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Brown midrib 6 and *12* Genes Introgression in Two Nigerien and One Malian Sorghum Varieties: A Practical Guide to Young Scientists with Limited Molecular Facility

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ABSTRACT

Introgression of *bmr* genes from less adapted donor parent to well adapted high yielding biomass varieties with poor nutritional value is very important for sustainable cattle feeding during pasture scare time in the Sahel. The main objective of this work was to introgress *bmr6* and *bmr12* genes in Nigerien and Malian sorghum varieties background for dual purpose grain and biomass potential. The plant material was composed of two improved sorghum varieties (Sepon82 and Kalla Kéné) and El mota a farmer preferred variety as recurrent parents. *bmr* donor parents were redlan *bmr6*, Tx630 *bmr12* and Wheatland *bmr12*. The hand emasculation technique was used to introgress *bmr* genes in recurrent parents to produce F3 and BC1F3 populations at Sotuba research Station in Mali from January 2016 to June 2017. Anthocyanin pigment and heterosis effects were key phenotypic traits to identify F1 and BC1F1 plants during the population development. Anthocyanin allowed the identification of F1 plants in a cross involving anthocyanin (purple plant) and tan plants, while for both tan plants cross, heterosis effect was major key to discriminate F1 from parental lines and *bmr* segregation in F2 to ascertain successful crosses. The χ^2 test was used to analyze *bmr* segregation ration. Segregation ratios of *bmr* plants in F2 and BC1F2 showed a good fit of a single recessive gene (3:1). *bmr 6* and *12* genes were successfully transferred to three recurrent parents varieties which are at F4 and BC1F3 generation for grain and biomass yields potential tests in Niger during the 2017 cropping season.
KEYWORDS: *brown midrib* genes, phenotypic marker, segregating populations.

INTRODUCTION

The brown midrid (*bmr*) trait is a phenotypic marker linked to a genetic mutation. The *bmr* plant type has been identified in maize, sorghum and millet. [1] reported that *bmr* mutants were first discovered in maize, with a total of four genes and described each gene segregating as a simple Mendelian recessive character. In sorghum, [2] claimed that the *bmr* abbreviation was adopted to distinguish it from *bm*, already in use for the sorghum bloomless mutants.

[3] produced 19 *bmr* sorghum mutants by chemically treating seed from two grain sorghum lines. These mutants were numbered *bmr* 1 to *bmr* 19. [3] suggested that the *bmr*-6, *bmr*-12, and *bmr*-18 should be selected for further evaluation. However, the *bmr*-6 and *bmr*-12 genotypes are more prevalent than the *bmr-18* [4].

[5] reported four allelic groups (*bmr19, bmr2, bmr6 and bmr12*) of which the latter mentioned here are involved in improving forage quality. [6] 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. [7] asserted that the *bmr19* appears to be of limited value for forage and bioenergy applications and is not publicly available. [8] demonstrated that the *bmr* phenotype is the result of a recessive mutation in the lignin biosynthesis pathway. While for ([9]; [10], the effect of *bmr* mutations on forage quality varies depending on the genetic background of recipient in which the mutation is introduced. Moreover, [11] emphasized on the importance of identifying a suitable genetic background to allow an optimal impact of the mutation.

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The *bmr* plants exhibit a reddish brown pigmentation (Photo 1) on its leaf midrib and stalk pith in sorghum associated with lignified tissues since the plants have about five expanded leaves [12]. [13] suggested that *bmr* plants have lignin which is less polymerized and contains less phenolic monomers that can affect digestion. [14] showed that the *bmr* mutant of sorghum have significantly lower levels of lignin content and estimated around 51% less in their stems and 25% less in their leaves; furthermore, *bmr* sorghum silage has 17% less lignin than regular sorghum silage making it more digestible. This property makes *bmr* stovers very attractive for cattle feeding in many countries to improve milk and meat production.

In South Saharan African (SSA) countries particularly in the Sahel region, cereal stovers constitute an important source of feed for livestock during feed scarcity time. Unfortunately, stover from cereals is often of low nutritional quality. [15] affirmed that the potential of cereal crop residues as animal feed is enormous if all the different types of cereal crops are considered and if appropriate methods of improving their nutritive value are employed.

In Niger and Mali research to improve sorghum stover feed value in recent years often emphasized postharvest treatment of crop residues ammonization. This method involves a good control of the technique; additionally, it can also be expensive due to the cost of fertilizers. On the other hand, stay green trait has been incorporated into new lines to enhance cereal stovers quality after harvest. Adding *bmr* trait to local cultivars and newly developed lines will be more sustainable and will bring a significant impact on livestock productivity in these two countries.

To date no studies have been reported on the *bmr* sorghum introgression in local cultivars in Niger and in Mali. The main objectives of this population breeding were to introgress *bmr6* and *bmr12* genes in two Nigerien and one Malian sorghum varieties using phenotypic markers and to validate a dominant Mendelian segregation ratio using χ^2.

Biology and methods of introgressing a new trait in sorghum

Sorghum has a perfect-flower and is a self-pollinated crop. Its level of outcrossing is contingent on the specific genotype being grown and the environmental conditions encountered prior to and during anthesis [16]. [17] estimated the outcrossing from 1 to 10% and can also reach 30-60% depending to the panicle compactness, while [18] claimed sorghum outcrossing is established from none to 30%.

Sorghum flowering starts few days after panicle exsertion from top to bottom. Based on the flowering habits, sorghum breeders have developed several methods of hybridization and outcrossing elimination (Table 2). Indeed, fertile line panicles' early selfing ensure pure line maintenance whereas several crossing methods were established for segregating populations' development. [16] reported four different methods of crosses:

Hand emasculation (HE)

Panicle chosen for HE is prepared 1-2 days before emasculation. 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. Three anthers are coaxed out of the enclosing lemma and palea by inserting a sharpened pencil or similar pointed instrument such as forceps. 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 [18].

Genetic male sterility: GMS

[19] reported that a series of nuclear recessive male sterility genes, designated as *ms1* through *ms7*, have been characterized in sorghum. These mutations, in the recessive condition result in a male-sterile plant that can be used for hybridization. For [20], the most commonly used is the *ms3* due to its stable expression over different environments. These mutations in the recessive condition result in a male-sterile plant that can be used for hybridization.

Cytoplasmic male sterility: CMS

CMS occurs in sorghum. The CMS system was identified and characterized by [21]. There are many different CMS systems, each caused by a different mutation in the cytoplasm and each is complemented by different nuclear restoration loci. [18] reported that for most CMS systems, the interaction of cytoplasmic and nuclear genes defines whether all specific lines are fertile or sterile. The CMS system (Table 1) is widely used in sorghum hybrid seeds production and the following designations were conventionally adopted: A-line=Male sterile line; B-line=Male fertile and an A sterility maintainer line; R-line=Male fertile and is A-line fertility Restorer line. The most commonly used CMS system is the A1 system. In the CMS system, line that has [A] cytoplasm must have a dominant allele

present in the nuclear genome to restore male fertility. If the line lacks the dominant allele for fertility restoration, the plant will be male sterile. Table1 contains information on genotypes and their corresponding phenotypes types in sorghum hybrid seeds production.

Table 1: The CMS system in sorghum

Line	Cytoplasm	Genotype	Phenotype
A-line	[A]	rf rf	Male sterile
B-line	[N]	rf rf	Male fertile
R-line	[A] or [N]	RF RF	Male fertile
Hybrid	[A]	RF rf	Male fertile

Source: [20].

Hot-water emasculation

This method of emasculation was developed by [22]. In practice, open florets of the selected panicle are removed and the entire panicle is enclosed in a waterproof sleeve of rubber or plastic tied securely around the peduncle. The panicle is immersed in water heated to 42-48°C for 10 min. This treatment kills the majority of the pollen grains but does not damage the ovary [18]. The panicle is allowed to dry and then covered with a paper bag. Pollen from the selected male parent is dusted onto the sterilized panicle 3-4 days after the hot water treatment [18].

Anther dehiscence control by use of humidity

This method was developed by [23] to control anther dehiscence using the humidity created from covering the panicle with a plastic bag prior to flowering. This method, is also known as plastic bag emasculation and/or poured crossing. It allows a breeder to make large numbers of crosses in a short amount of time. This emasculation method is described by [18] as follow: plants selected for use as females in poured crosses have flowered approximately 2.5-5 cm from the panicle apex. The portions of the panicle that have flowered are removed. The bottom florets in the panicle are also removed, so that 3-5 cm of the panicle remains. This panicle is covered with a plastic bag and tied firmly on its peduncle while covering it with a pollinating bag to shade and to reduce the temperature of the panicle under the plastic bag. The bag remains on the plant for 2-3 days during which the panicle completes anthesis. These bags create a highly humid atmosphere in which the moisture content inhibits anther dehiscence. Because all of the anthers are not removed, a certain level of self-pollination will occur in seed from a poured cross.

Table 2: advantages and disadvantages of emasculation techniques in sorghum for diversity development.

Method	Advantages	Disadvantages
Hand emasculation-HE	Require simple tools [17].	Varieties differ in ease of emasculation [17]. Produce small quantities of seeds [16]. Takes special skill and time consuming [16].
Genetic male sterility-GMS	Simple and breeders can make a large number of crosses in a short amount of time [18].	Cannot be used for commercial hybrids seeds production. Request the prior development of ms3 lines.
Cytoplasmic male sterility-CSM	Possibility of commercial hybrid seeds production [17]. Simple and breeders can make a large number of crosses in a short amount of time [18].	Request the prior development of A and B lines. Female parent limited to A lines and thus reduction of available cytoplasms
Hot-water emasculation	Simple to use in green house [18].	High level of seedlings from self- fertilization in the nurseries.
Anther dehiscence control with humidity	Simple and breeders can make a large number of crosses in a short amount of time [18].	A certain level of self-pollination can occur in seed from a poured cross [18]. 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 [18].

Sources: [17]; [18]; [16]

MATERIAL AND METHODOLOGY

Material

The plant material was composed of six Caudatum types (Table 3): Sepon82 (Niger); Kalla Kéné from the Malian national catalogue and El mota (Nigerien farmer variety) as recurrent parents (RP). Three *bmr* donor parents (DP), originated from Purdue University, Indianapolis (USA), composed of Redlan *bmr6,* Wheatland *bmr12,* and Tx630 *bmr12,* were used [24].

The RPs (Sepon82 and El mota) are largely grown by Nigerien farmers for their stover and grain yield potential. RPs stover yield potential data were collected from sorghum breeders of Institut National de la Recherche Agronomique du Niger (INRAN) and farmers during the 2016 Participatory Rural Appraisal (PRA) survey in Niger. Selected RPs were therefore very good candidates for the dual purpose sorghum breeding. Kalla Kéné is a stay green late maturing improved variety from Malian sorghum breeding program. Kalla Kéné is a dwarf and well appreciated for its stover quality. Twenty F1 selfed seeds (F2) and sixty (60) BC1F1selfed seeds (BC1F2) were used for χ^2 test.

Table 3: Origins, agronomic characteristics and environmental conditions of recurrent and donor parents involved in the *bmr* sorghum population development in Niger and Mali. [25]

Plant material	Agronomic characteristics of recurrent parents								
	Origin	**Race**	**Plant type**	**Midrib color**	**Plant Height (cm)**	**Days Maturity (day)**	**Grain yield (t/ha)**	**Biomass yield (kg/ha)**	**Lodging**
Sepon82	ICRISAT	Caudatum	Tan	Green	150-170	90-105	2-2.5	Good	Resistant
El mota	Landrace	Caudatum	Anthocyanin	White	200-250	80-90	0.8-1	Acceptable	Resistant
Kalla kene	IER	Caudatum	Tan	White	150-190	130-140	2.8	11553	Resistant
Donor Parents									
Redlan *bmr6*	Purdue Univ.	Caudatum	Anthocyanin	Brown	130-135	95-100	1.1-1.4	Poor	Resistant
Tx630 *bmr12*	Purdue Univ.	Caudatum	Tan	Brown	90-100	95-100	1-1.5	Poor	Resistant
Wheatland *bmr12*	Purdue Univ.	Caudatum	Anthocyanin	Brown	90-100	95-100	1-1.5	Poor	Resistant

Photo1 shows different types of midrib of RP and DP used in the *bmr* population development.

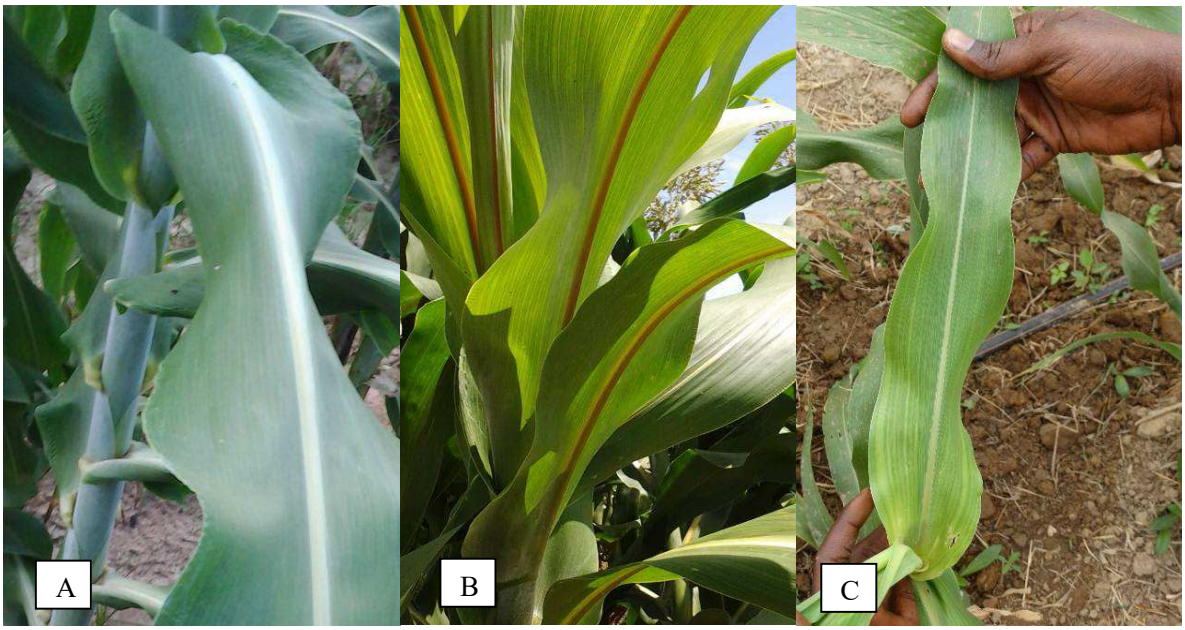

Photo 1: different types of sorghum leaves midrib color taken at Sotuba in 2016: A = white midrib (El mota); B = *brown midrib* (Wheatland*bmr12*, Redlan*bmr6* and Tx630*bmr12*) and C = dull green midrib (Sepon82)

Methodology

bmr populations development

Population development activities for the introgression of *bmr* trait into three recurrent parents 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 320m altitude.

January 2016-June 2016: F1 production

Crossing blocks were installed in early January 2016 off season under drip irrigation system (Photo 1). 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 DP parents and one date of planting for the 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 parental line.

At flowering stage, RPs were hand emasculated using tweezers/forceps (Photo 2 A). The hand emasculation 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 emasculation 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 (Photo 2 B). Other flowers on the same female plant were completely discarded to avoid seed pollution. Likewise, each donor parent was crossed to each recurrent parent to produce F1 seed for each population. Matured crosses were harvested and kept separately. Donor parent plant seed was also selfed. RP and F1 seeds were threshed independently to avoid any seed mixture and stored at 4°C for the next F2 or BC1 population development.

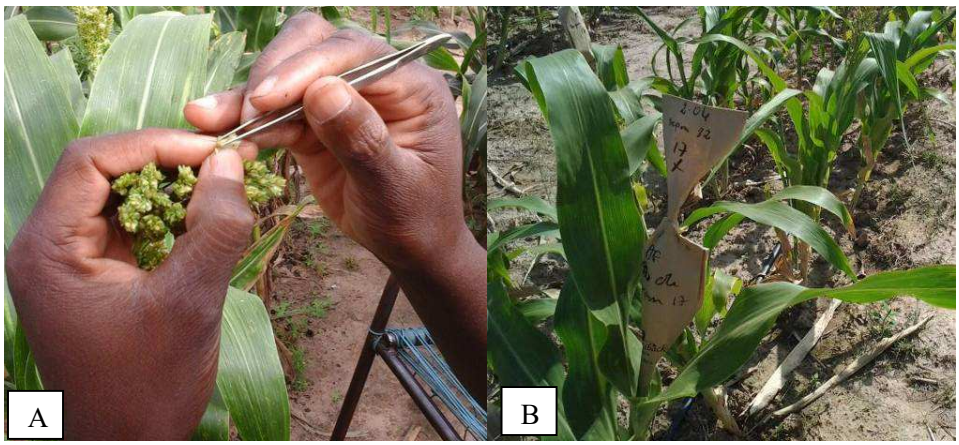

Photo 2: crossing of bmr donor lines to El Mota RP (A) and to Sepon82 (B). The bottom panicle bagged (B) is used to produce recurrent parent seed and the top is to produce F1, Sotuba, 2016 offseason.

June 2016-October 2016: BC1F1 production

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-germinate in Petri-dishes, and then were transferred in plastic pots containing 1 kg of compost for BC1F1 seed production to secure seed. After 20 days period, F1 were transplanted in one row of 3 m. Parental seed was planted next to F1 for conformity control. Heterosis and presence of anthocyanin were used to differentiate F1 from their parental phenotypes. Hybrid vigor 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 deriving from these two parents must be purple. Conversely, if F1 is tan plants, this is considered a failure. These two techniques allowed the identification of few F1s plants. From F1 deriving from two purple or tan plants, segregation of *bmr* plants in F2 or BC1B2 was a sure mean of verification of true F1 cross.

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October 2016-June 2017: BC1F2 and F2 segregation

BC1F1 seeds obtained during 2016 cropping season crosses between F1s and recurrent parents were planted for segregation study and generation advancement to obtain BC1F2. Each BC1F1 plant seeds were germinated in Petri dish (21-31 October), transplanted in plastic pots containing 1kg 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 and further DNA analysis. Harvest was done in early January for the first day short cool off season period.

At harvest, each *bmr* BC1F2 panicle and F3 panicles was 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 (Photo 2 A) with cages every day from 5PM to 8AM, during 30 days. This artificial shortening of day length induces reproductive phase and seed for day length sensitive plants. There is a black plastic layer under each white cotton tissue for firm shade to cover each sorghum plot. All panicles were selfed during all generation advancement. BC1F2 and F3 populations were harvested for adaptation, grain yield and stover potential under rain-fed conditions in Niger in 2017 cropping season.

Photo 2: shows the cage technique (A) and breeding population selfing (B) for generation advancement

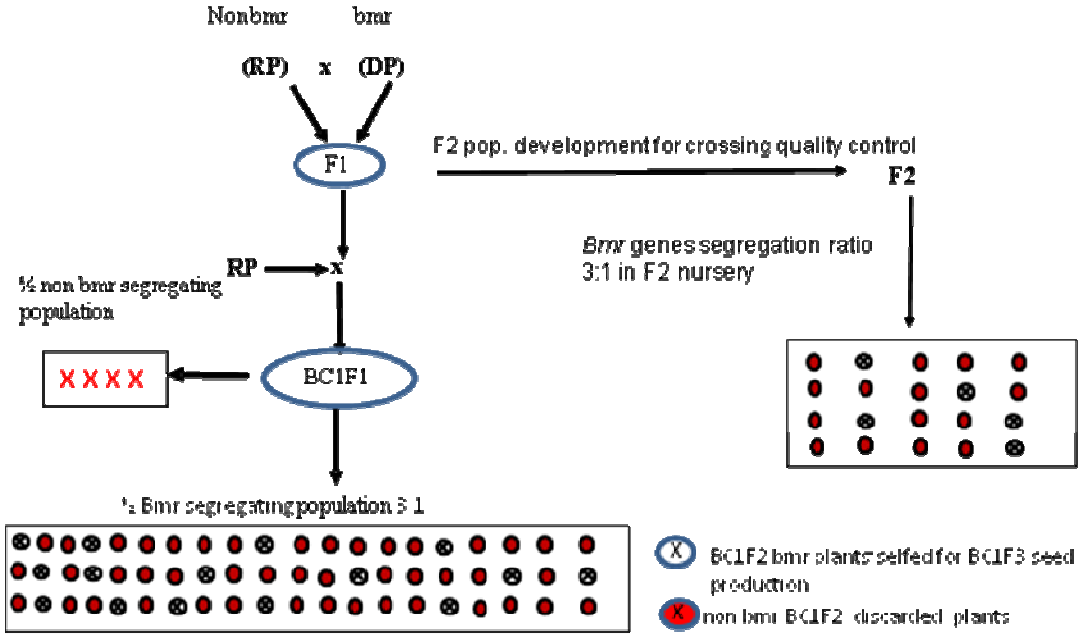

Figure 1: General scheme of the *bmr* breeding population development in Sotuba research Station, Mali.

All BC1F1 plants were planted and non *bmr* segregating BC1F2 populations were discarded. By discarding non *bmr* population in BC1F2, we had 1/4 (3:1) *bmr* plants in a segregating population instead of 1/8 from the total number of BC1F2 population planted. It is worth notifying that segregation ratio was 1:1 in BC1F2 population for *bmr* and non populations. Identification of wild types (non *bmr* plants) could have been done on F1 or BC1F1 plants using parental polymorphic marker close to *bmr 6* and *bmr 12* during parental survey. Another option is to plant 10-20 seeds from BC1F1 in plastic pot filled with compost and transplant seedling to open field only genotypes showing *bmr* trait to save time, money and space.

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 population. Chi-Square test is used in plant breeding for goodness of fit between expected and observed values. It is a statistical term which refers to how well numbers of what was expected and numbers of what is observed are the same. In this case, the number of expected *bmr* plants will be compared to observed ones as well as those expected non *bmr* to observed non *bmr* plants. This test accepts or rejects the segregation ratio based on one recessive allele (*bmr*) in our case. 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 - expected; d represents the deviation from e or the expected value, e = expected

RESULTS

F1 production

A total of 38 seeds were obtained as F1s (Table 4) in April 2016. During the following cropping season (June to September), the F1s and their parents were grown for F2 and BC1F1 seed production and generation advancement. All the F1s exhibited white midribs confirming the dominance of white midrib over the brown midrib type. Selection based on the anthocyanin inheritance combined with heterosis was done for progenies obtained in tan x anthocyanin crosses. Any tan plant was discarded due to self-fertilization seeds. These methods allowed an easy identification of 9 true F1s among 35 crosses (Table 4). This low number of 9 F_1 seeds out of 35 seeds indicates that certain selfing occurred during crossings. All progeny of possible crosses were examined:

- 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 F_1s.
- El mota *(purple, anthocyanin*) x Tx630 *bmr12 (tan plant)*: twelve (12) F1 seedlings were generated and among them 3 were selected as F_1s because of the heterosis observed.
- El mota (*purple anthocyanin*) x Redlan *bmr 6 (purple, anthocyanin)*: both parental lines were anthocyanin. 3 F1 seedlings were produced but 2 were selected as F_1s based on their visible heterosis effect.
- Kalla Kéné (*tan plant*) x Redlan *bmr6 (purple, anthocyanin)*: two (2) seedlings were generated. One exhibited anthocyanin character and was selected as true F_1.
- Kalla Kéné (*tan plant*) x Redlan *bmr12 (purple, anthocyanin)*: all seven (7) seedlings were tan plants and were all discarded from the breeding population development.

Table 4 indicates the number of successful crosses and Table 5 reports on the segregation ratios of *bmr* plant in F_2 population and respective Khi square value of each population.

Table 4: quantities of F_1 seed produced and F_1 seedling selected per cross at Sotuba April 2016.

Crosses	'F1s' seeds count	Seedlings count	True F1s count
Sepon82 x Redlan*bmr6*	10	9	1
Sepon82 x Wheatlan*bmr12*	2	2	2
El mota x Tx 630*bmr12*	14	12	3
El mota x Redlan*bmr6*	3	3	2
Kalla Kéné x Redlan*bmr6*	2	2	1
Kalla Kéné x Redlan*bmr12*	7	7	0
Total	**38**	**35**	**9**

F2 production and segregation ratios

Table 5: segregation in the F_2 families, Sotuba December 2016.

Population	Phenotype	Obs	Exp	df	Deviation (d)	d^2	d^2/e	Cal X^2	X^2 Critical value
Sepon82//Sepon82/Redlan*bmr6*	Dull green midrib	13	14	1	1	1	0.071	0.271	3.840
	Bmr	6	5	1	1	1	0.200		
Total		**19**	**19**	**-**	**-**	**-**	-	-	
Sepon82//Sepon82/Wheatlan*bmr12*	Dull green midrib	14	15	1	1	1	0.066	0.266	3.840
	Bmr	6	5	1	1	1	0.200		
Total		**20**	**20**	-	-	-	-	-	
El mota//El mota/Tx 630*bmr12*	White midrib	15	15	1	1	1	0.066	0.266	3.840
	Bmr	5	5	1	1	1	0.200		3.840
Total		**20**	**20**	-	-		-		
Kalla Kéné//Kalla Kéné /Redlan*bmr6*	White midrib	15	15	1	1	1	0.066	0.266	3.840
	Bmr	5	5	1	1	1	0.200		3.840
Total		**20**	**20**	-	-		-		

For a test with 1 df (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. Obs: number of *bmr* plants counted in the field out of a total of 20 plants. Exp: number of *bmr* plants derived from the single dominant gene segregation.

BC1F1 populations

Tables 6 gives the number of BC1F1 seeds produced per cross at Sotuba in September 2016.

Population BC1F1	Number of seeds produced
El mota//El mota/Tx630*bmr12*	15
Sepon82//Sepon82/wheatland*bmr12*	12
Sepon82//Sepon82/Redlan*bmr6*	15
Kalla Kéné//Kalla Kéné/Redlan*bmr6*	74
Total	**116**

BC_1F_2 and BC_1F_3 development

Four (4) BC_1F_2 *bmr* populations were obtained (Table 7) and advanced to BC_1F_3.

Table 7: Segregation ratios in the BC_1F_2 families

BC1F2 Populations	Phenotypes	Total plants	Obs	Exp	df	Deviation (d)	d^2	d^2/e	X^2	X^2 Critical value
Sepon82//Sepon82/Redlan *bmr6*	*bmr*	60	18	15	1	3	9	0.60	0.80	3.84
	Dull green		42	45	1	3		0.20		
Sepon82//Sepon82/Wheatland *bmr12*	*bmr*	60	13	15	1	2	4	0.26	0.35	
	Dull green		47	45	1	2		0.09		
El mota//El mota/Tx 630 *bmr12*	*bmr*	60	17	15	1	2	4	0.26	0.35	
	White midrib		43	45	1	2		0.09		
Kalla Kéné// Kalla Kéné/Redlan *bmr6*	*bmr*	60	15	15	1	0	0	0	0	
	White midrib		45	45	1	0	0	0	0	

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

DISCUSSION

After hand emasculation crosses 38 F1 seeds were obtained from almost 2000 expected seeds. This low level of success of crosses is mainly due to high temperatures occurring at this period of the year (almost 45°C) at Sotuba. In this view, [17] demonstrated that crossing sorghum at a temperature of 40°C (with low humidity) usually results in failure of seed set. Regarding the low success of crosses (only 9 true F1s over 38 seedlings), breakage of anthers and pollen pollution on tweezers tips during the emasculation process can be responsible. This hypothesis was

supported by [17] who prevented about anther rupture and pollen sticking to the emasculation tool combined with heat. In addition, [16] reported that the success of hybridization varies with personal skill, the amount of injuries sustained by the floret part during the emasculation.

The midrib trait segregation in the breeding populations was verified using the chi-square test and heterosis vigor. Every breeding population was constituted of two types of phenotypes (*bmr* plants and non *bmr* plants). For a test with 1 degree of freedom, the critical value of the chi-square statistic was 3.84 (chi-square table). Consequently, a very small chi-square statistic value obtained here means that the observed data fits the expected data extremely well. In this order, our results showed no statistic deviation from the ratio of 3:1 in the segregating populations (F2s and BC1F2). Indeed, two situations were observed from our data analysis: (1) a good fit of the segregation; (2) the Chi-square values were inferior to the critical values. Our results are in harmony with those found by [3]. For the same trait, [26] also found similar result in F2 *bmr* segregating populations. Furthermore, in a cross involving a rice wild type and a mutant, [27] observed in the following F2 population a ratio of 3:1 and suggested that each mutant phenotype was caused by a recessive mutation in a single locus.

Night time of 15 hours imposed on Kalla Kéné using cage technique was critical for inducing its reproductive stage. This cage is locally manufacture and is easy manipulated.

CONCLUSION-PERSPECTIVES

Conventional hand emasculation (HE) technique used to create diversity in self pollinated crop such as sorghum is still a very practical and worth technique in the absence of molecular makers for young breeders with resources limited to use molecular techniques facility. Mali and recently Niger breeders have been using HE technique which needs skill field technicians to pursue their breeding activities while out sourcing sometime their crossings when financial opportunity is available. Opportunity to train other institution technician with limited access to molecular laboratory facility is available at Sotuba in Mali for hand emasculation training. This technique is still very useful in genes introgression for sorghum segregating populations development. However, in such circumstance, the environmental conditions such as temperature and humidity are of great importance in sorghum hybridization. This was clearly observed during the *bmr* genes transfer. The *bmr6* and *bmr12* genes were successfully introgressed in Nigerien and Malian sorghum varieties background.

HE genetic transfer of the *bmr* genes laid out the dual purpose sorghum varieties with potential high stover quality populations for the two countries. Large populations *bmr* populations are developed and are under field evaluation for grain and yield potential in Niger and Mali, two Sahelian countries facing livestock feeding issue with quality stover during dry season. Furthermore, introgression of *bmr* trait in late maturing photoperiod sensitive varieties increase both biomass productivity and quality due to their lower lignin contents.

Identification of productive and quality stover lines, experimented in pilot study with livestock holders at farmer level, will set up the opportunity for intensive milk and beef production to pave the road for stabilized cattle husbandry and to end long term free grazing conflicts.

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