Bt cotton seed purity in Burkina Faso: Status and lessons learnt

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Research

Keywords: Conventional cotton, organic cotton, GM cotton, seed production scheme, seed control

Posted Date: July 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-44703/v1

Version of Record: A version of this preprint was published on November 11th, 2020. See the published version at https://doi.org/10.1186/s42397-020-00070-4.

Abstract

Background

Since the commercial release of Bt cotton the issue of seed purity in producers' fields has been little addressed and in an unbalanced way when it was. It is well documented that the loss of purity in conventional seeds has endangered the continuation of organic cotton production. However, studies are rare on the purity of Bt-cotton seeds despite its implications on the effectiveness and sustainability of their use.

This paper compensates for the mentioned lack of literature by analyzing data collected in 2015 in Burkina Faso, namely results of ELISA tests on samples of seeds from 646 fields grown with conventional or Bt varieties.

Results

According to the conservative criteria retained to declare the presence of Bt gene (more than 10% and 90% of controlled seeds for conventional and Bt variety, respectively), seed purity was very questionable for both types of varieties. For the conventional variety, the presence of Bt gene was observed on 63.6 and 59.3% of samples for Cry1Ac and Cry2Ab, respectively. Only 29.3% of samples corresponded to pure conventional seeds while 52.2% were double Bt seeds. Conversely, for the Bt variety, the presence of Bt gene was observed on 59.6 and 53.6% of samples for Cry1Ac and Cry2Ab, respectively. Actually BG2 seeds with both Bt genes were found in 40.4% of samples against 27.2% of samples of actually conventional seeds while the remaining of 32.4% of samples corresponded to single Bt gene seeds.

Two factors affected the severe lack of seed purity. As regard to conventional seeds, it clearly resulted from a phenomenon of contamination, indicative of a failure in adjusting the seed production scheme to the use of Bt-cotton. With regard to the Bt variety, the lack of purity of the original seeds provided to Burkina Faso accounted and should even be the major factor.

The observed lack of seed purity is a threat to the initiative of organic cotton production, albeit a very minor production mode in the country. It also calls upon the effectiveness and furthermore the sustainability of Bt cotton to control target pests.

Conclusion

Our results show the extent of purity loss when no especial attention is paid to the preservation of seed purity. Pure conventional seeds could totally vanish while Bt seeds become a combination of seeds of various types encompassing or not the expected Bt genes.

Any country willing to embark the use of Bt cotton, or to resume this use like Burkina Faso, must previously adjust its seed production scheme and enforce its operation. This is a condition to preserve pure seeds both to enable the launch or the continuation of identity-cotton production and to ensure a sustainable effectiveness of Bt-cotton. The mentioned condition implies that seed purity must be checked and the related information shared.

Background

Since the commercial release of genetically modified cotton, especially Bt cotton made resistant to some damaging insects, the issue of seed purity has been little addressed and mainly from the perspective of non-GM fields. Studies pointing out the loss of purity of conventional seeds are related to the concern of producers of identity-cotton, namely organic cotton, for not being able to meet the requirement of absence of GM at the imposed threshold (Cederholm 2014). In the USA, the coexistence of GM and non-GM crops is perceived as an illusion because the contamination by GM crops is thought to be impossible to escape (Food Decmocracy Now! 2014), so the continuation of organic cotton production is threatened. In Europe, like in France, coexistence is found to be possible (Vinck 2003), at least on theory as GM crops are little developed in this continent.

However, published studies dealing with the purity of GM, more precisely Bt-cotton seeds, are rare. Many research works have dealt with the expression of Bt genes in varieties, by measuring the concentration of Bt toxins. It was observed for instance variety effect or seed generation effect in India where F1 hybrids are released and where the F2 offprings might have been used by farmers (Singh et al. 2016). The issue of purity of Bt-cotton seeds was only specifically addressed in Pakistan, where discrepancies were found between farmers' declaration on the type of cotton they were growing and the real type checked through lab analysis of plant samples, in a context where only the single Cry1Ac Bt gene was encountered (Spielman et al. 2017). Even in works about the issue of coexistence, the rate of contamination of non-GM seeds by GM traits is addressed, from 0.1–5.0%, but the rate of GM purity of GM seeds is seldom mentioned just like if there is no issue about it. GM trait is not considered distinct from any other genetic trait for which the general genetic purity should be met, at a level generally established at 95.0% to 99.9% depending on the type of seeds in the USA or Europe. In China, the standard of GM purity rate is established at 90% (Seed world 2017), and 90–95% in India (Mohan and Sadananda 2019).

The lack of work on the seed purity of Bt-cotton varieties is regrettable because of the possible implications on the effectiveness and sustainability of Bt-cotton use. In case of insufficient seed purity, seeds used would pertain to in-pack seed mixture with debatable outcome. Sun et al. (2013) have shown that 20 to 50% share of conventional in seeds mixtures of Bt cotton led to poor if not very poor pest resistance effectiveness. Although the presence of conventional seeds in the mixture might procure kind of refuge within plot, it was documented that such type of refuge was less effective (Tabashnik 1994) and dominance of resistance was promoted (Brévault, Tabashnik, and Carrière 2015) while good stewardship is required for the success of its implementation (Mohan and Sadananda 2019).

The objective of this paper is to compensate for the mentioned lack to check how pure seeds were both for conventional and Bt varieties being used. It was based on the data collected in 2015 in Burkina Faso, at the eve of the decision to suspend the use of Bt cotton that had been legally released in the country in 2008 with BG2 varieties, e.g. stacking two Bt genes (Cry1Ac and Cry2Ab). The data corresponded to ELISA tests conducted to assess the presence of Cry1Ac and Cry2Ab genes in samples of seeds from 646 fields representing more or less equally the conventional variety (FK37) and the Bt variety (FK95 BG2, derived from the introgression of the two Bt genes into FK37). The knowledge gained with regard to the level of seed purity and influencing factors should be helpful to other developing countries willing to embark Bt-cotton use or even Burkina Faso in case of decision to resume such use.

Discussion

Literature lacks to confront our results about the purity of Bt-cotton seeds. There should be data resulting from control by seed production organizations but they are not disclosed. The reality of lack of seed purity is documented only in a few countries.

In Pakistan, the lack of seed purity for both conventional and Bt cotton has been assessed through a research work confronting farmers' declaration on the type of seeds they used and the biochemical control of plant leaves. It was found that only a single-Bt gene cotton was cultivated (Cry1Ac) and 11% of farmers believed they were cultivating Bt cotton while the Bt gene was not present, and 5% of farmers believed they were cultivating non-Bt cotton when, in fact, the Bt gene was present (Spielman et al 2017). The figures of discrepancies in our study are higher; the fact that a double Bt gene cotton was used should be an explaining factor, although the authenticity of the seeds supplied only by the cotton company should be better.

In China, through the measurement of Bt toxin contents, Pemsl, Waibel, and Gutierrez (2005) have suspected the lack of seed purity in Bt cotton varieties released in a very competitive context. Besides, in the particular case of hybrid varieties destined firstly for cultivation in more southern province of China, the lack of seed purity was also addressed indirectly through the assessment of Bt toxins (Xu et al. 2008). In both cases, the extent of purity imperfection was not estimated.

In Burkina Faso, the control of Bt nature of FK95 BG2 variety is implemented but data were not accessible. The only information available was obtained in an external initiative to check the Bt status of cotton plants in claimed Bt-cotton fields in this country. Out of the tests implemented on 45 samples, 24.4% of samples had no Bt status at all, 17.8% had a single Bt gene status equally distributed between the two Bt genes, and 57.8% had the double Bt gene status (Michel Fok et al. 2016). These figures are quite consistent with those in our present study that is based on a much higher number of samples.

More literature deal with the phenomenon of contamination of conventional seeds by Bt genes, but with much less, if any, quantitative assessment than in our study. The issue is more documented mainly because the phenomenon has endangered the continuation of organic cotton production, notably in the USA (Hershaw 2013) where it is claimed that no organic cotton producer could be meeting the purity criterion of conventional feature. The contamination status has become so much generalized and unescapable that Endres (2005) advocates a revision of federal and states laws governing seed purity. In India, in almost 30% of cases examined, conventional seeds supplied for refuge purpose contained Bt genes (S. Kranthi et al. 2017) although at non-specified extent. In Burkina Faso, a study mandated by promoters of organic cotton production pointed out that about 50% of organic cotton producers were provided with seeds containing Bt genes, however with the stringent criterion that Bt presence was declared when found on at least one out of 300 seeds and tests completed with lateral flow strips (Vognan and Bourgou 2014).

Our results clearly show that, at the eve of suspending the use of Bt-cotton, conventional seeds were contaminated at large extent. As we have retained rather conservative threshold of the presence of Bt genes in conventional seeds, the real situation of contamination was indeed worse than indicated in our figures.

The main reason of the observed contamination should be the lack of specific attention to prevent contamination when Bt-cotton was disseminated at large scale. No specific measures were implemented to delineate non-Bt cotton zone where conventional seed production could have taken place. In addition, one may suspect some arrangements between farmers in exchanging seeds, including in seed production area, so that some assumed conventional cotton fields were indeed Bt-cotton ones. The reverse case was also possible as there were farmers unwilling to grow Bt-cotton, particularly at the first years of the shift to this cotton type. Quality control with regard to the Bt traits in seed processing was quantitatively insufficient as it can be observed through the procedures of implementation of ELISA tests (Sofitex n.d.). In addition, these tests were conducted mainly to check the Bt nature and not the level of presence of Bt genes.

Since the suspension of Bt-cotton use in 2016, it is probable that the seed contamination of conventional cotton by Bt genes has not persisted at the quite high level found in our study, but it is hard to claim that Bt genes have totally disappeared from fields. After the suspension decision, seed control was implemented with measures that were more stringent. Burkina Faso also has shifted to using another conventional variety. This variety shift could nevertheless not be total in one campaign. There must remain some level of adventitious and unintentional presence of Bt genes in cotton fields. So, to some extent, cotton producers keep benefitting from some effectiveness of Bt genes to control targeted pests.

At the eve of the suspension of Bt cotton use, the GM nature of the Bt-cotton seeds could be acknowledged although it was not perfect. Our point is based on ELISA test indicating that Bt toxins were detected but not at the levels expected. The inference to the absence of Bt genes might be excessive

–because of various factors impacting on the expression of Bt genes (Huang et al. 2014; Iqbal et al. 2013; Rochester 2006; Wan et al. 2005)– but this argument little applies in our study as we observed this expression in falsely-assumed conventional seeds in the same growing conditions.

Our work is the first to quantify the loss of the BG2 status up to a poor level. The BG2 status (based on the double presence of Cry1Ac and Cry2Ab genes) was applicable only to 40% of the seeds supplied. Again, because of the conservative threshold retained for purity with regard to the presence of Bt genes in seeds, the real lack of BG2 status was even worse than indicated by our figures.

Two factors at least are beneath the observed lack of Bt purity in seeds. Because of the insufficient control of the BG2 status at the stage of seed production and processing, the lack of the adjustment of the seed production scheme is to blame. However, as the seeds for large scale release were provided by Monsanto in a very short delay (Bourgou et al., 2020; Fok, 2016), too short for stabilized and homogenous seeds (technically impossible to achieve in two years with at most four cycles), another factor of purity shortfalls dates back to the seeds originally supplied. The original lack of seed purity would have made ulterior quality control more difficult and costly, so this lack –not reported so far in other countries– could be regarded as the principal factor of the poor BG2 status of the Bt-cotton seeds.

The cultivation of Bt-cotton with defaulting purity of its seeds has worrisome implications with regard to the effectiveness and sustainability of the related Bt genes against target pests. A mixture of four cotton types –with regard to the presence of Bt genes (none, only Cry1Ac or Cry2Ab and both Bt genes)– implies disadvantages additional to those indicated in introduction and wipes out the rationale of having opted for stacked genes. The presence of single Bt gene has helped the emergence of resistance to single gene, hence facilitating the emergence of resistance to double Bt genes. It is likely that the process of resistance build-up against the two Bt genes had already started and that resistant alleles could already be encountered, at least at low frequencies. If so, the relaunch of Bt-cotton with the same genes in Burkina Faso would not be ensured of a lasting effectiveness, regardless of the risk of outbreak of secondary pests encountered in most (if not all) countries having adopted Bt-cotton long enough (Zhao, Ho, and Azadi 2011; B. K. Kranthi 2011; M. Fok 2010).

Conclusions

At the eve of suspending its Bt-cotton use, Burkina Faso has not escaped the phenomenon of conventional seeds purity loss and has suffered from a serious lack of seed purity of the disseminated Bt-cotton variety. Such a situation –which probably had prevailed several years before– has resulted from the defaulting adjustment of the seed production and distribution scheme upon the Bt-cotton release and the lack of purity in the original Bt seeds supplied to the country.

The case studied provides the lesson that any country willing to embark using Bt-cotton should ensure the purity of the Bt seeds and must adjust its scheme of seed production and distribution so as to preserve seed purity both for Bt and conventional varieties. Following the lesson learnt implies that seed purity must be regularly checked and the related data shared among the cotton sector stakeholders.

For Burkina Faso, the lesson learnt on checking seed purity and sharing the corresponding information also applies in case it resumes the use of Bt-cotton. In such a case, one might fear that the effectiveness of resuming the use of Bt cotton will be lower than expected because of the consequences of a period of seven years of imperfect seed production scheme. The comparative measured effectiveness will not be as strong as expected because conventional seeds are indeed already partially Bt ones. The effectiveness could also last less because the process of building up the pest resistance to the two Bt genes had probably been engaged before the Bt-cotton use was suspended.

Declarations

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not Applicable

Availability of data and materials

The datasets generated during and/or analysed during the current study are not publicly available due to their collation in the framework of a private study requested by the interprofessional body of the cotton sector in Burkina Faso (AICB) but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

The study was implemented on request of the interprofessional body of the cotton sector in Burkina Faso (AICB) with allocation of specific fund

Authors' contributions

LB conceives and supervises sample collection in fields, processes data of ELISA tests and co-write the manuscript ; EK supervises the implementation of ELISA tests, manages and processes the data obtained; MS conceives the study design and the sample collection in field; MF formats the results from the data processing of ELISA tests, conceives and write the manuscript. All have approved the manuscript before submission.

Acknowledgement

We are gratifull to DIANE K. Sidiki for helping in data handling and organisation. We also thank OUATTARA B. Marcel for helping to design and draw the map showing Sofitex cotton zone and sites sampled.

Methods

The data were collected in December 2015 in the framework of a study to access the gap in fiber quality between the conventional and Bt cotton varieties being cultivated at this period (Bourgou et al. 2020). The gap observed, at the expense of the Bt-cotton variety, has implied huge financial loss for the cotton companies (Michel Fok 2016) which led to decide in mid-2015 to suspend the Bt-cotton use (Dowd-Uribe and Schnurr 2016). The 2015 season was the last where both conventional and Bt, isogenic cotton varieties were grown in coexistence.

The conventional variety was known as FK37, developed by the national research in Burkina Faso while the transgenic variety was FK95 BG2 obtained via introgression of two Bt genes (Cry1Ac and Cry2Ab) implemented by Monsanto. It is worthwhile to note that FK37 was supplied for introgression in 2004, and seeds of FK95 BG2 BC2 were sent back in 2006 for demonstration of biological effectiveness before its release at large scale in 2008. The supplied seeds were obtained after at most four cycles (by cultivation in both northern and southern hemispheres), quite insufficient to claim for a stable and homogenous genetic material.

For the mentioned study, samples collection was conducted in districts over the whole intervention area of Sofitex, representing about 80% of total production (Figure 1). Three cotton producer groups (CPG) were randomly selected by district and then three producers by CPG. Within CPG, fields of producers growing both types of cotton were sampled preferentially; they were complemented by producers growing one of the two types. In 2015 season, most farmers planted Bt-cotton (62.53% of of the total 520,428 ha), consequently, the share of FK95 BG2 in sampled fields was higher (349 and 297 for FK95 BG2 and FK37, respectively).

In each field, a sample of one kilogram of seedcotton was collected randomly from 135 cotton plants across diagonal lines of the field. Three bolls were picked at the bottom, in the middle and on top of each plant. After ginning, eleven seeds were randomly isolated to undergo ELISA test and check the presence or not of Cry1Ac and Cry2Ab, according to the procedures followed at the seed quality control lab of Sofitex (Sofitex n.d.) and probably set up in line with Monsanto stewardship.

For each field sample, the presence of Bt genes was controlled for each of eleven seeds but the data we highlighted were the percentages of seeds being tested positive (e.g. 9.09% means one seed tested positive out of eleven). For each sample, separated figures were obtained for the presence of Cry1Ac and Cry2Ab.

In our study, we retained to declare that a so-called conventional cotton field was cultivated with seeds contaminated by a Cry gene when the corresponding sample showed an ELISA test result above 10% (or ELISA test was positive for more than one out of eleven seeds). Conversely, we opted to declare a so-called Bt-cotton field was cultivated with pure seeds with regard to a specific Cry gene when the ELISA result of the corresponding sample was above 90% (or test positive for at least 10 out of 11 seeds). In other words, we retained a maximum threshold of 10% of Cry gene presence to approve purity of conventional seeds and a minimum threshold of 90% for that of Bt cotton seeds. These thresholds are much higher than those commonly referred to in the USA or Europe, at 1.0-5.0% and 95.0-99.0%, respectively, and which are quite stringent and hard to achieve in practice. The thresholds we have retained are consistent with a conservative approach to shelter from false-positive or false-negative errors (Remund et al 2001). Our purpose is to show a status of loss of purity, which would be furthermore real when following a more stringent criterion of seed purity.

However, a field might be considered to have a Bt-cotton status although the seeds used were not pure as defined above. This is a situation seldom addressed as it is assumed that GM seeds being supplied are necessarily pure. For a first study taking into account the possibility of growing Bt cotton with seeds which are no pure, we have retained a minimal threshold of 46% of Bt gene presence in seeds (in our case, presence in at least 6 out of 11 seeds) to assume that supplied seeds were of Bt nature.

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Tables

Table 1: Presence of Bt genes in seeds from fields cultivated with conventional variety

	Cry1Ac	Cry2Ab	Cry1Ac + Cry2Ab
Number samples	297	297	297
Share of samples with presence* of the concerned Cry gene, %	63.6	59.3	52.2
Presence rate** of Cry1Ac, %			
Mean	41.5		43.1
Std. Deviation	23.2		22.5
Presence rate** of Cry2Ab, %			
Mean		39.3	40.3
Std. Deviation		23.2	23.3
* Presence was claimed when observed on at least one out of 11 seeds of each sample ** Rate of presence in samples where the presence was observed according to the retained criterion, calculated from the number of seeds with Bt gene detected out of eleven			

Table 2: Contamination status of conventional seeds in samples from conventional cotton fields (% of 297 samples)

Contamination* by Cry1Ac	Contamination* by Cry2Ab		Total
	No	Yes	
No	29.3%	7.1%	36.4%
Yes	11.4%	52.2%	63.6%
Total	40.7%	59.3%	100.0%
* Contamination was claimed when observed on at least one out of 11 seeds of each sample			

Table 3: Bt status of seeds in samples from fields of Bt cotton

	Cry1Ac	Cry2Ab	Cry1Ac + Cry2Ab
Number samples	349	349	349
Share of samples with presence* of the concerned Cry gene, %	95.7	94.3	92.6
Presence rate** of Cry1Ac, %			
Mean	88.1		88.8
Std. Deviation	11.9		11.2
Presence rate** of Cry2Ab, %			
Mean		86.5	86.6
Std. Deviation		12.2	12.1
* Presence is claimed when observed on no less than 6 seeds out of 11 of each sample ** Rate of presence in samples where the presence was observed according to the retained criterion, calculated from the number of seeds with Bt gene detected out of eleven			

Table 4: Purity status of Bt seeds in samples from Bt-cotton fields

	Cry1Ac	Cry2Ab	Double presence
Number samples	349	349	349
Share of samples corresponding to fields grown from pure* seeds for the concerned Cry gene, %	59.6	53.6	40.4
Rate** of Cry1Ac presence in the samples corresponding to fields grown from pure seeds for this gene, %			
Mean	96.0		96.7
Std. Deviation	4.5		4.4
Rate** of Cry2Ab presence in the samples corresponding to fields grown from pure seeds for this gene, %			
Mean		95.4	95.9
Std. Deviation		4.6	4.5
* Purity is claimed when the presence of Bt gene was observed on no less than 10 seeds out of 11 of each sample ** Rate calculated by the number of seeds with Cry gene detected out of eleven of each sample.			

Table 5: Bt status of seeds in Bt-cotton fields

Cry1Ac status*	Cry2Ab status*		Total
	No	Yes	
No	27.2%	13.2%	40.4%
Yes	19.2%	40.4%	59.6%
Total	46.4%	53.6%	100.0%
*Status refers to the use of pure seeds, i.e. presence on at least 90% of the tested seeds in samples, or on at least 10 out of 11 seeds of each sample			

Figures

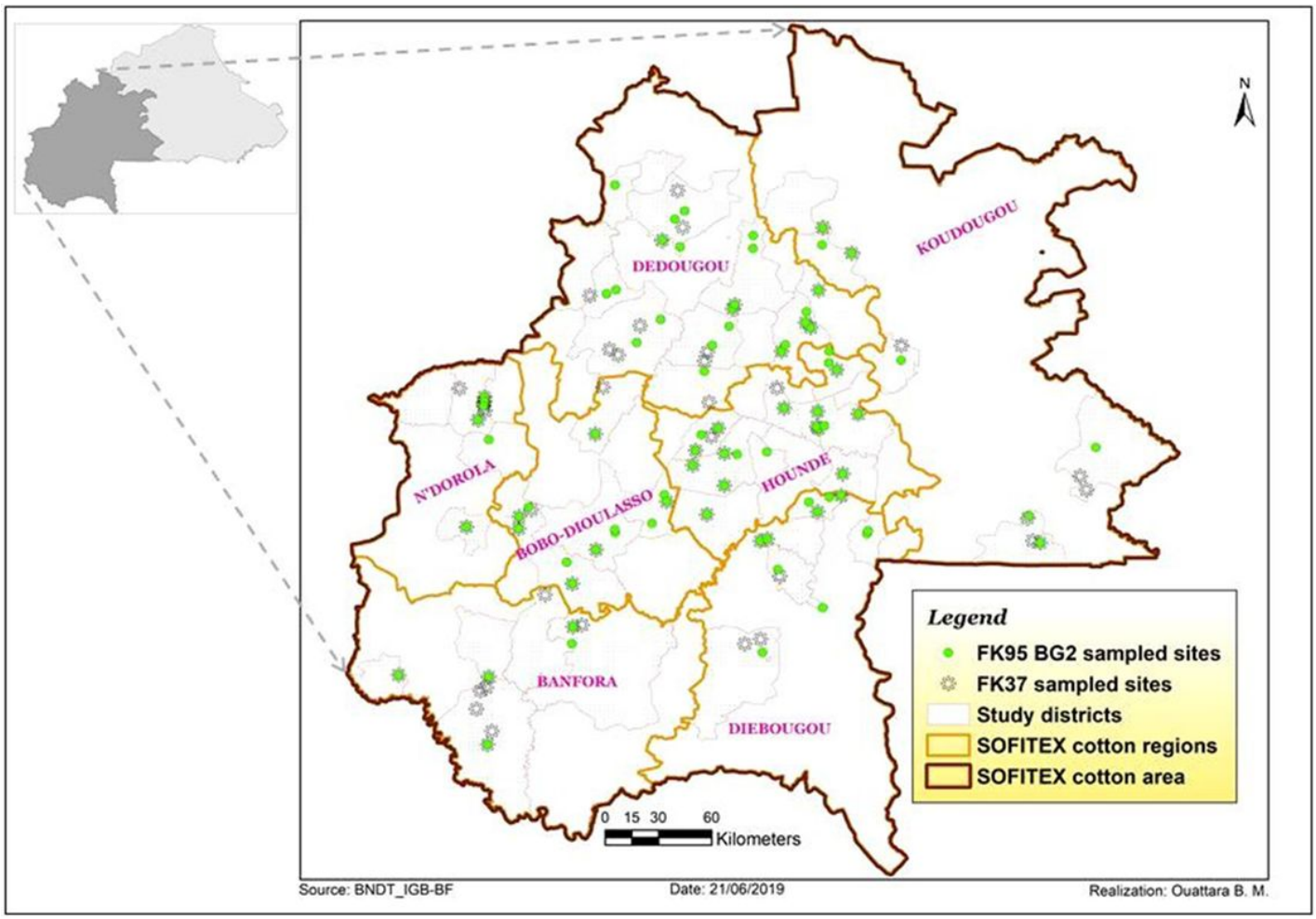

Figure 1

Sofitex cotton zone, showing study sampled districts and sites