GENOTYPIC DIVERSITY STUDY OF ADAPTED COTTON CULTIVARS AND FERAL POPULATIONS COLLECTED IN BURKINA FASO

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<u>Abstract</u>

For further enhancement of cultivated cotton varieties, a total of 358 traditional cotton accessions conserved by farmers were collected across Burkina Faso as genetic resources. Based on growth habit and phenotypic observations, the collection includes at least 3 known cultivated species i.e. *G. arboreum*, *G. barbadense* and *G. hirsutum*.

The genetic diversity and population structure of a core set of 121 accessions plus 3 cultivated varieties were assessed by SSR markers, 49 % polymorphic with an average PIC at 0.50 and revealing 2 to 12 alleles by SSR. Accessions clearly clustered into 3 groups corresponding to the 3 species (on the basis of the species and the ploidy level). Within the G. *hirsutum* group, perennials and annuals were clearly separated. Every group or sub-group was found to be highly diversified with intra-group dissimilarity at 0.24, 0.25, 0.20 and 0.26 respectively in the G. *arboreum* group, G. *hirsutum* perennial, G. *hirsutum* annual and G. *barbadense*. The *arboreum* group was found to be more diversified with diversity parameters established at 2.46 for allelic richness, 0.31 and 0.25 respectively for expected and observed heterozygosity.

The persistence of those anciently cultivated cotton species and their maintenance by villagers is remarkable and fairly unique. This genetic resource could possess promising attributes related to environmental adaptation or to traditional uses and it needs to be properly characterized and preserved either *in situ* or *ex situ*.

Introduction

Africa is an ancient home of cotton, especially for diploids species of *Gossypium* L. After the independence from colonial domination, industrial cotton cultivation became one of the success stories in many new African countries, with reference to GPD being raised and foreign exchange earnings as a main source, and offering benefits to around 16 million people. Producers remain essentially small holders growing in rainfed conditions with a low to medium level of technical intensification, best adapted varieties, agronomic practices and on-farm pest management are needed to continue to draw benefits from cotton. On the varietal level, the success was favored by genetic resource availability and significant adaptation research efforts by the African cotton breeder network under the leadership of the Research Institute on Cotton and Exotic Textiles (IRCT). Nowadays, after IRCT stopped its activities in Africa, the breeding gene pool has become aged and increasingly degenerated to face the current breeding challenges while the alternative of CGM is facing prejudice and controversy. It was suggested to take into account the local germplasm of traditional cottons still conserved by some populations as relics for pharmaceutical uses, ritual practices and more rarely for textile uses (Bourgou and Sanfo, 2012). The objectives were to collect local cotton germplasm, to estimate the extent of genetic diversity of the collected cottons and to determinate the opportunity to conserve or valuate them in feeding the breeding gene pool or further breeding/enhancing quality of African cultivated cotton varieties.

Methods

Collecting surveys covered the 13 administrative regions of Burkina Faso, and visited more than 500 villages with a wide rainfall range from 300 to 1200 mm. This geographic distribution goes beyond the regions of commercial cotton production as recommended by the cotton companies, especially for the samples from those most northerly and drier regions (Bourgou et al., 2014).

121 of the 124 plant materials analyzed are part of the 358 accessions collected across Burkina Faso between 2009 and 2012 while 3 are cultivated varieties from Togo, Chad, Burkina Faso. DNA extraction was done from seedling leaves at CIRAD (France) in 2014 by MATAB method (Coppens d'Eeckenbrugge and Lacape, 2014). 72 SSR markers were used, amplifying DNA from both the diploid A and the tetraploid AD genomes and showing an amplification of a single PCR product (Lacape et al. 2007). SSRs were genotyped in multiplex panels of 8 SSRs and PCR products were denaturated and size fractionated using capillary electrophoresis on an ABI 3500 Genetic Analyzer (Applied Biosystems) and GeneMapper 4.1 (Applied Biosystems) software was used for allele size estimation.

The software Genetix 4.04 (Belhkir et al. 2004) was used to determine basic statistics, such as number of alleles, observed (Ho) and expected heterozygosity (He) across loci and populations and the FSTAT 2.9.3 statistical package (Goudet 1995) to calculate allelic richness (Rs). The data matrix of bi-allelic codings for the 35 SSRs and 124 genotypes was imported into the DARwin 6.0.012 software (Perrier and Jaquemoud Collet 2006) to calculate genetic dissimilarities using the Sokal and Michener index. Additionally with DARwin package, unweighted trees without topological constraints were constructed using a neighbor joining (NJ) approach (Saitou and Nei 1987) to represent individual relations.

Results

The prospection/collection assembled over 350 accessions representing a wide range of diversity within the genus Gossypium, including *G. arboreum* and *G. herbaceum* (represented by a single specimen) as diploid species, and *G. barbadense* and *G. hirsutum* as tetraploid species. The geographical repartition within Burkina Faso showed that the species were frequently overlapping in their distributions as indicated the 121 accessions SSR-analyzed and represented in this map (Fig.1).

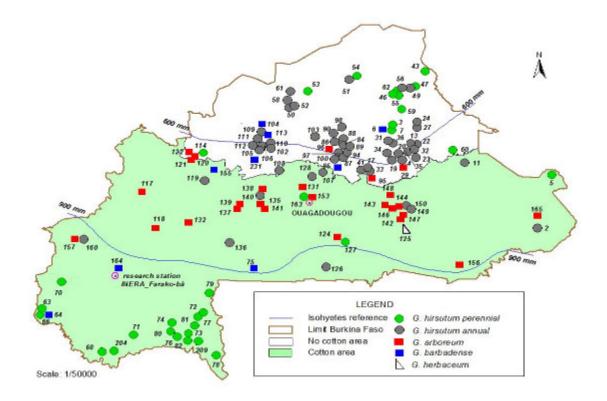


Figure 1: Geographic localization of the 121 accessions collected in Burkina Faso and SSR-based analysis. Species and genetic groups are indicated by the symbols and colors.

The results of SSR-based analysis of 121 collected accessions plus 3 cultivated varieties showed that 35 SSRs were polymorphic, with an average PIC of 0.50, revealing 2 to 12 alleles by SSR. The results after F_{ST} analysis confirmed that the marker-based genetic organization was strongly attributable to the species and annual/perennial status (Table 1).

Factor	No	At	А	Ass	Rs	He	Ho	F _{ST}
All collection	121	183	5.22			0.5	0.13	
Species/group								0,61 **
G. arboreum	25	108	3.09	37	2.40	0.32	0.26	
G. barbadense	11	81	2.31	13	2.24	0.25	0.12	
G. hirsutum annuals	52	78	2.23	14	1.64	0.19	0.09	
G. hirsutum perennials	32	123	3.51	18	2.27	0.28	0.12	
Cultivated	3	49	1.40	-	-	0.21	0.00	
Climatic regions								0,15 NS
< 600 mm/an	48	129	3.68	9	3.12	0.33	0.10	
600-900 mm/an	51	154	4.40	30	3.86	0.56	0.17	
> 900 mm/an	22	132	3.77	17	3.73	0.42	0.09	

 Table 1: Genetic diversity and differentiation of accessions according to species/group and to climatic (rainfall) conditions.

The resulting data matrix of dissimilarities was used to construct an overall distance-based rooted neighbor-joining tree. The studied material clearly clustered into 3 groups on the basis of the species. So, 25 were clustered as G.

arboreum accessions, 11 as G. *barbadense* accessions and the reminder as G. *hirsutum* accessions. The group of *hirsutum* clearly subdivided in 2 sub-groups, one of accessions with perennial tendency and the other of accessions with annual tendency including the cultivated varieties (figure 2).

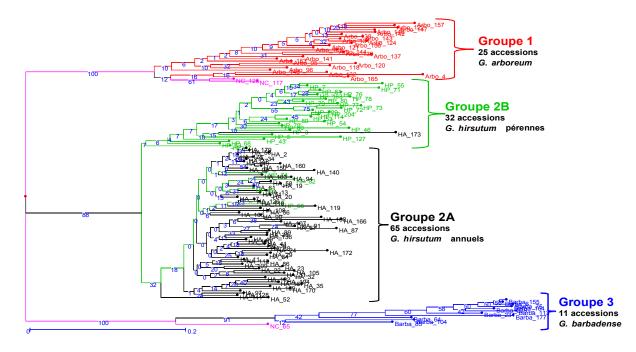


Figure 2. The 124 accessions obtained from Sokal and Michener distance matrix based on 35 SSR. Groups are different colored, *G. arboreum* in red, *G. barbadense* in blue, *G. hirsutum*-annuals in black and *G. hirsutum*-perennial in green.

<u>Summary</u>

It is assembled in Burkina Faso more than 350 locally-collected cottons including both feral populations and obsoletes cultivars. This collection is more important and better analyzed compared to previous ones in Africa, from Senegal (1988) and Cameroon (1991). The results by SSR-based analysis support that the collection is a valuable source of genetic diversity to be used in feeding local degenerated breeding gene pool, to enhance C4 countries cultivated varieties or to breed news ones. More accurate evaluations are ongoing or need to be initiated to determine the real potential of recognizably unique features – such as local adaptation – of the collection; for this purpose, we are looking for comparative similar collections and for collaborative interests in genetic resources evaluation and valuation. Right now, important efforts are taking to conserve the collection *ex situ* in Burkina Faso.

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