DISTRIBUTION AND POTENTIAL IMPACT OF FERAL COTTON ON THE REINTRODUCTION OF COTTON IN THE SOUTHERN HIGHLANDS, TANZANIA

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ABSTRACT

*Cotton (*Gossypium hirsutum*) production is limited by bollworms that cause declining yields and poor lint quality. Generally, farmers manage pests by employing Integrated Pest Management (IPM) strategies, which include biological, cultural, physical and chemical approaches. Pest management by quarantine and pesticide sprays reduce production area and lead to resistance build-up. The Red bollworm,* Dipsaropsis castanea *is an important cotton pest of significant economical importance to Tanzania. The pest invaded the Southern Highlands (SH) of Tanzania in 1960's from southern neighbour countries causing the Government to quarantine cotton production from 1968 as measure to limit the spread of the red bollworm. Transgenic Bt cotton with insecticidal properties presents a potential solution to the bollworm infestation in Tanzania. However, concerns associated with transgenic crops viz.; transgene flow to wild and feral relatives, increased potential for resistance evolution, need to be addressed prior to adoption of any transgenic crop. Information from national herbaria, research stations and a field survey established sparse distribution and diversity of feral cotton species* G. barbadense*, an exotic ornamental from Brazil though as isolated garden plants. Informal interviews revealed medicinal and fibre value of the ornamental. Diploid wild cotton relatives such as* G. longicalyx *and* Gossypoides kirkii *were also recorded but are incompatible to* G. hirsutum*. Field observations indicate continued red bollworm presence in the SH on feral cotton, but low in number as plants are few and isolated. Cluster analysis indicates presence of hybrid remnants of* G. hirsutum *and* G. barbadense *suggesting potential for gene flow.*

Keywords: Bt cotton, bollworms, quarantine, insecticides, insect resistance, feral cotton, refuge plant

INTRODUCTION

Cotton is the world most important fibre crop that is mainly produced by two species *Gossypium hirsutum* and *G. barbadense*. *G. hirsutum* accounts for over 95% of commercial cotton and *G. barbadense* also known as Pima cotton provides less than 5% (Lukonge *et al.* 2008). These two species are self-pollinating allotetraploids of the AD-genome. Other *Gossypium* species that also produce fibres are *G. arboreum* and *G. herbaceum* are diploid, A-genome species (Rana *et al.* 2007).

Cotton production is limited by insect pests and diseases that cause decline in yield and

poor lint quality in most producing countries. The yield losses from insect pests alone are estimated at 24% in Sub-Saharan Africa, 13% in South America and only 7% in Australia where control has been more effective but expensive (Oerke and Dehne 2004; Deguine *et al.* 2008). Of all the cotton insect pests, the bollworms are of particular economic importance as they cause significant destruction of crop (Kabissa and Nyambo, 1989). Bollworms are extremely destructive in their larval stages feeding on cotton leaves, flowers and boring into the bolls (Deguine *et al.* 2008).

In Africa, the red bollworm, *Diparopsis castanea* and other species *Helivicopera armigera*, *H. zea* and *Heliothis armigera* have caused cotton losses of up to 75% in Mozambique and Zambia, while in Malawi 70% losses is due to the red bollworm alone (Kabissa and Nyambo, 1989). In Makathini, South Africa in addition to *D. castanea* other bollworm species *H. armigera*, *Earias biploga*, and *E. insulana* have also caused considerable damage (Ismael *et al.* 2002; Morse *et al,* 2004). In Tanzania *D. castanea* infestation led to a quarantine of cotton production in the Southern Highlands (SH) of Tanzania to prevent spread to other growing areas since 1968 (Kabissa and Nyambo, 1989). Despite this cotton remains the second most important cash crop in Tanzania, contributing about 15% per year to the foreign currency exchange earning and approximately 40% of the population depend solely on cotton for their employment (Lukonge *et al.* 2005).

Insecticide sprays are the main means for bollworm control. Cotton utilizes more insecticides than any other single crop in the world (James 2003). Insecticide sprays have limited efficiency due to the development of resistance over time (Rashid *et al.* 2008). *H. armigera* for example developed resistance to pyrethroids in the U.S.A and likewise in Australia, Burkina Faso, Benin and Mali following a decade of use (Martin *et al.* 2002). Furthermore, insecticide use is discouraged due to side effects on human and animal health, contamination of the environment and cost (Aldridge, 1998; Martin *et al.* 2002; Sinzogan *et al.* 2004; Achaleke *et al.* 2009). Biotechnological options such as transgenic cotton genetically engineered to incorporate *Bacillus thuringiensis* (Bt) with insecticidal properties are currently commercially employed in several cotton growing countries (James C 2003). Bt cotton is widely grown in the United States of America, China and India where yields have been higher than non-Bt cotton (James C 2003).

Despite the potential benefits of Bt cotton, there are concerns regarding production of transgenic crops. These risks include undesirable transgene flow to wild and feral relatives that can in turn lead to increased rates of resistance development where wild and/ or feral relatives act as refugia to the pests. For Bt cotton, it is argued that transgenes through hybridisation may cross into wild *Gossypium* species and if the hybrids express the Bt toxin it may influence the selection pressure which may enable bollworms evolve resistance to the Bt toxin. This is a real concern as two crop pests; the *Plutella Xyllostella* (diamondback moth) and *Trichoplusia ni* have already evolved resistance to conventional Bt sprays in the field; and laboratory tests have shown selection of resistance in *Heliothis/ Helicoverpa* (Tabashink *et al.* 1994, 2003; Fitt, 2000; Chilcutt and Tabashnik 2004; Morin *et al.* 2004; Soberon *et al.* 2007; Raymond *et al.* 2007). Resistance to transgenic Bt cotton and other Bt crops has been observed in certain areas but it is argued that good integrated pest management practices and appropriate mitigation measures such as refugia can significantly delay resistance development

(Bourguet 2004; Tabashnik *et al,* 2003 and Tabashnik 1994a).

In the event, Tanzania considers introduction of Bt cotton in the SH this would revive farmer incomes, and make significant contribution to total cotton production. The present study was thus undertaken to establish the presence and distribution of feral cotton and socio-economic values/ issues associated with it in the SH. This information is necessary to provide premises for informed-decision making prior to adoption of Bt cotton.

MATERIALS AND METHODS

Wild and Feral Cotton Distribution in Tanzania

Wild and feral cotton distribution in Tanzania was established using documented information from two herbaria *viz.* National Herbarium of Tanzania (NHT), the University of Dar es Salaam and two cotton research centres i.e. Lake Zone Agricultural Research and Development Institute (LZARDI)-Ukiriguru and Agricultural Research Institute (ARI)-Ilonga in 2006. Information housed at these institutions provided specimen records, collection sites, and phenology. Selection of sites for the field survey in the SH were decided following information from collected specimen records and consultation with ARI staff.

Survey in the Southern Highlands (SH) Cotton Quarantine Zone

Selected villages in Ruvuma (East), Mbeya and Iringa (West) regions in the SH of Tanzania were surveyed from end of August to early September 2006 to establish localities of wild and/ or feral cotton plants. The specimens were collected from the following sites/locations of the SH; Ipinda, Ushirika, Lutusyo, Ikulu, Magamba, Ifumbo, Ifumbo-makona, Mbala, and in Mbeya region; Hanga-A, Hanga-B, Sinai-A, Sinai-B, Sinai-C, Luhimbililo, Likuyufusi, Namatuhi, Lilambo, Kitanda-A, Kitanda-B and Naikesi in Ruvuma region; and Mng'elenge-A and Mng'elenge-B in Iringa region.

Sampling and morphological characterisation was done using the International Board for Plant Genetic Resources (IBPGR 1985) and Union for the Protection of new Varieties, (UPOV 2001) targeting the flowering period for morphological character scoring. At each location four individual plants were sampled and presence of bollworm larvae recorded. Three different people noted the morphological characters using the designated codes that were later converted to a binary matrix system. Scored characters included; petal colour, basal petal spot, pollen colour, boll (shape, surface and colour), leaf (colour, hairiness and shape), seed (fuzzy/no fuzz, fuzz colour, nature, number per boll) and locules per boll. Principal component analysis (PCA) from the R-2.9.2 software program was used to test variation in specimen based on morphological markers. Twenty two herbarium vouchers were collected from the SH, fourteen of which had complete floral and vegetative information were sent to the Royal Botanical Gardens, Kew, United Kingdom for identification and the remaining eight that did not have complete information were deposited at the Botany Department herbaria, University of Dar es Salaam.

Informal interviews (open-ended questions) were conducted with four randomly selected villagers at each of the twenty sampling points (Table 2) to gather information on feral cotton. The questions were focused on use of the plants, whether the plants were planted intentionally or grew on their own and where seeds were sourced. The information was used to establish whether there was a single source or mixed seed source that would enable inference to be

drawn on the observed variations within the feral cotton surveyed.

RESULTS

Wild and Feral Cotton Distribution

Herbaria records indicated the presence of only one true wild diploid cotton species; *Gossypium longicalyx* (F-genome) and a species of the sister tribe Gossypieae; *Gossypioides kirkii* to occur in Tanzania. *Gossypioides kirkii* records are from the Coast, Arusha and Tanga regions and *G. longicalyx* records are from Dodoma and Iringa regions. The records show that both species are found in habitats ranging from forest edge to lowland and seasonally wet open *Acacia* bush land. Neither of the two species was observed in the SH.

Records at the ARI stations by Nyambo (1989) and Temu and Mrosso (1999) listed two feral species of cotton, introduced from the Americas, that is *G. barbadense* var. *brasilience* Macf and *G. hirsutum* var. *mariegalante* Watt. *G. hirsutum* may have established as feral populations after previous cultivation. *G. barbadense* var. *brasilience* was cited as being a common bush in East Africa also known as kidney cotton. In Tanzania the records indicated the distribution of *G. barbadense* to be Songea Highlands at the head of Lake Nyasa. *G. barbadense* was introduced by the Arabs during their colonial era as an ornamental. The two populations of cotton may have adapted to the unmanaged conditions and persisted in the environment as feral cotton.

Confirmation of Feral cotton identity

All the 14 specimens sent to the Royal Botanical Gardens, Kew were identified as feral *G. barbadense* using morphology as documented in the Flora of Tropical East Africa (FTEA) Family Malvaceae of 2007. The FTEA re-classified *G. barbadense* var. *brasilience* as *G. barbadense* and listed characteristics of the species as having a yellow corolla with dark red or purple centre (petal spot), free or fused seeds, 3-7 lobed leaves and glabrous, pitted, elongate bolls with 3 locules and broadly ovate bracts of epicalyx. The FTEA also discourages use of the variety names; *mariegalante* and *brasilience*. The eight voucher specimens that had not been sent to the Royal Botanical Gardens, Kew were also identified as *G. barbadense* using the FTEA in Dar es Salaam. The morphological characters used in the FTEA classification were adopted for this study with some exceptions that not all plants had a purple spot and some plant bolls were not elongated but rather oval (See Table 1). *G. barbadense* plants were found to be sparsely distributed in the SH with a few individuals in home gardens and no large populations.

Principal Component Analysis (PCA) using the morphological markers showed two main groupings of individuals collected from the Eastern villages (Ruvuma) and Western villages (Mbeya and Iringa) (See Figure 1). The distances between collecting areas ranged from 150Km to 270Km. The overall variation as explained by PCA is 66%, which infers that variations between individual plants were significant. Individuals grouped according to geographical region with some exception such as individuals from Lutusyo village which is in Mbeya region grouped with the Eastern individuals and those from Mng'elenge_A and Mng'elenge B grouped with the Western individuals from Ruvuma region. It is likely that some seeds have been dispersed between Ruvuma and Mng'elenge (Iringa) as these places border each other or moved by farmers from one location to the other as the feral cotton was primarily introduced as an ornamental.

Table 1: Morphological Markers Scored from different individual Plants

Village	Region	Petal colour	Petal spot	Pollen colour	Boll shape	Boll colour	Boll surface	Seed fuzzy	Seed natue	Leaf hair	leaf colour
Hanga A	**Ruvuma Region**	deep yellow	absent	deep yellow	oval	deep green	pitted	naked	fused-black	short	deep green
Hanga B		deep yellow	absent	deep yellow	oval	light green	pitted	naked	fused-black	short	deep green
Kitanda A		deep yellow	present	deep yellow	oval	deep green	pitted	naked	singly-black	short	deep green
Kitanda B		light yellow	absent	deep yellow	oval	deep green	pitted	green-fuzzy	singly	short	deep green
Likuyufusi		deep yellow	absent	cream	conical	light green	pitted	naked	singly-black	short	deep green
Lilambo		deep yellow	present	cream	conical	deep green	pitted	naked	singly-black	glabrous	deep green
Luhimbililo		deep yellow	present	deep yellow	oval	light green	pitted	naked	singly-black	glabrous	deep green
Naikesi		light yellow	absent	pale yellow	oval	deep green	pitted	naked	singly-black	glabrous	deep green
Namatuhi		deep yellow	present	deep yellow	oval	light green	smooth	naked	singly-black	long	deep green
Sinai A		light yellow	absent	deep yellow	oval	deep green	pitted	naked	singly-black	short	deep green
Sinai B		deep yellow	absent	deep yellow	oval	deep green	smooth	naked	singly-black	short	deep green
Sinai C		deep yellow	present	deep yellow	oval	deep green	pitted	green-fuzzy	singly-black	short	deep green

Ushirika		light yellow	absent	deep yellow	conical	deep green	pitted	naked	fused-black	short	deep green
Ikulu	**Mbeya Region**	light yellow	absent	deep yellow	conical	light green	pitted	naked	fused-black	short	deep green
Ipinda		light yellow	absent	deep yellow	conical	light green	pitted	green-fuzzy	fused	short	deep green
Lutusyo		deep yellow	present	deep yellow	conical	deep green	pitted	naked	singly-black	glabrous	deep green
Ifumbo-makona		light yellow	absent	deep yellow	conical	light green	pitted	naked	fused-brown	short	deep green
Ifumbo		light yellow	absent	deep yellow	conical	light green	pitted	naked	fused-black	short	light green
Magamba		deep yellow	present	deep yellow	conical	deep green	pitted	naked	fused-brown	short	deep green
Mbala		light yellow	absent	deep yellow	conical	light green	pitted	naked	singly-brown	short	deep green
Mng'elenge A	**Iringa Region**	deep yellow	absent	deep yellow	conical	deep green	pitted	naked	fused-black	long	deep green
Mng'elenge B		deep yellow	absent	deep yellow	conical	deep green	pitted	naked	fused-brown	long	deep green

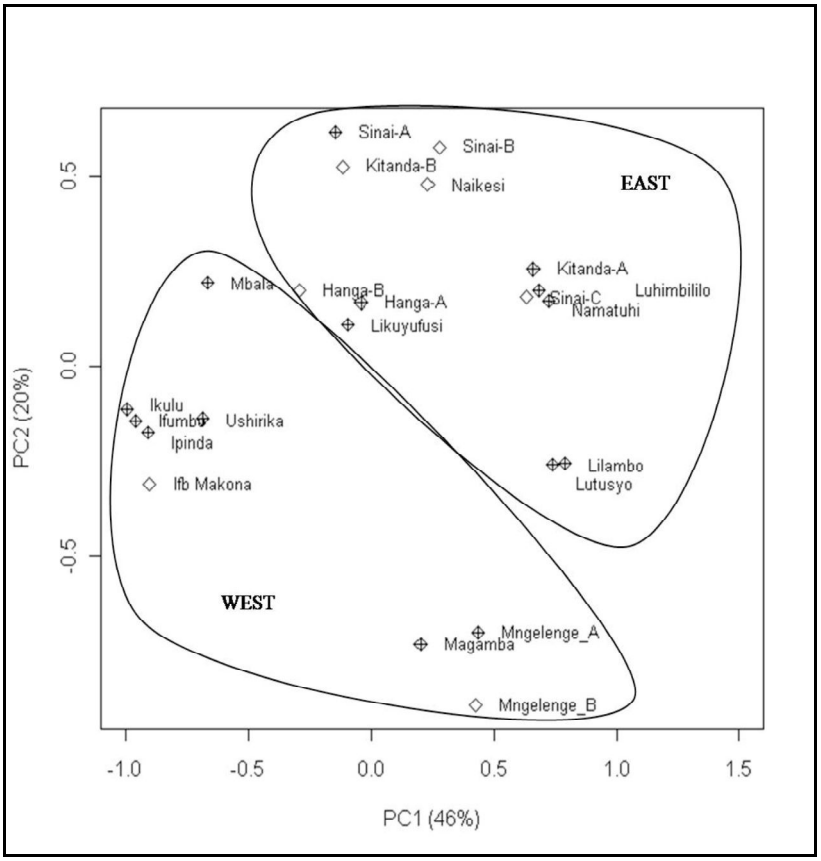


Figure 1: PCA analysis of the *G. barbadense* individuals collected in the East and West SH; crossed squares indicate the accessions identified at Kew

Red bollworm larvae were observed on cotton plants at two different locations; Ipinda (4 larvae) and Ushirika (3 larvae) all in Kyela district. In other areas dry bored bolls indicated presence of larvae, as the organisms were not observed.

Consulted communities and individuals in the SH indicated medicinal and lint value of feral *G. barbadense*. *G. barbadense* serves as an antiseptic for cleaning wounds, leaf and root concoctions treat convulsions and stomach ache and the oil from seeds cures ear aches. *G. barbadense* fibre is considered stronger than that from G. hirsutum. Furthermore the lint was used to make local mattresses and wicks. Local names for feral *G. barbadense* varied between regions, in 'Undendeule' in Ruvuma region it is called 'Litonje', in the Kyela district in Mbeya region it is called 'Masapa' while in several other regions it is known as 'pamba pori'in Kiswahili.

DISCUSSION

The present study established the presence, distribution and the reasons why feral *G.*

barbadense plants are maintained by communities in the SH.

Identification by experts from Kew Royal Botanical Gardens and the FTEA Malvaceae (2007) using keys indicate that all the collected voucher specimens to be feral *G. Barbadense,* the PCA grouping using the characterised morphological markers separates individuals from different geographic areas. PCA groupings suggest subtle phenotypic variations within the recorded *G. barbadense* individuals. For instance, some plants lacked purple spots, there was variation in petal yellowness ranging from deep to pale yellow and boll shape which was recorded as oval or conical (see Table 1). The observed variations can be plausibly argued on two fronts; firstly environmental factors that are known to influence morphological markers may account for the different groupings (Lihova *et al.* 2007). Secondly, the variations may have arisen from natural intraspecific crossing, where offspring deviate from their parents for some of the characters. Lastly, the informal consultations revealed that seeds from good lint producing plants have been exchanged between villages and regions with minimal monitoring and this may explain variation of characters such as the colour and spots. Furthermore, intraspecific crossing is also a strong possibility taking into account that these plants have co-existed together for a significant period. Under normal circumstances the intraspecific crosses in cotton is very low (1-5%) in the absence of large pollinators like bees as cotton pollen is heavy.

The findings of this study complement that of Fontes *et al.* (2005) who observed two distinct *G. barbadense* populations based on seed form; one with unattached seed and the other with attached seeds (kidney cotton). Other studies also show similar distinctions in seed form for example Singh *et al.* (2003) found that the tree cotton of *G. barbadense* had a characteristic feature of naked seeds fused to form a typical kidney structure in each locule of the capsule, whereas Acland (1971) and Purseglove (1968) showed that *G. barbadense* has dark brown and pitted bolls, usually 3 locules with 5 to 8 seeds per locules. It can be argued that the morphological grouping observed in the PCA diagrams is due to these differences as examination of the individuals from Magamba, Mng'elenge-A and Mng'elenge-B show grouping based on shared characters of yellow petals, deep yellow pollen; conical and pitted bolls with non-fuzz fused seeds, deep green leaves lacking a petal spot. The distinction between *G. barbadense* populations using morphological characters provides some insight to parent origin but for purposes of taxonomic grouping all individuals collected from the SH are considered one species i.e. *G. barbadense.*

The presence of the Red bollworm larvae on feral *G. barbadense* suggests that the pests continue to thrive on other plants in the absence of the quarantined *G. hirsutum.* Temu and Mrosso 1999 postulate *G. barbadense* to be the main host for the bollworm as it was not found on any other plant species, in this survey similar observations were made though the examination of all species was not comprehensive.

Implications to *G. hirsutum*

G. barbadense and *G. hirsutum* have the same ploidy level and related genomes. This suggests that the two species can hybridise giving rise to the probability of cultivar-to-feral out crossing and the likelihood of gene flow (Brubaker *et al.* 1999). Transgenic *G. hirsutum* with the Bt gene insert is physiologically and genetically similar to conventional cultivars. The presence of *G. barbadense* in the SH that continues to thrive showing relative tolerance to the Red bollworm may be of some concern for

resistance evolution against Bt toxin if the *G. barbadense* is allowed to remain in the area. However as the distribution of *G. barbadense* is sparse with no distinct populations at any one point this may be a barrier to pollen mediated gene flow. This postulation however, requires confirmation through surveys in other parts of the country that will inform on the potential for gene flow.

A lack of clear differences in geographically isolated individuals suggests that there has been movement of genes between the locations, as confirmed by the consultations. For introduction of Bt cotton management strategies that ensure seed movement between growing areas is monitored to avoid spread of transgenes are necessary.

Public participation in implementing management options is vital since the study has shown that feral plants are of value to some of them and that is why plants are found not very far from farmyards and along the roadsides. Furthermore, if Bt cotton is to be introduced in the SH apart from gene flow other pest management and socio-economic concerns would need to be addressed in the risk assessment such as the capacity for adoption, need for refugia among others.

CONCLUSION

Based on the findings of the study it can be concluded that feral *G. Barbadense* distribution in the SH does not pose a significant threat to the introduction of Bt cotton. This is because the isolated occurrence of the feral individual plants would not facilitate significant gene flow between the transgenic and feral plants. Likewise despite the occurrence of the red bollworm on the feral cotton, individual plants do not offer sufficient refugia to allow the pest to grow populations that would facilitate resistance build up to Bt. However, prior to Bt cotton introduction it is necessary to consider management options that would safeguard the minor likelihood of hybridisation between the two species. Furthermore as the survey was limited to the SH it would be of added value if similar studies were conducted in other cotton growing areas to establish the potential for gene flow and resistance development where introduction of Bt cotton may be considered. The value attached to the feral cotton by the communities may be due to the lack of *G. hirsutum* and or access to modern clinics, an extended study in other parts of the country would serve to confirm this assumption.

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