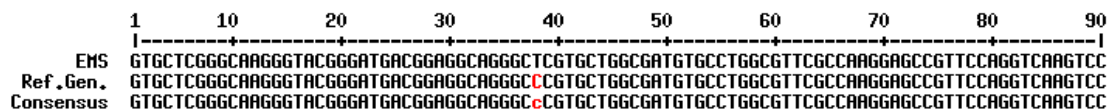


Figure 7. 4. The T:C mutation contrasting the EMS2354 mutant to the reference genome.

7.3.2.3. Family 3: SbEMS 3779 mut-1 - Niger 27 x Tx623

The sequence alignment of the SbEMS 3779 mut-1 - Niger 27 with the sorghum bicolor v3.1.1 reference genome showed a Guanine:Adenine (G:A) nucleotide change in the Sobic.004G071000.2 on the chromosome 4 at the genetic position 5730127 base pair. The reference genome contains Guanine while Adenine was present on the mutant's sequence at this position for one read depth. The initial TGG from the reference genome was converted to TAG*, therefore the initial amino acid (Proline) became a stop codon. This nonsense mutation in the protein coding sequence places the mutant as a candidate for the *bmr6* phenotype.



Figure 7. 5. The G:A mutation contrasting the EMS3779 mutant to the reference genome.

7.3.2.4. Family 4: SbEMS 4465 mut-1 - Niger 29 x Tx623

The sequence alignment of the SbEMS 4465 mut-1 - Niger 29 with the *Sorghum bicolor* v3.1.1 reference genome showed a C:T mutation in Sobic.004G071000.2 sorghum *bmr* gene. This mutation at the genetic position 5732336 base pair with six read depth on the chromosome 4. Indeed, a TCC/TCT change was observed in an intron close to the protein coding sequence. This

mutation suggested a possible splicing site change and indicates the mutant as a candidate *bmr6* phenotype.



Figure 7. 6. The C:T mutation contrasting the EMS4465 mutant to the reference genome.

7.3.2.5. Family 5: SbEMS 4791 mut-2 - Niger 31 x Tx623

The sequence alignment of the SbEMS 4791 mut-2 - Niger 31 with the *Sorghum bicolor* v3.1.1 reference genome showed two candidate mutations in two different *bmr* genes.

➤ In the Sobic.004G062500 on the chromosome 4 at the genetic position 5042074 base pair, a C:T mutation was found. Indeed, while the reference genome exhibited a Cytosine; a 100% Thymine was observed on the mutants' sequence at the corresponding genetic position with 4 read depth. As a result of this nonsense mutation in the protein coding sequence, the initial triplet CAG coding for the amino acid glutamine was changed into a stop codon (TAG*). This mutation resulted to a *bmr2* candidate phenotype for this mutant.

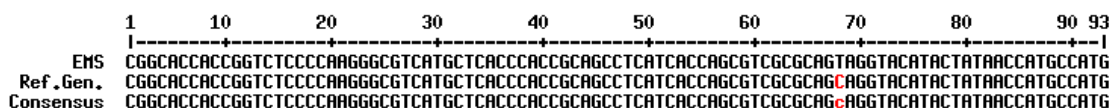


Figure 7. 7. The T:C mutation contrasting the EMS 4791 mutant to the reference genome.

➤ In the Sobic.004G071000.2 on the chromosome 4 at the genetic position 5730873 base pair a C:T mutation was found. Indeed, an alternative T nucleotide was observed instead of C from the reference genome. This possible splicing site change with four read depth may also be a candidate mutation responsible of a *bmr6* phenotype.

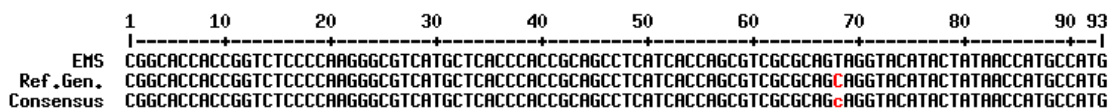


Figure 7. 8. The C:T mutation contrasting the EMS 4791 mutant to the reference genome.

7.3.2.6. Family 6: SbEMS 4848 mut (#1)-1 - Niger 32 x Tx623

The sequence alignment of the SbEMS 4848 mut (#1)-1 - Niger 32 with the *Sorghum bicolor* v3.1.1 reference genome revealed an Guanine:Adenine (G:A) nucleotide change on the chromosome 4 at the genetic position 5731386 base pair for the Sobic.004G071000.2 gene. For the four read depth, a 100% Adenine was observed whereas the reference genome presented Guanine. This missense mutation in the protein coding sequence resulted a change GCG to ACG. Consequently, the expected arginine amino acid was changed to threonine and the mutant was classified as a candidate *bmr6* phenotype.

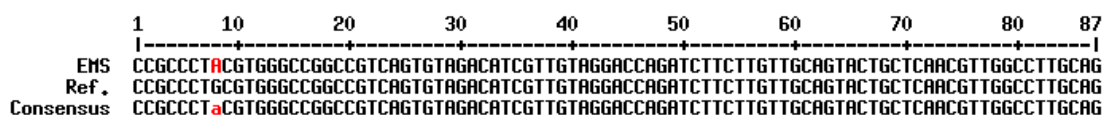


Figure 7. 9. The A:G mutation contrasting the EMS4848 mutant to the reference genome.

7.3.2.7. Family 7: SbEMS 5095 (#1)-1 - Niger 34 x Tx623

The sequence alignment of the SbEMS 5095 (#1)-1 - Niger 34 with the *Sorghum bicolor* v3.1.1 reference genome revealed three different mutations on the chromosome 7 for the Sobic007G047300 gene. The mutations were found in the introns. This suggest a possible splicing site change. Their read depth were respectively of 7, 9 and 12 at the genetic position 4722728 base pair, 4722588 base pair and 4722669 base pair with the following nucleotide change T:C, T:A and C:T. The results of those mutations in the Sobic007G047300 gene suggested this mutant as a candidate for the *bmr12* phenotype.

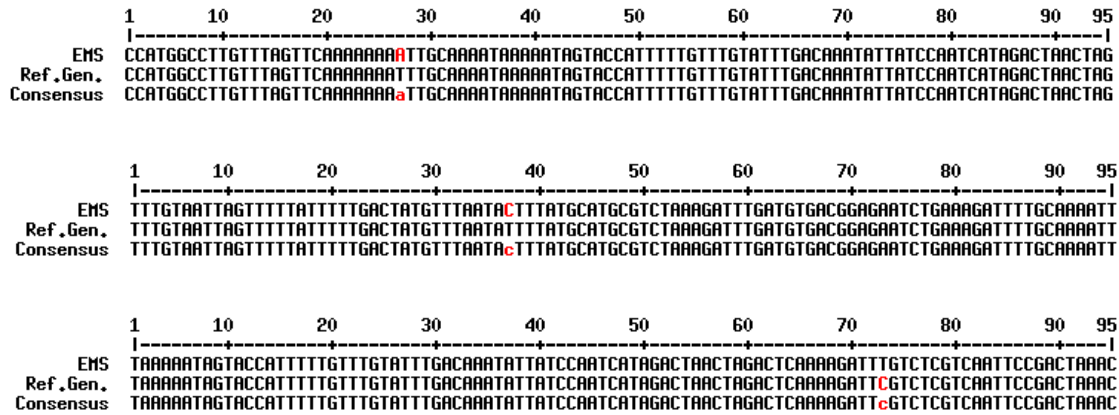


Figure 7. 10. The different mutations contrasting the EMS 5095 to the reference genome.

7.3.2.8. Family 8: SbEMS 5199 mut (#1)-1 - Niger 38 x Tx623

The sequence alignment of the SbEMS 5199 mut (#1)-1 - Niger 38 x Tx623 with the *Sorghum bicolor* v3.1.1 reference genome did not reveal any homozygous SNP for all the known sorghum *bmr* genes.

7.3.2.9. Family 9: SbEMS 5398 mut-1 - Niger 39 x Tx623

The sequence alignment of the SbEMS 5398 mut-1 - Niger 39 x Tx623 with the *Sorghum bicolor* v3.1.1 reference genome did not reveal any homozygous SNP for all the known sorghum *bmr* genes.

7.3.2.10. Family 10: SbEMS 932-1-1 - Niger 553 x Tx623

The sequence alignment of the SbEMS 932-1-1 Niger 553 x Tx623 with the *Sorghum bicolor* v3.1.1 reference genome revealed a G:A nucleotide change on the chromosome 4 in the Sobic.004G062500 at the genetic position 5042945 base pair. Two read depth was observed and

GAG/AAG change in the protein coding sequence resulted in glutamic acid to lysine change. This mutation places the mutant as a candidate *bmr2* phenotype.

	1	10	20	30	40	50	60	70	80	90
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----									
EMS	ATGTACCAAGATCCCRACTGCTCGGGCAAGGGTACGGGATGACGAGGGCAGGGCCCGTGCTGGCGATGTGCCTGGCGTTCCGCCAAGGGAG									
Ref. Gen.	ATGTACCAAGATCCCRACTGCTCGGGCAAGGGTACGGGATGACGGAGGCAGGGCCCGTGCTGGCGATGTGCCTGGCGTTCCGCCAAGGGAG									
Consensus	ATGTACCAAGATCCCRACTGCTCGGGCAAGGGTACGGGATGACGAGGGCAGGGCCCGTGCTGGCGATGTGCCTGGCGTTCCGCCAAGGGAG									

Figure 7. 11. The A:G mutation contrasting the EMS932-1-1 mutant to the reference genome.

Table 7. 3. Summary SNPs results.

Mutant ID	Gene	Read	Chr	SNP Position (bp)	Candidate Phenotype			Ref. Allele	Alt. Allele	Sequence	Amino Acid	Mutation type
					W	<i>Bmr6</i>	W					
SbEMS1453	Sobic.004G071000.2	4	4	5731439	W	<i>Bmr6</i>	W	C	T	tgC/tgT	A(Alanine)/Cysteine	Missense
SbEMS2354	Sobic.004G062500	3	4	5042955	<i>Bmr2</i>	W	W	C	T	cCc/cTc	P(Proline)/Leucine	Missense
SbEMS3779	Sobic.004G071000.2	1	4	5730127	W	<i>Bmr6</i>	W	G	A	tGg/tAg*	P(Proline)/Stop Codon	Nonsense
SbEMS4465	Sobic.004G071000.2	6	4	5732336	W	<i>Bmr6</i>	W	C	T	tcC/tcT	SSC	
SbEMS4791	Sobic.004G071000.2	4	4	5730873	W	<i>Bmr6</i>	W	T	C	gTa/gCa	SSC	
SbEMS4791	Sobic.004G062500	4	4	5042074	<i>Bmr2</i>	W	W	C	T	Cag/Tag*	Q(Glutamine)/Stop Codon	Nonsense
SbEMS4848	Sobic.004G071000.2	4	4	5731386	W	<i>Bmr6</i>	W	G	A	Gcg/Acg	R(Arginine)/Threonine	Missense
SbEMS5095	Sobic007G047300	9	7	4722588	W	W	<i>Bmr12</i>	T	A	aTt/aAt	SSC	
SbEMS5095	Sobic007G047300	7	7	4722728	W	W	<i>Bmr12</i>	T	A	aTt/aAt	SSC	
SbEMS5095	Sobic007G047300	12	7	4722669	W	W	<i>Bmr12</i>	C	T	tCg/tTg	SSC	
SbEMS932-1-1	Sobic.004G062500	2	4	5042945	<i>Bmr2</i>	W	W	G	A	Gag/Aag	E(Glutamic acid)/Lysine	Missense
SbEMS5199	No homozygous SNP data available											
SbEMS5398	No homozygous SNP data available											

W=Wild type; Chr=Chromosome; Ref.=Reference; Alt=Alternative; SSC=Splice Site Change

7.4. Discussion

According to Kharkwal (2012) mutant forms showing both large and small effects on the phenotype arise for all kinds of traits. In Purdue University, Addo-Quaye *et al.* (2016) induced EMS mutations in Tx623 and developed a large population for SNPs discovery and gene function study. Among the 554 EMS families tested in Niger in 2015, ten (10) families exhibited clearly the *bmr* phenotype. This level of *bmr* phenotype exhibition (1.8%) is in agreement with Sattler *et al.* (2014). Those authors isolated 46 *bmr* mutants from M₂ generation of EMS mutagenized TILLING population of BTx623 out of approximately 3,000 M₁ lines. In this view, Sarada (1989) reported that the effectiveness of the mutagen agent depends on several conditions such as the properties of the tissues treated, the treatment conditions. Furthermore, Konzak *et al.* (1965) observed that pre-soaking results in higher efficiency compared to dry seeds treatment.

DNA from the 10 EMS *bmr* sorghum families were sequenced at 8.33x genome coverage and a wide variety of mutations were identified across their genome. In the majority the SNPs mutations observed were C:T and A:G. In this view, Greene *et al.* (2003) affirmed that chemical mutagenesis by soaking sorghum seeds for example in diethyl sulfite or ethyl methane sulphonate (EMS: C₃H₈SO₃) can induce point mutations in plant genome leading to phenotypic changes, which could be desirable traits in crop improvement. Ali *et al.* (2012) indicated that EMS alkylates guanine bases and leads to mispairing-alkylated G pairs with T instead of C, resulting in primarily G:C to A:T transitions. Bradley *et al.* (2007) concluded that 70-99% of the changes are G:C to A:T base pairs transitions. The results of the current study showed mainly point mutations with transition mutations C:T (64%); G:A (27%) and transversion mutation T:A (9%). Lee *et al.* (2011) reported that transition mutations occurred more frequently than transversion in mutational events. The

SNP mutations observed resulted in: amino acid changes (36%); stop codons (18%) and possible splice site change (45%). The current study found for the *bmr6* phenotype amino acid change (Alanine to Cysteine, Arginine to Threonine), a possible splice site change and a change of Proline to a premature stop codon. Saballos *et al.* (2009) reported an amino acid change to a premature stop codon for the *bmr6* phenotype. For the *bmr12* phenotype, the current study identified a possible splice site change as a causative SNP while Sattler *et al.* (2012) reported missense mutations in the coding region responsible of amino acid change contrasting with the premature stop codon previously reported. The results of the SNP identification of the current study revealed amino acid change (Proline to Leucine, Glutamic acid to Lysine) and Glutamic acid to a premature stop codon for the *bmr2* phenotype. Saballos *et al.* (2012) also reported an amino acid substitution for the *bmr2* phenotype.

The results of this study indicate the following EMS 1453, EMS 3779, EMS 4465, EMS 4848 as good candidate for the following *bmr6* phenotype; the EMS 2354, EMS 932-1-1 for the *bmr2* phenotype and the EMS 5095 for the *bmr12* phenotype. The EMS 4791 was found as candidate for the stacked *bmr2/bmr6* phenotype. A complementation test conducted at Purdue University (laboratory 2304 unpublished data) also classified the EMS mutants 1453, EMS 4848, EMS 5398 for the *bmr6* phenotype; EMS 3779, EMS 4791, EMS 5095, EMS 932-1-1 for the *bmr2* phenotype and the EMS 4465 for the *bmr12* phenotype. The results of the SNP identification are therefore in agreement with the complementation test for the mutant EMS 1453, EMS 4848, EMS 932-1-1.

For the EMS 4791 the sequencing data revealed a SNP mutation in the Sobic.004G062500 resulting in a premature stop codon and a mutation in the Sobic.004G071000.2 (splice site change). According to Per Sikora *et al.* (2011) that mutations in coding regions can be silent, missense or nonsense whereas in noncoding regions, mutations can change promoter sequences or other

regulatory regions resulting in up-or downregulation of gene transcription; in addition, aberrant splicing of mRNA, altered mRNA stability and changes in protein translation may also occur as a result of mutagenesis. In this view, Black (2003) stated that alternative pre-mRNA splicing is a central mode of genetic regulation in higher eukaryotes and variability in splicing patterns is a major source of protein diversity from the genome. The SNP identification results for the EMS 4791 suggest a stacked *bmr2/bmr6* phenotype. For forage quality trait, Pedersen *et al.* (2008) suggested that double *bmr* mutant may contain lesser lignin and may be more digestible than the single *bmr* mutant. However, they revealed a reduction in the total biomass yield of the double mutant compared with the single mutants and closest wild-type used. For the EMS 5398, no homozygous SNP was found for the reported *bmr* genes, however the complementation test classified the mutant as a *bmr6* phenotype. The result from the current study disagrees with the complementation test for this mutant.

For the EMS 5199, no homozygous SNP was found for the reported *bmr* genes. The complementation test also did not classify this mutant for the known *bmr* phenotypes. The SNP identification result is in agreement with the complementation test for this mutant. The findings are in agreement with Sattler *et al.* (2014). Indeed, for those authors, among a set of 20 EMS mutants, six *bmr* mutants were not allelic to *bmr2*, *bmr6* or *bmr12* and a further complementation testing showed these mutants represent four novel *bmr* loci.

7.5. Conclusion

The *bmr* phenotype is obviously visible from seedlings to mature plants, thus forward genetic was used to identify the candidate mutations. The comparison of the EMS mutants' DNA to the *Sorghum bicolor* reference genome showed some candidate SNPs mutations. The missense mutations resulted in an amino acid changes while the nonsense mutations resulted to premature stop codon. The transition mutations observed resulted in general into amino acid change whereas the transversion mutation led to a possible splice site change.

Two of the mutants were found to be different from the reported *bmr* genes. Further investigation (allelism test) may be useful for novel *bmr* loci identification.

The EMS *bmr* mutants will undergo in a backcrossing program before their utilization in the sorghum stover improvement program. However, the candidate SNPs discovered needs to be confirm.

CHAPTER EIGHT

8.0. GENERAL CONCLUSION AND RECOMMANDATIONS

8.1. GENERAL CONCLUSION

Participatory rural appraisal revealed mixed crop-livestock was the main production system for all farmers involved in the study zones. Farmers were well aware of the impact of the feed shortage on animal performance. Feed shortage during the hot and dry season (March-July) is recurrent. Cattle (milking cows and bullocks) were the first to be fed with sorghum residues. Stover with greener and enough biomass was preferred by traders and buyers for feeding cattle.

Three populations composed of 94 families (BC1F3) from three different *bmr6* and *bmr12* donor parents were developed using hand emasculation technique.

Bmr derived populations (BC1F3) were phenotyped at Tillabery and Konni in Niger for earliness, grain yield, dry matter yield, resistance to lodging and foliar diseases. Several *bmr* BC1F3 families exhibited a great potential in grain and dry matter yields better than their recurrent and donor parental lines. High yielding dual purpose lines were identified showing stability across locations for dry matter and grain yields. *Bmr6* and *bmr12* genes in locally adapted sorghum varieties with higher stover yield were identified with improved harvest index.

Bmr derived elite families showed significant improvement in dry matter, NDF, ADF and ADL contents.

EMS *bmr* lines were tested in the Nigerien environment and their genetic analysis was performed at Purdue University. The genotyping revealed *bmr2*, *bmr6*, *bmr12* and stacked *bmr2/bmr6*

phenotypes as results of the EMS mutagenesis. *Bmr6* and *bmr12* are already used for sorghum stover improvement. The stacked *bmr2/bmr6* may result in a useful *bmr* donor line. For two other *bmr* EMS families, no homozygous SNP were found. This indicate a new mutation in a non-reported *bmr* gene due to the EMS mutagenesis. Further investigations may identify the nature of their candidate mutations.

8.2. RECOMMENDATIONS

Breeding effort should continue to identify elite lines from promising families (BCS1-102, BCS1-66, BCS1-60, BCS2-138, BCS2-201, BCS2-203, BCS2-145, BCS3-192, BCS3-210).

More field phenotyping of derived elite lines should continue to study GxE effect.

Additional backcrossing is needed to decrease recurrent parent background for better digestibility.

Digestibility study should be undertaken to bring in the market nutritionally valuable lines.

Develop dual purpose sorghum varieties well adapted with high stover quality.

Establish stover business in the country by assisting traders with appropriate technics of stover conservation and bunching.

Feeding milking cow, draught animal, small ruminants, etc. for improved productivity should be undertaken.

Establishing pilot study for intensive milk cows farming by feeding them with *bmr* stover is required for efficient use of *bmr* lines.

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APPENDICES

Appendix 1. Descriptive Statistic of 20 best *bmr* derived families and checks conducted at Tillabery in 2017 for chemical contents.

DM	Mean	SEM	SD	Mini	Maxi
Pop1 (Elmota//El mota/Tx630 <i>bmr</i> 12))	95.80	0.20	0.69	94.05	96.67
Pop2 (Sepon82//Sepon82/Redlan <i>bmr</i> 6))	95.37	0.24	0.58	94.42	95.98
Pop3 (Sepon82//Sepon82/Wheatland <i>bmr</i> 12))	94.82	0.34	0.47	94.48	95.15
T1 (Redlan <i>bmr</i> 6)	95.25				
T2 (Wheatland <i>bmr</i> 12)	95.35				
T3 (Tx630 <i>bmr</i> 12)	95.00				
T4 (El mota)	95.32				
T5 (Local Tillabery)	95.32				
T6 (Sepon82)	95.02				
Ash %	Mean	SEM	SD	Mini	Maxi
Pop1 (Elmota//El mota/Tx630 <i>bmr</i> 12))	7.53	0.38	1.33	5.05	9.92
Pop2 (Sepon82//Sepon82/Redlan <i>bmr</i> 6))	8.71	1.15	2.81	5.56	13.30
Pop3 (Sepon82//Sepon82/Wheatland <i>bmr</i> 12))	6.77	0.74	1.05	6.03	7.51
T1 (Redlan <i>bmr</i> 6)	4.99				
T2 (Wheatland <i>bmr</i> 12)	8.36				
T3 (Tx630 <i>bmr</i> 12)	6.86				
T4 (El mota)	10.98				
T5 (Local Tillabery)	11.09				
T6 (Sepon82)	11.10				
Cellulose %	Mean	SEM	SD	Mini	Maxi
Pop1 (Elmota//El mota/Tx630 <i>bmr</i> 12))	31.06	0.34	1.19	28.56	32.56
Pop2 (Sepon82//Sepon82/Redlan <i>bmr</i> 6))	28.33	1.17	2.87	24.20	31.75
Pop3 (Sepon82//Sepon82/Wheatland <i>bmr</i> 12))	27.25	1.05	1.49	26.20	28.31
T1 (Redlan <i>bmr</i> 6)	34.70				
T2 (Wheatland <i>bmr</i> 12)	30.05				
T3 (Tx630 <i>bmr</i> 12)	31.58				
T4 (El mota)	33.31				
T5 (Local Tillabery)	30.53				
T6 (Sepon82)	33.83				
Crude Proteins %	Mean	SEM	SD	Mini	Maxi
Pop1 (Elmota//El mota/Tx630 <i>bmr</i> 12))	4.18	0.28	0.98	2.75	5.63
Pop2 (Sepon82//Sepon82/Redlan <i>bmr</i> 6))	4.76	0.64	1.57	2.63	7.00
Pop3 (Sepon82//Sepon82/Wheatland <i>bmr</i> 12))	4.10	0.04	0.05	4.06	4.13
T1 (Redlan <i>bmr</i> 6)	3.75				

T2 (Wheatland <i>bmr12</i>)	4.25				
T3 (Tx630 <i>bmr12</i>)	5.31				
T4 (El mota)	3.94				
T5 (Local Tillabery)	3.81				
T6 (Sepon82)	5.44				
Lipids %	Mean	SEM	SD	Mini	Maxi
Pop1 (Elmota//El mota/Tx630 <i>bmr12</i>)	1.41	0.14	0.49	0.89	2.82
Pop2 (Sepon82//Sepon82/Redlan <i>bmr6</i>)	1.64	0.16	0.39	1.25	2.29
Pop3 (Sepon82//Sepon82/Wheatland <i>bmr12</i>)	1.19	0.03	0.04	1.16	1.21
T1 (Redlan <i>bmr6</i>)	0.58				
T2 (Wheatland <i>bmr12</i>)	0.47				
T3 (Tx630 <i>bmr12</i>)	1.37				
T4 (El mota)	1.68				
T5 (Local Tillabery)	2.20				
T6 (Sepon82)	2.32				
Ca %	Mean	SEM	SD	Mini	Maxi
Pop1 (Elmota//El mota/Tx630 <i>bmr12</i>)	0.19	0.03	0.10	0.10	0.39
Pop2 (Sepon82//Sepon82/Redlan <i>bmr6</i>)	0.10	0.02	0.05	0.05	0.15
Pop3 (Sepon82//Sepon82/Wheatland <i>bmr12</i>)	0.10	0.06	0.08	0.04	0.15
T1 (Redlan <i>bmr6</i>)	0.10				
T2 (Wheatland <i>bmr12</i>)	0.17				
T3 (Tx630 <i>bmr12</i>)	0.13				
T4 (El mota)	0.15				
T5 (Local Tillabery)	0.05				
T6 (Sepon82)	0.21				
P %	Mean	SEM	SD	Mini	Maxi
Pop1 (Elmota//El mota/Tx630 <i>bmr12</i>)	0.19	0.02	0.06	0.10	0.32
Pop2 (Sepon82//Sepon82/Redlan <i>bmr6</i>)	0.17	0.04	0.09	0.06	0.27
Pop3 (Sepon82//Sepon82/Wheatland <i>bmr12</i>)	0.13	0.11	0.15	0.02	0.23
T1 (Redlan <i>bmr6</i>)	0.21				
T2 (Wheatland <i>bmr12</i>)	0.25				
T3 (Tx630 <i>bmr12</i>)	0.13				
T4 (El mota)	0.17				
T5 (Local Tillabery)	0.05				
T6 (Sepon82)	0.12				
NDF %	Mean	SEM	SD	Mini	Maxi
Pop1 (Elmota//El mota/Tx630 <i>bmr12</i>)	60.66	0.87	3.02	55.66	65.17
Pop2 (Sepon82//Sepon82/Redlan <i>bmr6</i>)	57.24	1.32	3.23	53.99	62.15
Pop3 (Sepon82//Sepon82/Wheatland <i>bmr12</i>)	61.31	2.02	2.85	59.29	63.32
T1 (Redlan <i>bmr6</i>)	67.15				
T2 (Wheatland <i>bmr12</i>)	64.90				
T3 (Tx630 <i>bmr12</i>)	62.58				

T4 (El mota)	62.45				
T5 (Local Tillabery)	62.77				
T6 (Sepon82)	64.82				
ADF %	Mean	SEM	SD	Mini	Maxi
Pop1 (Elmota//El mota/Tx630bmr12))	34.07	0.53	1.82	31.43	37.60
Pop2 (Sepon82//Sepon82/Redlanbmr6))	30.73	0.93	2.27	28.08	33.40
Pop3 (Sepon82//Sepon82/Wheatlandbmr12))	32.94	1.09	1.55	31.85	34.04
T1 (Redlanbmr6)	38.24				
T2 (Wheatlandbmr12)	34.85				
T3 (Tx630bmr12)	33.38				
T4 (El mota)	33.57				
T5 (Local Tillabery)	33.95				
T6 (Sepon82)	37.61				
ADL %	Mean	SEM	SD	Mini	Maxi
Pop1 (Elmota//El mota/Tx630bmr12))	2.72	0.21	0.73	1.70	4.45
Pop2 (Sepon82//Sepon82/Redlanbmr6))	2.80	0.30	0.74	1.93	3.93
Pop3 (Sepon82//Sepon82/Wheatlandbmr12))	3.05	0.40	0.56	2.65	3.45
T1 (Redlanbmr6)	4.67			4.67	4.67
T2 (Wheatlandbmr12)	2.41				
T3 (Tx630bmr12)	2.83				
T4 (El mota)	4.06				
T5 (Local Tillabery)	2.56				
T6 (Sepon82)	5.41				
Energy kcal/kg DM	Mean	SEM	SD	Mini	Maxi
Pop1 (Elmota//El mota/Tx630bmr12))	3404.30	83.30	288.40	2893.40	3755.00
Pop2 (Sepon82//Sepon82/Redlanbmr6))	3607.80	35.90	88.00	3506.80	3732.30
Pop3 (Sepon82//Sepon82/Wheatlandbmr12))	3683.00	9.46	13.40	3673.60	3692.50
T1 (Redlanbmr6)	3611.40				
T2 (Wheatlandbmr12)	3714.30				
T3 (Tx630bmr12)	3664.30				
T4 (El mota)	3525.20				
T5 (Local Tillabery)	3392.80				
T6 (Sepon82)	3282.60				

Appendix 2: Questionnaire SSI sorghum farmers

Title: Improved forage quality of sorghum stover

General objective: Generate information on the production and use of sorghum biomass in animal feed in the village of Soumarana in Niger

1. Identification

Full name of the manager: _____

Age of manager: _____ Sex Male Female

Village _____ Commune of: _____

Interviewer: _____

Date: _____

Are you a member of an organisation? YES NO

If YES which one? _____

Have you received any training? _____

In what field? _____

Farm equipment: _____

2. Activities

What are your activities? _____

Among these which ones are the most important? (*NB: from the most important to the least important*)

How do you manage these activities? _____

How many hectares do you have for your farming activities? _____

3. Land tenure

Use /soil type	Crops	Land Tenure: (TLBMCP) ¹	ha
-Irrigated Plot	Sorghum		
	Millet		
	Cowpea		
	Others		
-Non irrigated Plot			

¹**T** = traditional (belongs to extended family, distributed between members or not under the responsibility of the head of the family);

L = legally owned (Obtained from a legal structure (administrative, town hall, etc.) ;

B = lease /Rent;

M = Share-cropping (with part of the crop each year);

C = Land owned by a parent;

P = Lent land (no reward: borrow).

What is the soil condition of your plots in terms of fertility: _____?

Do you use organic fertilizers? YES NO

If YES for which crop? _____

Where is the manure from? _____

What quantity did you use last year? _____

In case of purchase specify the price: _____

3.1.Sorghum production

Do you grow sorghum each year? YES NO

Are you interested in new varieties developed by research? YES NO

Do you buy improved varieties each year? OUI NO

What are your production objectives?

1. Grain yield only.....Why?

2. Biomass only Why?
 - o *If sorghum forage crop; specify harvest period*

3. Double usage Why?

Uses	Quantities (kg)
Grain	
Biomass	
Marketing	
Others	

4. Animal breeding and milk production

Do you any animals? YES NO

If yes how many:

-Cattle _____

-Sheep_____

-Goats _____

-Camels_____

-Donkeys_____

Do you produce any milk? YES NO

How much do you produce per day? _____

Do you sell milk? YES NO

Why? _____

Where is the herd? In the dry season: _____

In the rainy season: _____

How many cows do you milk on average per day? _____

How many dairy cows do you have on average per year in the herd? _____

4.1. Animal Feed

Do you have forage constraints in feeding of your animals?

List them:

-
-
-

Quantity of animal feed purchased per season? _____

Purchase price per unit of measure (bag, bundle, etc.):

Type of animal feed	Quantity	Price
Concentrated animal feed		
Cereal bran		
Cultivated fodder		
Crop Residues		
Sorghum		
Millet		
Cowpea		
Other		

According to you what criterion influences forage quality?

-
-

-

Food quantity produced on the farm:

Type of food	Quantity	Price
Crop residues		
Cultivated fodder		
Others		

4.2. Animal Health

Costs of veterinary treatment _____

Number of veterinarian visits per year? _____

4.3. Reproduction

Did you do any artificial insemination? YES NO

If YES Number of inseminated cows? _____ How much? _____

Success rate? _____

What are the difficulties in milk production? _____

What are the solutions according to you? _____

Are you doing fattening? YES NO

Why? _____

What benefits do you derive from breeding your animals?

-
-
-

What is the share of the income obtained with the marketing of the animals?

-Important

-Average

- Weak

Appendix 3: Questionnaire SSI stover traders

Title: Improved marketing of sorghum fodder

General objective: Generate information on the marketing of sorghum straw and other fodder in Lossa, Dolli, and Soumarana

4. Identification

Full name of the manager: _____

Age of manager: _____ Sex Male Female

Village _____ Commune of: _____

Interviewer: _____

Date: _____

Are you a member of an organisation? YES NO

If YES, which one? _____

Have you received any training? _____

In what field? _____

5. Types of marketed fodder

Crops	Quantity (kg)
Sorghum	
Millet	
Cowpea	
Peanut	
Others	

6. Supply

- What is your period of supply? _____

- What is the purchase price? _____

- Who are your main suppliers? _____

- What quality standards are required to purchase? _____

7. Storage

- What are the storage conditions? _____

- How long is storage? _____

- List the main constraints encountered during storage: _____

- How do you manage these constraints? _____

8. Sale

- What is the sales period of the straw?

- What is the peak sales period? _____
- What is the selling price of the bundle of straw throughout the year? _____
- What are the quality standards required by agro-pastoralists for sale?

9. Profitability

- Do you have a rolling capital? YES
- What benefit do you derive from this commercial activity? _____
- What are your prospects in this business? _____