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How an improved sorghum variety evolves in a traditional seed system in Mali: Effects of farmers' practices on the maintenance of phenotype and genetic composition

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ABSTRACT

In Africa, it is mostly the informal seed system that ensures farmers' seed supply. This is partly because the formal seed systems are not always effective in meeting demand for new seed varieties. Sometimes informal seed recycling and exchange of improved sorghum varieties will take place alongside formal initiatives, as is the case in southern Mali. Focusing on one particular village in the Dioïla district, we analyze the efficacy of farmers' strategies for preserving varietal seed purity and genetic integrity of an improved inbred-line (Soumba variety). Six seed lots of Soumba, recycled for two to six years by farmers using different practices, were collected and assessed in on-station trials in order to compare their agronomic performance and phenotypic purity (off-type plant frequencies) with control versions of the variety. Additionally, 30 panicle samples were randomly collected from five farmer fields sown with recycled Soumba and assessed for phenotypic purity in a progeny nursery and investigated for molecular diversity using 12 SSR markers. A total of 150 panicles from five other non-Soumba varieties were collected in the village in order to investigate eventual gene flow and its potential genetic consequences for the Soumba variety. In fields sown with recycled Soumba seed, between 2% and 14% of plants showed phenotypic deviations from the typical Soumba variety. The progeny nursery and SSR marker analysis verified the presence of the off-type plants observed in the field. The STRUCTURE program revealed admixtures with other varieties in 23% of Soumba plants, confirming the presence of gene flow. Gene diversity values in Soumba samples ranged from 0.006 for the commercial sample to 0.257 for recycled samples. Introgression and contamination were best minimized when (1) farmers had received specific training in seed production, (2) they could take advantage of isolated fields and (3) they could practise true-to-type panicle selection. Farmers were generally able to maintain the phenotype, as well as sustain or even improve yield performance of their Soumba variety while at the same time genetically enriching their seed stock.

1. Introduction

With a view to enhance food security within the context of demographic pressure and climate variability, African countries have been expanding their efforts in recent years to promote intensified agricultural practices, including improved varieties. These efforts have taken various forms, including production-marketing projects, promotion of technology packages, government programs and seed relief programs, as elucidated by Kaboré et al. (2010); Baquedano et al. (2010); Warburton et al. (2010). Unfortunately, in most African countries sustainable seed

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provision for improved varieties is often hindered by the complex steps and regulations required for producing and commercializing seed (Guéi et al., 2011). Purchase of seed can also be hampered by lack of funds (low investment capacity of the subsistent farmer), lack of knowledge about the workings of modern markets, or even by socio-cultural restrictions; for example, monetary exchange of traditional cereals seed is a taboo in Mali (Siart, 2008).

Once a new or improved variety has been obtained, further supply often takes place through informal channels, such as farmer-to-farmer exchange of on-farm recycled seed (Bentley et al., 2011). In Syria, for instance, Aw-Hassan et al. (2008) demonstrated an effective diffusion of new barley varieties after an initial seed injection from research institutes through farmer-to-farmer seed trade (i.e., without public support from extension services). In most of Africa these informal systems still ensure between 80 and 100% of farmers' seed supply, as highlighted by Louwaars and de Boef (2012). Seed is saved on-farm (recycled), with the aim of reproducing the variety phenotype or adapting a population to the farmer's needs, whether it be maize in Central Mexico, pearl millet in India or organic bread wheat varieties in France (Louette and Smale, 2000; Perales et al., 2003; vom Brocke et al., 2003; Dawson et al., 2012; Westengen et al., 2014). Louette and Smale (2000) showed that farmer phenotype-based selection in traditional maize systems was the key to maintaining the typical ear characteristics of a maize variety under the presence of gene flow. In European organic farming systems, Dawson et al. (2012) concluded that farmers' varieties could retain distinctive multiple agro-morphological traits even after several years of on-farm production. Duupa farmers in Cameroon are likewise able to maintain sorghum landraces in mixtures through ideotype selection, in spite of pollen flow and relatively high outcrossing rates (Barnaud et al., 2008). Malian farmers have a long tradition of maintaining their varieties true-to-type by selecting panicles for specific phenotypic traits, such as grain, panicle and glume attributes and flowering dates, although it has been reported that some farmers nowadays favor food grain for sowing over the time-consuming panicle selection method (Siart, 2008).

Farmers' seed selection and management can especially influence the genetic composition of the variety in open-pollinated crops such as pearl millet and maize (Morris et al., 1999; vom Brocke et al., 2003; Warburton et al., 2010; Lakis et al., 2011; Westengen et al., 2014). Using SNP markers, Westengen et al. (2014) recently showed that the formal system seed of improved open pollinated maize varieties differentiated significantly from farmer recycled seeds of the same varieties in Tanzania. The authors attributed the changes in farmer recycled seed to possible hybridization and directional selection for drought tolerance. Even though sorghum is predominantly self-pollinating, outcrossing levels have been found to vary between 5 and 40%, with up to 30% for guinea landraces (Ollitrault et al., 1997; Barnaud et al., 2008). Using microsatellite markers, Rabbi et al. (2010) revealed considerable diversification for an improved sorghum variety among different farmer seed lots at a national scale in Kenya albeit much less than in Sudan. The authors attributed these differences to farmers' practices in traditional seed systems.

To ensure that the farmers who depend on informal seed systems can also access quality seed of improved varieties, it is important to recognize the effects of farmers' seed recycling practices on the variety's desired properties. For this purpose, we analyzed the evolution of the phenotypic purity and genetic integrity of an improved sorghum (*Sorghum bicolor* (L.) Moench) inbred line (of caudatum-guinea race) within a family-based agricultural system in a village of southern Mali. The article provides a brief description of the varietal dynamics in the study village and an assessment of farmers' practices and channels through which an introduced variety is diffused. Further, the success of farmers in maintaining phenotypic and genetic characteristics of the improved inbred-line within this system is analyzed through a combination of in-situ evaluation of variety "off-type plants" and diversity analysis with SSR markers. The effect of seed recycling on agronomic performance of farmer seed lots was investigated by means of on-station field trials. This paper also explores the genetic structure of other varieties grown in the study village in order to assess an eventual preferential direction of gene flow and its potential genetic consequences for the improved inbred line.

2. Materials and methods

2.1. The study area

This study was carried out in the Dioïla district (cercle de Dioïla), Koulikoro region, in southern Mali, where sorghum is an important staple next to maize and pearl millet. With mean annual rainfall ranging between 800 and 900 mm during a 4–5 month rainy season extending from May/July to September/October, the district is situated in the Sudano-Sahelian climate zone. In this region, as in most of Mali and neighboring Burkina Faso, farmers still grow primarily sorghum landraces belonging to the guinea race, whereas the adoption and commercialization of modern varieties remains low (Siart, 2008; vom Brocke et al., 2013). The Dioïla district is also an established cotton producing area where farmers have access to fertilizers and other production technologies. Since 2003, the International Crops Research Institute of the Semi-Arid Tropics (ICRISAT), along with its national partner, the Institut d'Economie Rurale (IER) and a local farmer organization, the Union Locale des Producteurs de Céréales de Dioïla (ULPC), together have tested around 60 improved sorghum lines in about 28 villages of the Dioïla district through participatory variety evaluation and breeding programs (Weltzien et al., 2007). From 2008 onwards, the six most preferred lines from this program were incorporated into the formal seed production and commercialization programs managed by the ULPC.

The present study focuses on Magnambougou (latitude 12.82N, longitude 8.21W), a highly representative village for the district (Falconnier, 2009). This village comprises approximately 26 farming households, of which 22 grew sorghum in 2008 (with one to four varieties per household). Sorghum is cultivated in rotation with cotton and maize, either in pure stand or intercropped with cowpea. Vom Brocke et al. (2012) have described an important dynamic of varietal changes for sorghum in this village: 50% of the varieties cultivated in 2003 were no longer grown in 2008 while 70% of the varieties noted in 2008 were introduced after 2003. Only about 30% of varieties grown during this period were considered as "constant" varieties (local landraces produced consistently over five years by at least 8% of the households interviewed).

2.2. The Soumba variety

One particular improved sorghum variety that is popular in the region, especially in the village of Magnambougou, is the Soumba (CIRAD 406) variety from the CIRAD/ICRISAT breeding program. Formal seed production of certified Soumba seed in the Dioïla district by the ULPC began in 2004. Today, Soumba is purchased and produced for household consumption and for commercialization, either at the local marketplace or with the ULPC within the framework of a production–marketing project (Baquedano et al., 2010). Soumba is a tan, white-grained, photoperiod insensitive inbred line derived from a cross between a guinea landrace from Uganda and an improved caudatum line from Senegal. Its plant type is similar to the caudatum parent with a medium height

Table 1
Number of years of on-farm production, farmers' seed replacement and/or recycling practice, as well as the nature (panicles or bulk seeds) of farmers' Soumba seed lots and samples collected in April 2009 and November 2009.

Name	Years	Farmers' practice[a]	Seed lots	Soumba samples
M_6	1	Replacement with CS in 2009	–[b]	Panicles (30)
M_2	2	PS in field pile	Grain stock (bulk)	Panicles (30)
M_3	2	Use of GR	Grain stock (bulk)	–
M_4	2	PS	Selected panicles (bulk)	–
M_7	5	1st year GR, PS in sheaves	Selected panicles (bulk)	Panicles (30)
M_1	5	GR, 5th year PS	Grain stock (bulk)	Panicles (30)
MS_1	5	1st year GR, PS	Selected panicles (5)	–
W_1	6	PS in field pile	–	Panicles (30)

[a] CS: commercial seed; GR: seed taken from food grain; PS: seed taken from selected panicles.
[b] Sample not available.

(240 cm) and an erect semi-compact panicle. The white to yellowish grains possess some guinea traits as open glumes and rather corneous endosperm with high amylose content – traits that are important for local grain processing and food preparation. Its growing cycle is between 105 and 110 days from sowing to maturity without photoperiodic sensitivity. Its phenotype (or plant type) stands in strong contrast to the largely photoperiodic sensitive guinea landraces of the region, which are of a tall height (up to 5 m) with characteristically loose and drooping panicles (De Wet, 1978).

2.3. Survey and choice of households

Preliminary, semi-structured interviews were conducted before the start of the cropping season in April 2009 with 22 of the 26 farmer households in Magnambougou. Household members responsible for seed management (in most cases the head of the household) were asked about the number of sorghum varieties cultivated between 2003 and 2008; their origin, morphological characteristics, performance, and uses. The interviews revealed eleven households cultivating the Soumba variety in Magnambougou. Members from these households were questioned during a second round of semi-structured interviews at the end of the cropping season in October 2009. In the course of these interviews, two further households in neighboring Wéla (W1) and Gouligan (GL1) villages were identified and subsequently interviewed as they were found to have an important role in the informal seed flow of Soumba into the village of Magnambougou. The interviews focused on Soumba seed management i.e., the area being cultivated with Soumba, the provenance of their Soumba seed stock, their own seed production practices and if they exchanged their seed grain with farmers in other villages.

2.4. Collection of genetic material

Three sets of materials, including Soumba bulk seed lots, Soumba panicle samples and panicle samples from other varieties grown in Magnambougou and in the two neighboring villages, were collected for the purpose of studying the consequences of seed recycling on the purity and integrity of the Soumba variety (Table 1and S1, Fig. 1).

1. Soumba seed lots*:* Six seed lots, representing Soumba seed retained by farmers for their 2009 plantings, were collected during the preliminary interviews in April 2009 from six of the eleven interviewed farmer households (Table 1). Five farmers gave seeds (500 g) from threshed panicles (M_1, M_2, M_3, M_4, M_7) while one farmer provided five panicles (MS_1). Four households were not included in the collection as they all used the same commercial seeds from the 2008 ULPC stock (M_5, M_6, M_{14}, M_{18}).
2. Soumba panicle samples: 30 panicles (20 g of seeds per panicle) were randomly harvested in each of five fields at crop maturity in November 2009. Seeds from 150 panicles in total were collected. The thirty panicles collected from the same

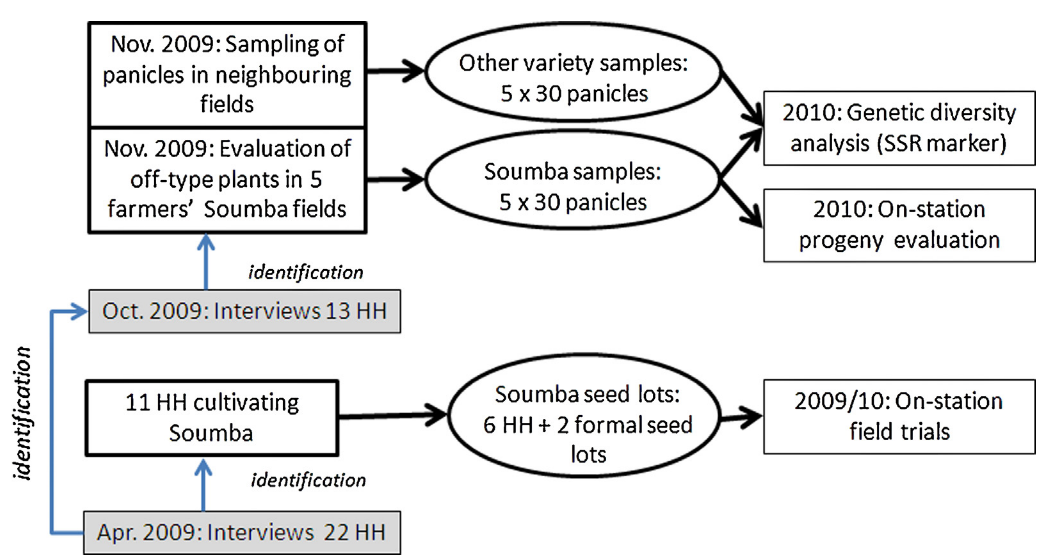

Fig. 1. Sequence of interviews, collections and samplings of seed stocks and plants, and their subsequent analysis and testing. HH: households.

field are further referred to as one "Soumba sample". A condition of the study was to select Soumba fields that were not being used for re-sowing or variety mixtures or different Soumba sources, and where the farmer had not yet eliminated off-type plants. As samples MS_1, M_3 and M_4 did not correspond to these criteria they were not included in the sampling. Five households possessed Soumba fields that corresponded to the criteria, notably households M_1, M_2, M_6, M_7 and W_1. Four households had been recycling Soumba seed on their farms for at least two consecutive years using different recycling practices (M_1, M_2, M_7 and W_1) while one household, M_6, was chosen to represent commercial seed after one year of production (Table 1). In the case of the M_1 household, the personal field of the wife of the household head was chosen for sampling, as she was the only one who continued to use the recycled Soumba seeds of this household (she selected panicles during the harvest in 2008 for sowing in 2009). The household head replaced the Soumba seed stock for the 2009 sowing with new, commercial seeds from the ULPC, as he felt that there were too many off-types in his own recycled seed stock. In all fields, Soumba was sown from mid to late June. Field areas varied between 0.3 and 2 ha for the five fields. The proximity of the sampled fields to adjoining sorghum varieties is given in Table S1.

3. Panicle samples of other varieties: In order to detect potential cross-pollination between Soumba samples and other varieties, seed from 30 panicles from five varieties grown in Magnambougou (further referred to as "non-Soumba samples") were collected during crop maturity in 2009. The non-Soumba samples represent the most popular variety in the village (Bamtouklabé), in addition to four other varieties grown near sampled Soumba fields (1–50 m distance) in 2009 and/or 2008 (Table S1).

2.5. Assessment of phenotypic purity and agronomic performance of recycled Soumba

Phenotypic purity in the Soumba variety was based on the frequency of Soumba off-type plants present in the Soumba seed lots, in the Soumba fields, and the Soumba panicle samples collected from these fields. On the basis of farmers' and breeders' criteria, off-type plants were defined as either wild-weedy sorghum plants (manure used to fertilize fields often contains seed of wild sorghums) with unfavorable grain productivity, or as plants with taller height and longer internodes than the Soumba variety, with longer and looser panicles and red grains of low quality. Red glumes and grain color whiter than the original Soumba were also mentioned by farmers during interviews and field visits.

2.5.1. Assessment of seed lots in field trials

The six farmer-recycled Soumba seed lots collected in April 2009 were used as entries to test agronomic performance compared to two formal seed lots (commercial seed of ULPC and breeder seed of ICRISAT). The seed lots were grown over two years in a randomized complete block trial with four replications at the ICRISAT Samanko research station (12°32′N, 8°04′W), which lies in the Sudanian zone of Mali (1000 mm annual rainfall). Sowing dates were July 8th in 2009 and June 30th in 2010. The field trials received 1069 mm and 1230 mm rainfall during the 2009 and 2010 cropping seasons respectively. Each plot comprised 100 hills in 2009 and 50 hills in 2010, sown in rows with 75 cm distance between rows and 30 cm distance between hills (thinned to two plants per hill). Fertilization of the 2009 trials was in accordance with national recommendations for sorghum cultivation in the target zone, which is 100 kg ha^{-1} of DAP (diammonium phosphate, 18–46–0) before planting and 50 kg ha^{-1} of urea at the stem elongation stage. In 2010, the trial was conducted under low phosphorous field conditions (Leiser et al., 2012), comparable to conditions in farmer fields, receiving a mere 50 kg ha^{-1} of urea. Agronomic observations comprised: days to 50% heading, plant height (cm), panicle length (cm), grain yield (kg ha^{-1}) and 100-grain weight (g). Phenotypic purity of a seed lot entry was observed each year by counting off-type plants in each plot. In order to learn if off-type plants in farmers' seed lots affect productivity, grain yield was firstly evaluated by including all plants (total plot) and then by considering only typical Soumba plants in each plot (TSP plot). Individual analysis of the low and high phosphor trials was performed using seed lots as a fixed factor and replications as a random factor. A combined analysis of the two years for the quantitative observed traits was performed by applying a linear model, with seed lot, year and blocks within years as factors, and only the error term taken as random, using GENSTAT release 14.1. For the off-type trait, which is categorical, a generalized linear model/logistic model was adjusted on the off-type and TSP counts, again with years and blocks within years as factors, and provision for over-dispersion using the GENMOD procedure of SAS (release 9.3.). Confidence intervals for off-type proportions were computed following Wilson's method, as described by Agresti and Coull (1998) and modified to allow for over-dispersion (Agresti, 2011) using the BINOM package of R (R Development Core Team, 2008). This modification is tantamount to dividing the observed counts by the estimated over-dispersion for obtaining an effective sample size.

2.5.2. Assessment in farmers' fields and collected panicles

The frequency of off-type plants was assessed at maturity time in the five farmers' Soumba fields where the Soumba panicle samples were derived from. The counting was performed in three different strips of the fields, on the sides, keeping four rows from the field border and in the centre. Between 161 and 471 plants were counted per field strip in each field. Again, a logistic model was fitted, with the farmer as the sole factor and with provision for over-dispersion. Confidence intervals were calculated using the same method as above. Off-type plants were also identified and counted within each Soumba sample.

2.5.3. Assessment of progenies in a nursery

At the onset of the rainy season in July 2010, seeds from each panicle of the five Soumba samples (30 panicles each) were sown as an individual progeny in a non-replicated, non-randomized nursery at the Samanko research station. Each progeny consisted of five plants (one plant per hill) with 75 cm spacing between rows and 30 cm between hills. In order to facilitate the detection of traits deviating from the typical Soumba phenotype, one row of formal Soumba seed (breeder seed bulk), produced at ICRISAT through self-pollination, was sown after every 15 progenies. Observations included the farmer-relevant traits of plant height (cm), plant, glume and grain color, presence of awns, and panicle compactness. The percentage of progenies showing deviation from the formal control or segregation for traits (further referred to as "off-type progeny") was determined for each 30-panicle sample. Since most of the farmers indicated during interviews that they remove off-type plants at maturity before harvesting, the frequency of off-type progenies derived from non-off-type plants, i.e., "typical Soumba plants (TSP)" was additionally assessed. In accordance with the random sampling scheme of the panicles, confidence intervals were computed according to Wilson's method, assuming a binomial distribution. Box plots were generated for heading date, plant height and panicle length observations in order to visualize trait expression variation among the Soumba samples and compared to the formal Soumba control. These traits are also used by farmers when describing off-type plants.

2.6. Molecular analysis

Seeds from 150 Soumba panicles and from 150 non-Soumba panicles, along with commercial Soumba seed (bulk seed provided by ULPC), were sown in a greenhouse at the CIRAD Centre in Montpellier. Leaves were collected from one seedling (3–4 weeks old) for each mother panicle and from 30 separated seedlings of the commercial seed for the purpose of lyophilizing overnight. DNA extraction was performed using the modified MATAB method (Risterucci et al., 2000). A set of 28 SSR markers previously used to assess in situ sorghum genetic diversity in Burkina Faso and Niger (Deu et al., 2008; Barro-Kondombo et al., 2010) was applied to a subset of 80 individual plants representing both Soumba and non-Soumba samples. Among these 28 SSRs, three gave weak amplifications, two SSRs did not reveal polymorphism while all the 23 remaining ones revealed contrasted alleles between Soumba and non Soumba samples. We selected 12 SSRs that (1) gave unambiguous bands easy to read, (2) could be multiplexed according to the size of the alleles and (3) were distributed along the chromosomes. They were: gpsb89 (chr. 1), Xcup63 (chr. 2), Xcup61 (chr. 3), gpsb151 (chr. 4), Xtxp65 (chr. 5), Xtxp57 and Xtxp145 (chr. 6), Xtxp295 (chr. 7), gpsb67 (chr. 8), Xcup2 and Xtxp289 (chr. 9), and Xcup7 (chr. 10). Genotyping was carried out at the Languedoc Roussillon Génopole platform located at the CIRAD Campus (Montpellier, France) using the methods described previously by Barnaud et al. (2007); which are currently used in the laboratory (Deu et al., 2008; Sagnard et al., 2011).

2.7. Genetic data analyses

Genetic diversity parameters were calculated for each sample or seed lot with the FSTAT software (Goudet, 2002): Nei's unbiased gene diversity or expected heterozygosity (H_e), observed heterozygosity (H_o), and mean allelic richness across loci (R_s). The significance of differences in H_e, H_o and R_s between samples was tested using Wilcoxon paired-rank tests. Genotype richness (also called polyclonality) was estimated as the percentage of unique genotypes in the sample. Fixation index (F_{IS}) for each sample and pairwise F_{ST} between varieties were computed with the FSTAT software. The significance of differences was assessed using a permutation procedure (55,000 permutations) and defined after adjustment for multiple comparisons using standard Bonferroni corrections.

A dissimilarity matrix between all pairs of individual plants collected from fields (299 individuals) was computed using the shared allele distance. Next, a principal coordinate analysis (PcoA) was performed in order to realize a synthetic representation of the diversity. Analyses were performed with DARwin v5 software (Perrier and Jacquemoud-Collet, 2006).

The Bayesian model-based clustering method developed by Pritchard et al. (2000) and implemented in the STRUCTURE v2.2 software was also applied to the same data set. The admixture model, with correlated allele frequencies, was employed without prior population information while other parameters were set to their default values. We ran 20 replicate analyses for each K value ranging from one to fifteen, with a burn-in period of 500,000 followed by 1.10^6 iterations. To assess the number of populations supported by the data set, we used both the log likelihood and the change in the second order of likelihood (ΔK), as suggested by Evanno et al. (2005). We verified the congruence between runs for each K value by visual observation of the admixture coefficient (Q_i) for each individual plant in each cluster.

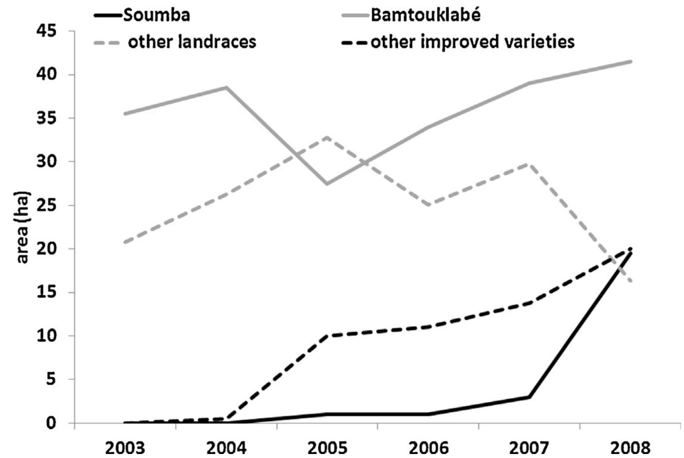

Fig. 2. Five-year evolution of area planted with Soumba variety and other improved varieties compared to area cultivated with the local landrace Bamtouklabé and other local landraces reported by farmer households in Magnambougou.

3. Results

3.1. Soumba production in Magnambougou

Between six and eleven different sorghum varieties were grown in Magnambougou during 2003 to 2008; with ten different varieties in 2008 (data not shown). The Soumba variety is one of three modern varieties introduced in Magnambougou in 2003. By 2008 this variety covered almost 19% of area allocated to sorghum, around 20 ha of the total sorghum area (114 ha) in the village. While local landraces other than the variety Bamtouklabé experienced a slight decline, the modern varieties claimed increasingly more area (Fig. 2). According to farmers, the Soumba variety offers some specific advantages over the local varieties, such as good grain quality in a wide range of conditions, good yield production even in poor soils, improved fodder quality, bird resistance (glumes difficult to separate from the grain) and resistance against plant lodging. They are traits particularly favored by Soumba growers in the context of changing agricultural conditions. Soumba presents a useful varietal option for farmers because it tolerates delayed sowing and harvesting, not to mention bird damage in fields near rivers.

3.2. Soumba seed system and recycling practices

In 2008, around a quarter of the Soumba fields in Magnambougou were sown with commercial (certified) seed from the ULPC cooperative. The remaining Soumba plots were planted with seed that had been recycled by farmers on their own fields or seed that had been accessed through informal channels. The predominant seed exchange was by the way of seed presents (Fig. 3). From 2003 to 2008 at least nine (69%) of the interviewed households were involved in informal seed exchange of Soumba (being either receivers, providers or both, depending on the year) and did not buy commercial seed. Some farmers (23%) were using both channels (formal and informal) to access or distribute Soumba seed in the same year. This is illustrated by M_4, who bought seed from the ULPC in 2008 and completed his seed stock, in the same year, with seed provided by M_1 or W_1, who, in addition to his official formal seed production for the ULPC, was also recycling Soumba seed on the family field and donating seed annually from his own production (Fig. 3).

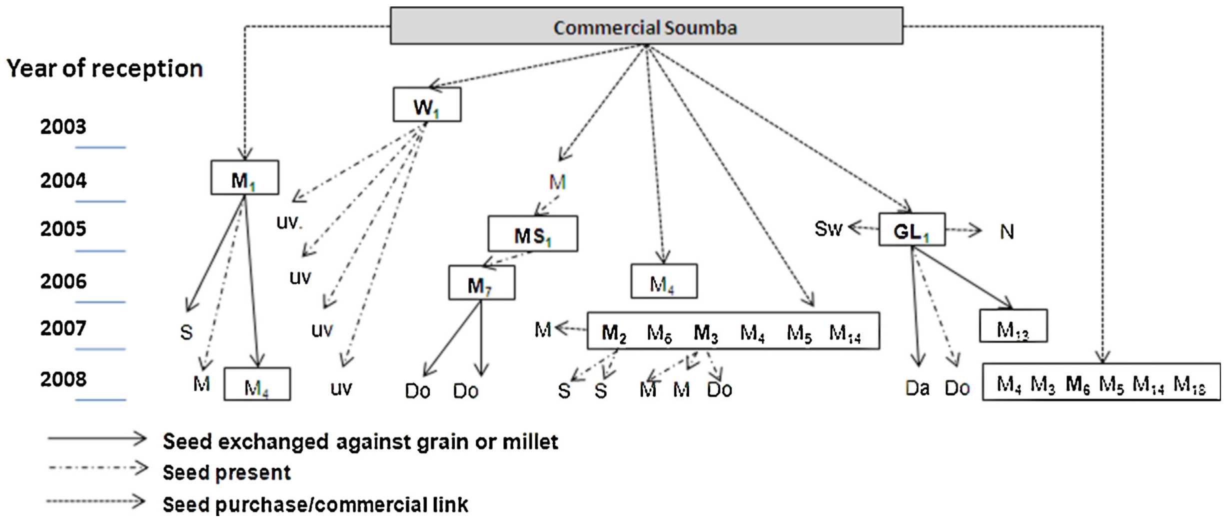

Fig. 3. Seed sources, reception years; seed exchange method for Soumba seed for households in Magnambougou and neighboring villages. Letters followed by numbers represent the location of the households in the different villages: Da: Dandougou, Do: Doukassola, GL: Gouligan, M: Magnambougou, N: Nangola, MS: Soumailabougou-local hamlet in Magnambougou, Sw: Sekouwéré, W: Welia, uv: unidentified village (each year 3–4 households received seed). Framed letters are households interviewed in 2009 and bold letters are households which provided seed samples.

Interviews revealed that Soumba was either recycled by farmers from selected panicles or taken from the food grain stock. Nine (69%) of the interviewed farmers recycled Soumba seeds on their farm in 2008; seven (53%), namely M_1, M_2, M_4, M_7, MS_1, GL_1 and W_1, practiced panicle selection while only two (15%) farmers (M_3 and M_{13}) used food grain as seeds (Table S2). It should be noted that the M_1 seed stock was only submitted to panicle selection in 2008; the farmer using food grain for seed in the preceding years. Farmers further reported that the uniformity or typical phenotype of the Soumba variety was menaced by the presence of off-type plants. They resorted to different measures for counteracting this “contamination”: planting in isolated fields (the W_1 Soumba field was surrounded by pearl millet and cotton fields, about 2000 m from the next sorghum field), practising panicle selection either in sheaves or in separate field piles (M_2, M_7, W_1), replacing their seed stock with commercial seed each year (M_6, M_{13}, M_4) or after the own seed stock is considered to have lost its integrity (M_1). All households reported that they regularly eliminated off-type plants (including wild sorghum types) before harvesting. Seed lots and samples collected for the present study were thus categorized whether they represent recycled or commercial seed, what recycling practices were used (panicle selection or taken from food grain stock) and for how many years they were recycled (Table 1).

3.3. Performance of recycled Soumba and occurrence of varietal off-type plants

Seed lots, including the farmer versions and the control versions, differed significantly in regard to their off-type frequency ($p < 0.001$, data not shown). Seed lots representing breeder seed and commercial seed showed 1% and 2% of off-type plants, respectively (Table 2). Seed lots recycled once showed 1–4% of off-types (M_4 and M_3, respectively) while seed lots recycled four times or more (M_7 and M_1) showed 11 and 4% of off-types, respectively. With 21% of plants showing morphological deviations from the typical Soumba plant, the twice recycled seed lot M_2 showed the significantly highest number of off-type plants in the field trial plots. MS_1 had no visible off-types, probably due to the fact that the farmers had provided only a limited number (5) of panicles. Confidence intervals indicate that only M_2 and M_7 differed significantly from the other seed lots for their off-type frequencies.

Table 2
Assessment of off-types (OT): total number of plants, frequencies and confidence intervals of off-type plants assessed in randomized field trials (seed lots) and in farmer fields. Total number of progenies, frequencies and confidence intervals of off-type progenies derived from Soumba (total) and frequencies and confidence intervals of off-type progenies derived exclusively from plants identified as typical Soumba plants (TSP) in the progeny nursery.

Samples	Seed lots sown in randomized trial		Farmer fields		Progeny nursery			
	N (plants)	OT (%)	*N* (plants)	OT (%)	*N* (progenies)	OT progenies (%)	N (TSP progenies)	TSP-derived OT progenies (%)
Soumba M_6	–[a]	–	772	2.0 (0.9–4.2)	30	10.0 (3.5–25.6)	28	3.6 (0.6–17.7)
Soumba M_2	627	20.6 (16.8–25.0)	1300	14.1 (11.2–16.6)	30	36.7 (21.9–54.5)	24	20.8 (9.2–40.5)
Soumba M_3	581	1.2 (0.5–3.0)	–	–		–		–
Soumba M_4	604	3.6 (2.1–6.1)	–	–		–		–
Soumba M_7	582	11.2 (8.3–14.9)	177	8.5 (5.9–12.0)	30	33.3 (19.2–51.2)	26	23.1 (11.0–42.1)
Soumba M_1	603	4.1 (2.5–6.7)	1097	12.7 (9.4–16.8)	30	40.0 (24.6–57.7)	24	25.0 (12.0–44.9)
Soumba MS_1	607	0.0 (0.0–1.1)	–	–		–		–
Soumba W_1	–	–	757	8.4 (5.7–12.4)	30	33.3 (19.2–51.2)	24	16.7 (6.7–35.9)
C. Soumba	647	1.9 (0.9–3.8)	–	–	–	–	–	–
B. Soumba	630	0.6 (0.2–2.1)	–	–	–	–	–	–
Over-dispersion	1.7		2.8					

N: total number of plants or progenies; C. Soumba: commercial seed lot of Soumba; B. Soumba: breeder seed lot of Soumba. Confidence intervals are given in brackets.
[a] Sample not available for analysis.

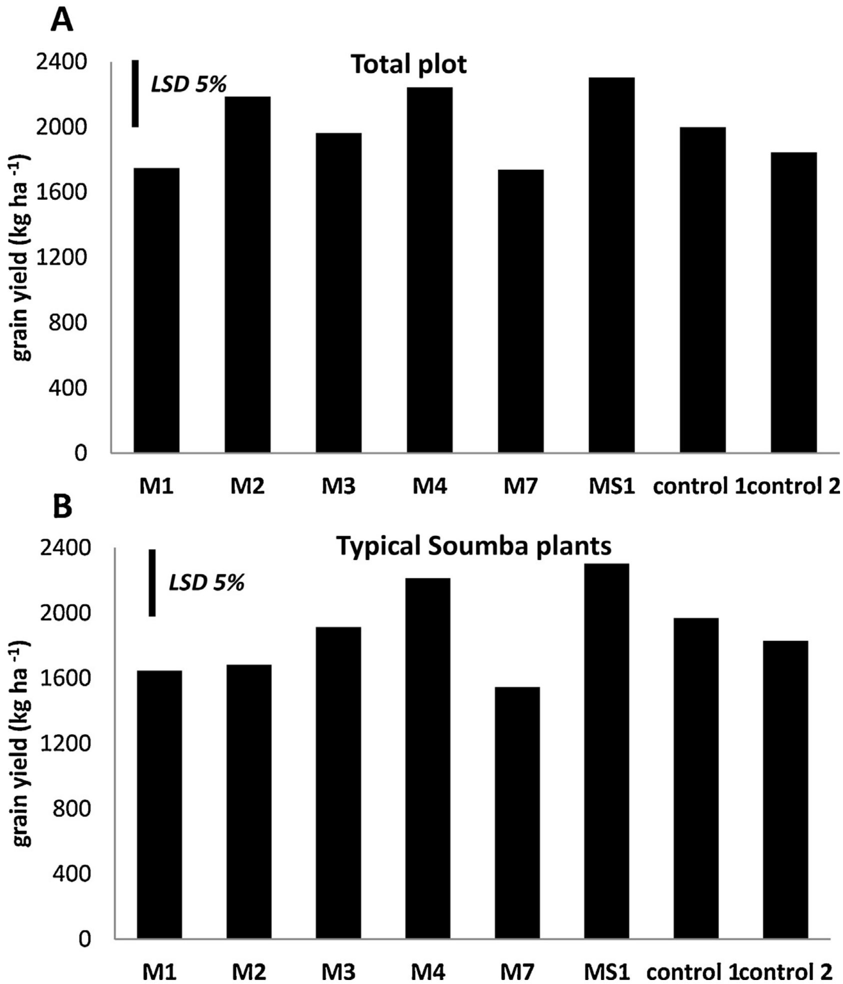

Fig. 4. Grain yield performance on the basis of all plants in a plot (total plot, A) and only typical Soumba plants (B) from six farmers' Soumba seed lots (M_1, M_2, M_3, M_4, M_7, MS_1) compared to formal controls of the Soumba variety (control 1: commercial seed, control 2: breeder seed), tested at two locations at the Samanko research station. The LSD (least significant difference) at 5% values are 402 kg ha^{-1} for total plot yield and 403 kg ha^{-1} for typical Soumba plant yield.

Field trials in 2009 and 2010 were conducted under high and low phosphor conditions. As rainfall conditions and sowing dates were relatively similar and favorable in both years, the effect of phosphor availability in the soil is considered more relevant when explaining differences in agronomic performance rather than the year effect. The analysis of the individual trials at Samanko showed significant seed lot effects and considerable higher total grain yield in the high phosphor environment (342 kg ha^{-1} total average grain yield compared to 2194 kg ha^{-1} with high phosphor input, data not shown). The combined analysis of both field trials shows expectedly significant effects for environments for all traits (Table S3). Significant seed lot effects were detected only for panicle length and for grain yield. Fig. 4 indicates that the farmer-recycled seed lots of M_4 and MS_1 produced the highest grain yields, which significantly exceeded samples M_1 and M_7 and which tended to exceed (non-significantly) the breeder and commercial seed lot controls. The M_7 seed lot shows the lowest grain yield regardless if "total plot" grain yield or of grain yield only from "typical Soumba plants (TSP)" was measured. This was the case in both environments for M_7 (data not shown). Off-types seem to contribute positively to grain yield for sample M_2.

The assessment in farmers' Soumba fields in October 2009 at the physiological maturity stage showed that between 2% (M_6) and 14% (M_2) of plants were off-types (Table 2). Even though the differences among Soumba fields were globally significant ($p = 0.04$, data not shown), computed confidence intervals for the different fields were relatively large (Table 2). Off-type plants with semi-loose panicles and red grains (and sometimes white grains) were perceived relatively frequent in M_2, whereas M_7 and W_1 had mainly off-types resembling wild-weedy sorghums. This difference however was not documented.

Among the 30 panicles collected in these fields, between 7 and 20% showed morphological deviation from the typical Soumba phenotype (Table 2). The progeny nursery confirmed the presence of off-type plants observed in the field, with 10–40% of progenies deviating from the typical Soumba type. Even when progenies derived from plants identified as off-types during the panicle sampling are excluded, there is still a high frequency of progenies (between 4 and 25%) deviating from the Soumba type (Table 2). Differences in frequencies of off-type progenies among Soumba samples were non-significant for both, total and only TSP derived progenies. Box plots for heading date, plant height and panicle length indicate a relatively high variability for these traits for the farmer recycled Soumba samples compared to the control seed lot (Fig. S1). This was especially so for M_1. The box plot for heading date reveals a trend towards a late flowering of off-type progenies, especially in W_1. Outliers, visualized in the box plots, generally correspond to previously identified progenies derived from off-type plants. Most variability outside of the quartiles and/or individual outliers concerning these traits was detected in the M_1, M_2 and the W_1 samples.

Table 3
Genetic diversity parameters assessed via 12 SSR markers for each Soumba sample (M_1, M_2, M_6, M_7, W_1), the commercial Soumba seed lot (C. Soumba) and for non-Soumba varieties. Parameters for Soumba samples were calculated on the basis of total samples, after excluding identified off-type plants in the Soumba panicle samples (considering only typical Soumba plants, TSP).

Samples	Total sample[a]							TSP sample[a]				
	N	H_e	H_o	A^t	R_s	G	F_{IS}	N	H_e	A^t	G	F_{IS}
Soumba M_6	30	0.076	0.033	26	2.14	0.20	0.56	28	0.041	20	0.14	0.86
Soumba M_2	30	0.186	0.081	31	2.55	0.30	0.57	24	0.098	24	0.13	0.68
Soumba M_7	30	0.089	0.031	26	2.14	0.30	0.66	26	0.029	17	0.19	0.67
Soumba M_1	30	0.257	0.079	34	2.80	0.40	0.69	24	0.127	27	0.25	0.83
Soumba W_1	30	0.252	0.061	41	3.57	0.27	0.76	24	0.014	14	0.08	1
C. Soumba	30[c]	0.006	0	13	1.08	0.03	1.00	–[b]	–	–	–	–
Niantjitiaman	29	0.127	0.026	26	2.15	0.34	0.80	–	–	–	–	–
Niogomé	30	0.274	0.143	31	2.54	0.93	0.48	–	–	–	–	–
Bobojé	30	0.324	0.189	31	2.57	0.96	0.42	–	–	–	–	–
Algerie	30	0.200	0.075	35	2.84	0.50	0.63	–	–	–	–	–
Bamtouklabé	30	0.342	0.175	38	3.12	0.96	0.49	–	–	–	–	–

[a] N: total number of plants genotyped; H_e and H_o: unbiased gene diversity and observed heterozygosity; A^t: total number of alleles; R_s: average allelic richness in each sample or seed lot (estimated for 27 individuals according to the rarefaction method); G: genotype richness; F_{IS}: fixation index.
[b] Sample not available for analysis.
[c] Commercial seed lot of Soumba.

3.4. Genetic diversity and structure of Soumba and non-Soumba samples

All 12 SSR markers were polymorphic, revealing a total of 65 alleles across the Soumba and non-Soumba samples that were collected. Both the Soumba and non-Soumba samples displayed variation for the genetic parameters. The lowest intra-varietal gene diversity value (<0.01) was found in the commercial Soumba seed lot, as expected, followed by Soumba samples M_6 and M_7 and the improved variety Niantjitiaman (Table 3). The two Soumba samples of M_1 and W_1 demonstrated the highest gene diversity (0.25), which were not significantly different to the four guinea varieties (H_e from 0.20 to 0.34), including the local landraces, such as Algerie and Bamtouklabé. A similar allelic richness was found in both Soumba and non-Soumba samples, with most pairs not displaying significant differences (4 out of the 25 pairs showed significant differences). Among the Soumba samples, the highest allelic richness was detected in W_1 (P-values from 0.003 to 0.007). Three non-Soumba samples (Niogomé, Bobojé and Bamtouklabé) had higher observed heterozygosity (H_o from 0.14 to 0.18) and genotype richness ($G > 0.90$) compared to the Soumba (H_o: 0.03–0.08; G: 0.20–0.40).

The Soumba samples displayed non-significant and low genetic differentiation at the threshold of 5% for P-values obtained after Bonferonni correction; pairwise F_{ST} estimates ranging from 0 to 0.05 (data not shown). A significant but low differentiation ($F_{ST} = 0.11$) between the commercial Soumba seed lot representing pure, certified Soumba seed and the M_1 farmer-recycled Soumba samples was found. A strong differentiation was detected between non-Soumba and Soumba samples (pairwise F_{ST} from 0.63 to 0.85) and among the former (pairwise F_{ST} from 0.21 to 0.81). However, the differentiation between Soumba and non-Soumba samples could be underestimated, as we left aside markers that did not reveal polymorphism between them. Genetic diversity parameters were markedly lower for the different Soumba samples (after excluding off-types plants) than for the non-Soumba samples (Table 3). In the TSP Soumba samples, F_{IS} values, especially for W_1 increased while total number of alleles, values for gene diversity and genotype richness decreased further (Table 3).

Axes 1 and 2 of the PCoA analysis account for 59% of total variability (Fig. S2). The two axes separate three clusters of individuals: Niantjitiaman individuals, individuals belonging to the Soumba samples and the four guinea non-Soumba samples. Two Soumba plants of the W_1 sample were very close to the non-Soumba group (at the right of the plane). Altogether six plants from Soumba samples W_1, M_1 and M_2 were intermediate between the Soumba and non-Soumba group, and two plants from non-Soumba samples (Algerie and Niantjitiaman) were close to the Soumba group.

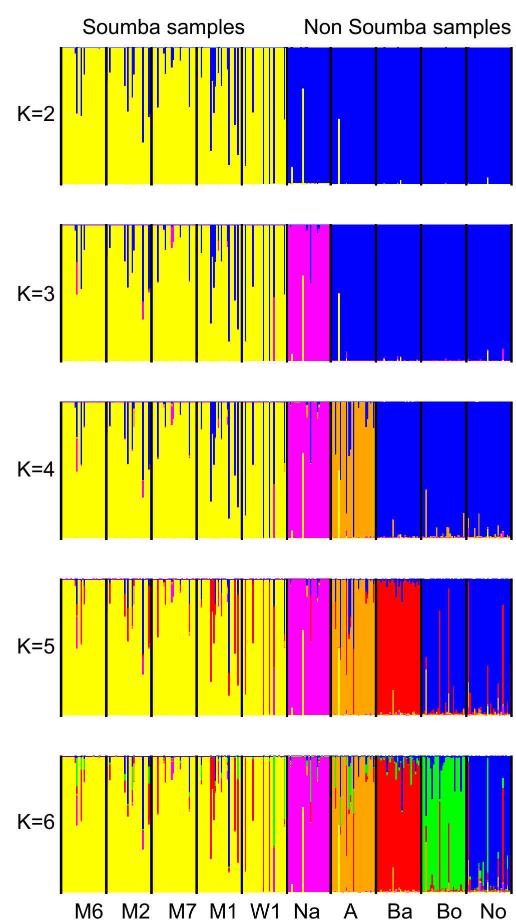

Fig. 5. Sequential identification of the genetic clusters through the Bayesian model implemented in STRUCTURE analysis within the whole data set including Soumba and non-Soumba samples (299 individual plants). The highest posterior probability of data for each K value is presented. The genome of each individual is represented by a thin vertical line, which is partitioned into K colored fragments that represent the admixture coefficient (Q_i), i.e., the estimated proportions of membership of its genome in each of the K clusters. Bold, vertical lines in black are separating individuals of different samples.

The Bayesian model-based clustering method, implemented in STRUCTURE, did not allow us to identify without ambiguity the putative number of clusters (K). The log likelihoods increased steadily until $K=5$, ΔK was maximum for $K=2$ and the best congruence between runs was observed at $K=3$ (18 of the 20 runs gave similar memberships in the three defined groups). Different clustering results across runs were observed, typical of a real multimodality (Rosenberg et al., 2002). For example, at $K=2$, 12 runs showing the highest probability of data clearly discriminated Soumba from non-Soumba samples. The other runs clustered together most Niantjitiaman and Soumba samples and assigned the remaining guinea samples to the second cluster. We thus presented in Fig. 5 the results obtained for the different K values (from 1 to 6), considering the run with the highest probability of data. At $K=2$, STRUCTURE indicated a clear differentiation between Soumba and non-Soumba samples, with two Soumba W_1 plants (previously detected by the PCoA) having admixture coefficients (Q_i) exceeding 90% in the non-Soumba cluster. At higher K values, most Soumba plants (116 among the 150) were consistently recognized as belonging to their cluster ($Q_i > 90\%$ in the Soumba cluster). Concomitantly (at once), the other varieties were iteratively distinguished: the improved, newly introduced variety Niantjitiaman (at $K=3$), followed by the traditional varieties Algerie (at $K=4$), Bamtouklabé (at $K=5$) and finally, at $K=6$, the remaining varieties were recognized.

When considering six clusters, 25% of the plants (74 plants of which 32 were Soumba) showed admixture (at the admixture threshold of 90%) and two Soumba W_1 plants were attributed to the Bamtouklabé cluster. In total 23% of Soumba plants showed admixtures with, or attribution to, non-Soumba samples. They mostly belonged to the M_1, M_2 and W_1 samples. Soumba M_6 and M_7 were less admixed with non-Soumba varieties. Soumba plants seemed to be admixed by Bamtouklabé, Niogomé and Bobojé, depending on the sample or on the different plants within a sample. Four plants in each one of the W_1 and M_1 samples showed high Q_i in the Bamtouklabé cluster (>40%). For the non-Soumba plants, admixture was essentially between non-Soumba varieties. However, Niantjitiaman and Algerie showed some admixture with Soumba samples (mainly due to one plant in each case, already revealed by the PCoA).

4. Discussion

4.1. The need for integration of improved varieties in the traditional system

The successful integration of improved varieties in the village of Magnambougou is the result of a participatory and decentralized breeding approach that exposed farmers to a new varietal diversity and an initiated seed system development (Weltzien et al., 2007). Recent upheavals in the agricultural system, such as declining cotton production, expanding areas of cereal cultivation and commercialization of traditional cereals (sorghum and millet), have undoubtedly contributed to the adoption of these improved varieties. Yet it was their varietal properties that corresponded to the newly emerging needs: high fodder quality, good market quality of grains and integration into the farming itinerary (Falconnier, 2009). This recent development adds to a global transformation that can be observed in West African family farming systems as this population segment attempts to adapt to evolving political, environmental and economic challenges (Toulmin and Guèye, 2003). The adoption of Soumba, which was facilitated by a production–marketing project that specifically promoted this variety between 2007 and 2008 (Baquedano et al., 2010), shows that better yield and market advantages can be a driving force behind varietal changes, as has been asserted by Perales et al. (2003) or Manu-Aduening et al. (2006). Altogether, three improved varieties were added to the farmers' portfolio in the study area between 2003 and 2008. The increasing area cultivated with newly introduced and improved varieties in Magnambougou confirms the need for new varietal options. Local landraces alone, apparently, are not satisfying the evolving needs of farmers in this region. In Ethiopia, Mekbib (2012) describes the introduction of new varietal diversity as genetic enrichment (opposed to varietal erosion) as an effort to adjust to evolving production systems and climate variability. In the present study, the introduction of improved varieties in Mali not only promotes varietal diversity, but also new racial diversity, as Soumba is a caudatum–guinea intermediate. Increased racial diversity can contribute new plant traits that might correspond to farmers' needs. Farmers favor the fodder traits—shorter height, seemingly better stem/leaf ratio, stay-green trait, less lignification—brought in by the caudatum parent of the Soumba variety compared to the tall and woody guinea varieties. Differences due to racial affiliation for biomass quality have been confirmed by Trouche et al. (2014). Availability of livestock feed is becoming an issue in this region due to declining cotton production (cotton seed was used as fodder) and decreasing natural grazing grounds (Falconnier, 2009).

4.2. New formal seed system that overlaps with the traditional system

Similar to descriptions of Louwaars and de Boef (2012) concerning integrated seed system development in Sub-Saharan Africa, the seed system of Dioïla presents a complex yet more or less successful integration of formal and informal seed system components characterized by dynamic interaction between the farmers, the ULPC seed cooperative and the breeders. Nevertheless, the purely informal system of exchanging farmer-recycled seeds remains an important driver for the dissemination and production of new varieties. In Magnambougou, one could say that the two systems are delicately balanced, if not complementary: the farmer can replace his seed stock with commercially certified Soumba seed from the ULPC or he can directly exchange for Soumba seeds via traditional channels, within the village or with neighboring villages. This complementarity allows farmers to respect their cultural obligations (i.e., giving seed presents) or to access Soumba seed at short notice (on account of unforeseen difficulties). This flexibility was demonstrated by various farmer households, including M_4, which not only procured commercial seed but also exchanged seed for re-sowing; by farmer M_1, who produced his own seeds but replaced his seed stock with commercial seeds when too many off-types were observed; or by W_1, who formally produced Soumba seeds but kept his own recycled seeds for household use and for the purpose of gift giving. In short, the coexistence and overlapping components of the formal and informal seed systems leads to stable and secure availability of seed, and thus improved food security. This is supported by Almekinders et al. (2007) who concluded that livelihoods can be improved by promoting the integration of formal and farmer-based seed systems (enhancing farmer awareness), building up trust in the quality of improved varieties, and simply increasing the diffusion of new varieties.

4.3. Varietal integrity and gene flow

Considering the reproductive system of sorghum and the fact that sorghum landraces vary greatly in their mating system within a single year (Barnaud et al., 2008), it is not surprising that the integrity of improved varieties cultivated under village conditions in non-isolated fields (less than 100 m distance) can be quickly compromised (Rabbi et al., 2010; Warburton et al., 2010). Apart from W_1, Soumba fields for food grain production are not spatially isolated from other sorghum varieties in Magnambougou. Furthermore, temporal isolation from neighboring varieties (mainly guinea types) would only be effective if Soumba was sown early (June); in which case, Soumba would flower at the beginning of September while the anthesis of the photoperiod-sensitive traditional guinea varieties would occur towards the end of September (Kouressy et al., 2008). Admixture revealed by SSRs in Soumba samples indicated that spatial and temporal isolation was not effective for all the samples. We found that the F_{IS}-values in most of the local guinea varieties were lower than that in the Soumba samples and consequently that derived outcrossing rates were higher. These results are in line with the findings of other authors, who estimated that outcrossing rates for guinea type varieties can amount to 30% (Ollitrault et al., 1997; Barnaud et al., 2008). Nevertheless, the outcrossing rates estimates in the total Soumba samples ranged between 14 and 28%, and declined quite considerably for M_1, M_6 and W_1 after off-types were excluded. Progenies of off-types which did show admixtures with other varieties were found to be more heterozygous than typical Soumba plants (data not shown). This could hint at a higher outcrossing rate in the off-types plants.

In order to gain a clearer picture of the suspected seed contamination (unwanted pollination, as defined by Morris et al., 1999) in the Soumba crops, the present study combined observations of phenotypic traits (in farmers' Soumba seed lots, farmers' Soumba fields and in progenies derived from plants collected in Soumba fields) with genetic analysis of the Soumba progenies. Seven traits related to morphology and phenology (plant, glume and grain color, plant and panicle length, panicle form and heading date) were used to distinguish between the true-to-type plants and off-type plants. The traits correspond to farmers' criteria for identifying off-type plants. SSR markers confirmed the identified off-type plants at a molecular level: all but one progeny (M_7) derived from off-type plants revealed admixture and could not be assigned to the Soumba cluster. Growing the same progenies in a nursery also allowed us to verify introgression events not "visible" to farmers during plant selection, albeit only in the subsequent generation e.g., for trait introgressions that are masked by dominance in F1. This underscores the need for combining observations of the maternal plants grown in the fields, and of their progenies, with both SSR markers and phenotypic traits in order to better assess varietal purity of a farmer's seed stock.

The incidence of contamination during seed recycling carried out by farmers can be inferred from the increased genetic diversity in Soumba seed samples compared to the commercial seed. The SSR markers confirmed gene flow between Soumba and other varieties: off-type plants in recycled Soumba mainly contained admixture with frequently cultivated varieties, such as Bamtouklabé or other varieties introduced through participatory variety testing in the village (Bobojé and Niogomé). The genetic analysis showed a tendency of gene flow from the guinea type varieties (such as Bamtouklabé, Bobojé and Niogomé) towards the Soumba plants. This may have been favored by the tall plant architecture of these varieties (up to 2 m higher than the Soumba variety) and by their higher occurrence in the village (for Bamtouklabé). This is in line with Barnaud et al. (2008) who described stronger pollen flow from the tall and frequent guinea type varieties towards rarer landraces.

If not eliminated before flowering, wild-weedy sorghum plants can be an additional source of contamination (Sagnard et al., 2011). Seeds for these plants is often disseminated in the fields via animal manure and it can survive for several years as seed banks in the soil (Jacques et al., 1974; Adugna, 2013). Farmers indicated during interviews that they generally eliminate weedy types before harvest; however, our observations suggested the presence of off-types resembling wild-weedy sorghums at the maturity stage, especially in M_7 and W_1 Soumba fields. Even if farmers actively select against weedy types, the resemblance of the latter with cultivated sorghums before maturity prevents their complete elimination. Barnaud et al. (2009) reported bidirectional gene flow between cultivated and wild-weedy sorghums in a traditional farming system in Cameroun dominated by the guinea sorghums and intermediates of this race. The resemblance of some off-type plants to wild sorghums in the progeny nursery supports potential gene flow from wild-weedy forms to the Soumba variety in the present study.

Gene flow due to proximity to neighboring sorghum fields in 2008 was found to be rare. This was indicated by the lowest admixture detected by SSR markers for the non-recycled M_6 sample, which was grown close to fields occupied by the Algerie and Niogomé varieties. Flowering periods of these varieties may not have coincided enough to cause gene flow, especially for the late and photoperiodic Niogomé variety. It is also possible that the detection of gene flow with SSR markers was not maximized on account of the limited number of plants analyzed (1 grain/progeny) and the strategy employed for the genetic sampling, where one-third of the panicles were collected in the centre of the fields (far enough away from other varieties in adjacent fields to prevent gene flow).

In a review focused on farmer maize recycling, Morris et al. (1999) suggested that unintentional mixing of grain or seed of different varieties during seed selection conditioning or storage is a potential source of genetic change. We could not exclude the possibility of unintentional mixing during seed storage, however, seldom it was for Soumba samples. One such example could be found within the W_1 sample, which was characterized by small Q_i in the Soumba cluster (<5%) and homozygosity at all loci. Phenotypic observations of the red glume color of this particular plant underlined a strong association to the Bamtouklabé variety (Q_i > 95% in the Bamtouklabé cluster) revealed by the STRUCTURE analysis. It should be noted that the increase in intra-varietal diversity through gene flow and/or contamination during seed recycling can in turn enhance population buffering in unpredictably and variable environments, as stated by Haussmann et al. (2012), and would thus contribute to yield stability in farmers' seed stocks.

4.4. Farmers' strategies for maintaining varietal purity and agronomic performance

It is generally acknowledged that farmers' selection practices are an effective means for maintaining the phenotype of their local varieties. These practices lead to morphological distinction regardless of the high genetic intra-varietal diversity (Barnaud et al., 2008; Rabbi et al., 2010). On the other hand, Siart (2008) demonstrated that the morphological identity of a variety can be somewhat dynamic, as farmers do not always consider panicles with different glume or grain color (different shades of white) as a separate variety. This was revealed by this author in the Dioïla district of Mali through panicle selection exercises. Siart demonstrated further that panicle selection in this region is only carried out to a small extent. This phenomenon, however, could not be confirmed for the Soumba variety in the present study, where the majority of farmers are using panicle selection. Variations in trait expressions may be perceived more strongly and to be more of a hindrance for the market value of the grain, for example, due to the homogenous and strongly different phenotype of Soumba compared to the more heterogeneous guinea varieties. This situation may persuade farmers to turn to the more time consuming panicle selection method. The analyzed Soumba samples showed that, irrespective of obvious signs of gene flow and seed contamination, diversity parameters generally stay clearly below those of most of the local and introduced guinea landraces, with the exception of Algerie. The relatively limited genetic diversity found in the early maturing landrace Algerie may be the result of temporal isolation from other varieties and its low frequency in the village. The fact that Soumba samples remain significantly distinct from other varieties grown in the village (F_{ST} on average 0.74) also points to the effectiveness of farmer strategies.

The applied practices ranged from replacing seed with commercial seed to the selection of panicles. The effectiveness of these methods in maintaining the identity of Soumba depends largely on farmers' resources (availability of isolated fields), skills (training in formal seed production, as was the case for W_1) and time (removing off-type plants, performing panicle selection). Seed recycling from food grain over the course of more than three years increased genetic diversity more than other methods. The relation was less obvious for the observed frequencies of off-types. Besides the five years-recycled M_7 and M_1 seed, the M_2 Soumba seed, recycled for only two years, also tended to show increased number of off-types. The high presence of off-types resulted in the rejection of the seed lot for further use by the farmer (M_1).

The high allelic richness detected in the even "older" W_1 sample was mainly due to a few extreme off-types, that is, wild types or plants strongly associated to the Bamtouklabé variety. The recycling practices applied by the W_1 farmer ensure that these off-type plants are "easily" eliminated. However, the isolated fields used by W_1 together with temporal isolation of the detected off-types most likely limited gene flow. Diversity values similar to the commercial seed lot, after excluding off-type plants from the analysis, validate this scenario. Low genetic diversity levels after exclusion of off-types are likewise found in the M_7 sample, which was also recycled via panicle selection for more than three years. In this case, genetic diversity was similar to that of the M_6 sample, which represents only the first year of production. Practicing regular panicle selection thus seems to be more effective against the relatively rapid increase in heterogeneity of the Soumba variety rather than the elimination of off-types alone.

Still, one particular sample (M_2) recycled through panicle selection over two consecutive years showed similarly high percentages of off-type plants and genetic diversity values to that of the M_1 sample. This may be attributed to the farmers' broader selection criteria concerning varietal purity i.e., M_2 took into consideration panicles with preferred characteristics deviating from Soumba. Given the relatively high total plot grain yield in the agronomic trial for the M_2 seed lot, the farmer might focus his selection on heterozygous plants with a probable heterosis advantage. Farmers' selection in favor of heterozygotes has been previously suggested by Ollitrault et al. (1997) when analyzing evolution of intra-population diversity in sorghum landraces in Burkina Faso. The relatively low yield performance of the M_7 seed lot compared to the M_2 seed lot underlines the diversity of farmers' strategies and objectives when selecting for the most preferred panicles.

With the exception of M_7, recycling Soumba over a number of years using different practices in the seed lots did not negatively affect grain yield performance relative to commercial seed. This is in line with the review of Morris et al. (1999) where recycled versions of the improved maize OPV "Azam" in Pakistan did not differ from their commercial version when tested in on-station trials. However, the authors reported that the recycled versions were out-yielded when tested in on-farm trials, probably due to less favorable management in on-farm trials which increased seed-related performance differences. In our study, a tendency of more favorable yield compared to the formal controls (commercial and breeder seed) was measured in three recycled seed lots, notably M_2, M_4, and MS_1. Whereas a positive trend in yield performance in M_2 may have been influenced by a favorable contribution of off-type plants, off-types were absent and/or low-yielding in M_4 and MS_1. The higher yield performance of the MS_1 seed lot may have been influenced by the seed lot sample, which was a limited number of panicles permitting easier control of the grain seed quality compared to bulk samples for the other seed lots. According to Morris et al. (1999) no correlation was detected between the number of recycling years of the maize OPV Azam and the performance of traits of interest such as grain yield and plant height. This is confirmed by our findings where the most recycled seed lots (M_1 and MS_1) display similar or higher performance than the commercial seed lots.

5. Conclusions

Formal seed systems in Sub-Saharan West Africa are not meeting demand for seed of new improved varieties. It is this reality that has led to the informal systems reinforcing the diffusion of improved varieties parallel to formal initiatives. Regarding the significance of this informal seed access, and farmers' preference for the typical Soumba characteristics and properties within the present study, the success of farmers' seed selection strategies are crucial to the preservation of varietal seed quality and integrity. The present study attempted to measure the effect of these strategies by combining phenotypic observations and assessment of off-type plants with the analysis of genetic diversity via SSR markers.

Results indicate that seed recycling affects genetic diversity and number of off-type plants in the improved variety. Recycling seed from food grain as well as farmers' individual selection criteria can especially contribute to an increase in off-type plants and diversity. The number of recycling years alone was not perceived as a major effect. Other important factors that can increase seed contamination include the use of animal manure, regrowth of other varieties, and possible impurities in commercial seed lots.

Introgression through gene flow and field contamination are best minimized when (1) farmers have received specific training in seed production, (2) they can take advantage of isolated fields and (3) they practice true-to-type panicle selection. Farmers who took part in this study were generally able to maintain the phenotype of their Soumba variety while at the same time genetically enriching their seed stock and bringing about a "more heterogeneous version" of the Soumba variety. They created seed stocks that sustained, or even improved, yield performance compared to commercial seed. In view of the frequency of off-type plants at maturity, however, farmers' recycled seeds does not meet the strict requirements for certified seed production in a formal system. Yet if the frequency of off-types becomes too high, farmers do not hesitate in resorting to commercial seed. Thus, the two systems complement each other in the dissemination of improved varieties. Creating awareness of the effects of recycling and seed management practices, especially for farmers with key roles in the traditional system of seed exchange, could help to increase local seed system security and enable farmers with limited access to the formal system to benefit from improved variety seed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2014.06.021.

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