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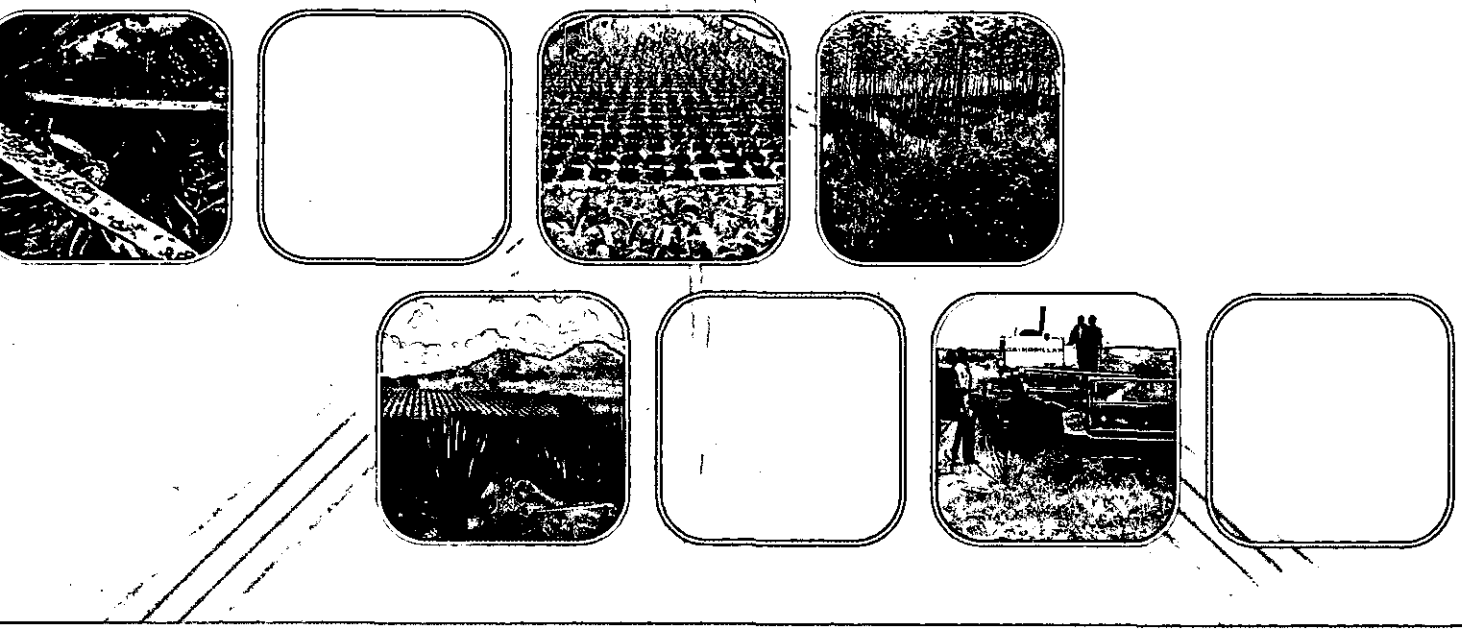
Product and market development of sisal and henequen



Multiplying Sisal by Meristematic Tissue Culture

Project completion report/Addendum A.5
Part One: Kenya

Kenya, October 1998 – September 2004

UNIDO

cfc COMMON FUND FOR COMMODITIES

FAO FIAT PANIS

KENYA [illegible] BOARD

COMMON FUND FOR COMMODITIES

Product and market development of sisal and henequen

Project completion report, Addendum A.5—Part One: Kenya

Multiplying Sisal by Meristematic Tissue Culture

Kenya
October 1998 – September 2004

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna, 2006

Project Completion Report preparation: National and international experts and consultants involved with the project, with contributions and support from the national teams.
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Project Completion Report

Sub-component A.5 – Part One: Kenya
"Multiplying Sisal by Meristematic Tissue Culture"

CONTENT

Abbreviations and acronyms

ARI	Agricultural Research Institution
BAP	Benzyl Amino Purine
CFC	Common Fund for Commodities
CICY	Centro de Investigación Científica de Yucatán
COMFAR	Computer Model for Feasibility Analysis and Reporting
FAO	Food and Agriculture Organization
ITTA	International Institute of Tropical Agriculture
KEPHIS	Kenya Plant Health Inspectorate Service
KES	Kenyan Shilling
KSB	Kenya Sisal Board
MT	Metric Tonne(s)
MTC	Meristematic Tissue Culture
psi	Pounds per square inch
spp	[Agave] Species
TC	Tissue culture
UNIDO	United Nations Industrial Development Organization
2,4-D	2,4-Dichlorophenyl Acetic Acid

I. Project sub-component summary

1. Title: "Multiplying Sisal by Meristematic Tissue Culture"

2. Location: Muguga (Kenya) at the KEPHIS-Plant Quarantine Station

3. Starting Date: October 1998

4. Completion Date: September 2004

5. Sub-component external financing – excluding counterpart contribution:

Total Subcomponent Cost:	US$ 145,206
Of which:	
CFC Financing	US$ 77,044
Belgium Government:	US$ 36,373
UNIDO	US$ 31,789

II. Background and context in which the sub-component was conceived

II.1 Background and context

The production of sisal fibre in East Africa has been declining from a production high of around 318,000 metric tonnes (MT) in 1967 to slightly above 40,000 MT over the last decade. At present the combined production of Kenya and Tanzania is approximately 42,000 MT per year. The reduction in tonnage has been primarily due to:

- Competition from other fibers, mainly synthetic fibers that are available at very competitive prices.
- Conversion of estates bordering big towns into residential areas.
- Replacement of Sisal with other agricultural enterprises for example high value horticultural crops like flowers and vegetables in high potential areas. Such crops are normally grown under irrigation.

Despite the changes in the global arena, sisal remains a key crop especially in marginal areas, since it withstands stress better than most crops. A renewed interest in the crop has arisen because of the realization that non-biodegradable synthetic fibres, which had replaced sisal and other natural fibres, are major environmental pollutants. As demand for sisal rises, a major constraint has been quality-planting materials, mainly due to the degeneration of mother plants caused by diseases, pests and genetic drift. The project sub-component A-5 was geared to:

- Multiply plants carefully selected by farmers for starting nucleus materials that are uniform and rapid in growth.
- Develop the capacities to collect and store germplasm.
- Enable international exchange and crop improvement. International exchange of vegetative propagated under the international plant protection safe movement of germplasm.

Sisal crop has been neglected for a long time and has not benefited from research and development. The genetic base available in the region has been poor and has led to the continued reduction of yields in most farms. Improvement has to be multi-faceted through the introduction of breeding materials and exchange with other countries. Vegetative propagated Sisal has to be exchanged as pathogen-tested *in vitro* cultures or as true seeds.

It is in light of the above that the tissue culture component was initiated both at the Agricultural Research Institution (ARI) at Mlingano, Tanzania and at the Kenya Plant Health Inspectorate Service (KEPHIS) at Muguga. The work at both laboratories involved refinements of media, multiplication of selected genotypes and field trials with culture plants. Replication of sisal gene-bank plants envisaged by the project was not achieved. Consultations towards attainment of this objective are still going on between the Government of Kenya and the Government of Tanzania.

II.2 Objectives, outputs and targeted beneficiaries

Sisal (Agave spp) can be propagated sexually or vegetatively. Traditionally, commercial sisal in East Africa has been propagated through the use of subterranean shoots that grow away from mother plants (rhizomes), suckers or bulbils. Bulbils originate from buds in axils of the stems of fruits. Continuous propagation of sisal through the use of the above propagules has resulted in gradual reduction in the yield of sisal due to genetic drift. A lot of chimeras have been observed in fields raised from bulbils. For this reason it was felt there was a need for enhancing productivity through the adoption of modern biotechnological applications.

The sub-component objectives were as follows:

- To increase sisal productivity by carefully selecting sisal plants which are distinct, uniform and stable;
- To develop a cost effective system for increasing sisal shoot regeneration from auxiliary shoots (direct organogenesis);
- To develop an efficient *in vitro* based system for rooting sisal plants;
- To establish an *in vitro* conservation protocol for selected sisal genotypes;
- To monitor performance of tissue culture plants particularly in terms of uniformity and genetic stability through "on station" trials and collaborative trials with sisal growers.

The final practical objective was to develop an efficient system to clone elite sisal materials through tissue culture applications. The strategy utilized to meet the objective was the regeneration of shoots from sections of rhizomes from which shoots were induced through direct organogenesis.

Mother plants were carefully selected from plantations taking into consideration the absence of diseases, the plant productivity, as determined by the number of leaves produced during the productive life of the plant, the time span before poling, and the absence of abnormalities including chimeras, stunting, attack by diseases etc. Selection of mother plants was done in consultation with growers/farmers.

The targeted beneficiaries were the various stakeholders involved in sisal:

- Farmers and smallholder farmers
- Farmers living near sisal estates, workers in the sisal estates and people living in urban areas interested in investing in sisal farming
- Companies involved in sisal growing and processing into finished products
- Traders involved in sisal fibre and sisal products locally and overseas
- Village and District Councils where sisal can be grown.

III. Implementation and results achieved

III.1 Establishment of an MTC laboratory

III.1.1 Refurbishing the laboratory

Although the Plant Quarantine Station, Muguga had an operational tissue culture laboratory prior to the initiation of the project, the facility was very small and inadequately provided with facilities for rapid *in vitro* multiplication of sisal and other crops.

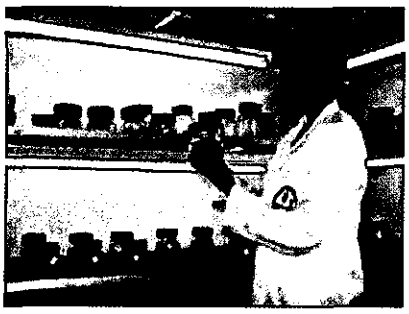
The project provided funds for up-grading the laboratory and for creating additional bench space in it starting in the year 1999. In 1999, one tissue culture growth room with capacity to hold 2450 magenta vessels was completed. This was in addition to the purchase of a new autoclave and air-conditioner. A further modernization /expansion program was approved in the years 2000-2001. Three green houses for acclimatization of tissue culture plants were also refurbished. In 2002 a further expansion of the laboratory in which two new growth rooms with lighting and air conditioning whose combined capacity can accommodate 2700 magenta vessels was completed. In total three additional growth rooms were added, this brought up the total bench capacity to 513,486 cm^2.

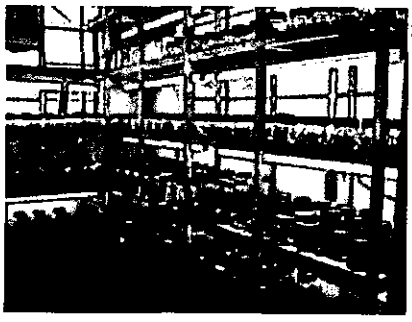
Unfortunately the laboratory suffered from enormous losses due to contamination during the refurbishment works, but in 2003 it recovered quickly.

Figure 1. Exterior view of the plant quarantine tissue culture laboratory.

The tissue culture laboratory also benefited from purchase and installation of a new 75-liter capacity vertical autoclave and laminar flow hood from the project in 2002.

Figure 2. A view of the interior of improved tissue culture growth rooms





A further improvement of the facilities was made possible through additional funding from the Food and Agriculture Organization (FAO) and from the International Institute of Tropical Agriculture (IITA). The funding from IITA was used for repairing the laboratory roof whereas FAO provided additional equipment. The KEPHIS counterpart funding has been used to pay for staff salaries, payment of water charges, electricity, security, purchase of additional consumables, installation of a more efficient soil sterilizer, back-up generator (64 KVA) and sinking of water borehole. These facilities together with those bought by the project have greatly improved the laboratory.

III.1.2 Equipping the laboratory

The project has, over the years, supplied chemicals and other equipment (Annex 1) to allow for laboratory operations.

III.1.3 Training and development of laboratory staff

Staff development was a major component of the sub-component. During the implementation period the following was achieved:

- Training for Mr. Njoroge of KEPHIS and Mr. Shabani of ARI Mlingano at the tissue culture laboratories of the "Centro de Investigación Científica de Yucatán" (CICY), in September 1998. The Training was geared at equipping the managers of KEPHIS and ARI Mlingano with technical and hands-on training on handling tissue culture of Agaves;
- One-month tissue culture training at the Plant Quarantine Station, in 1999, in which threee technicians from KEPHIS and Mr. Mrombo from Teita Estates were trained;
- COMFAR (Computer Model for Feasibility Analysis and Reporting) training held at Katani's premises in Tanga, Tanzania on 6 -10 October 2003, in which Mr. Njoroge participated;
- Exchange of consultative visits for both KEPHIS and ARI- Mlingano teams were facilitated throughout the project period

The international consultant, Dr. Manuel L. Robert, provided technical assistance and monitoring services during his missions, which have been very instrumental in improving the operations of the laboratory.

III.2 Experimental activities and establishment of a protocol

Multiplication of sisal through tissue culture targeted genotypes Hybrid 11648, Agave Sisalana, Agave Hildana, 1300, 1200 and Teita hybrid (farmers' selection). All these genotypes are traditionally multiplied through use of bulbils that are produced once the mother plant poles. Some farmers also use suckers, which are normally not uniform. Due to the use of inferior planting materials the sisal production has been steadily declining. Most producers report non-uniformity in the materials currently in production. The aim of the sisal tissue culture project was to rapidly multiply clone lines of the genotypes in order to increase uniformity, reduce the period from nursery to first leaf harvest and increase average leaf production per hectare.

III.2.1 Tissue culture materials and methods

The protocol used is based on Murashige and Skoog`s technique using the experience gained at CICY (Mexico) with henequen. More details on meristematic tissue culture are included in the CFC Technical Paper no. 38, *"Manual for the in vitro Culture of Agaves"*, October 2004. Reference should be made to this publication for technical and scientific details on the method.

a. Culture medium

Initiation media consisted of modified Murashige and Skoog salts supplemented with 30 g/l sucrose, BAP (Benzyl Amino Purine) levels (0; 5; 10; 12.5; or 15 mg/l) and auxin 2,4-D at 0.025 mg/l and pH was adjusted to 5.7 ± 0.01 with either 0.1 percent N HCl or 0.1 percent N NaOH. The media was solidified with 8g/l agar. The media was heated while stirring until the agar dissolved after which 25ml or 50mls aliquots were dispensed into magenta media vessels and ½ liter capacity Kilner jars, respectively. Media was autoclaved at 15 pounds per square inch (psi) i.e. (121°C) for 15 minutes. Autoclaved media was allowed to cool in sterile environment after which it was ready for use.

Growth media contained BAP at $1mgl^{-1}$ combined with 2,4-D at 0.025 mgl^{-1}. The multiplication medium used contained $10mgl^{-1}$ BAP and 0.025 mgl^{-1} of 2,4-D, respectively.

b. Preparation of explants materials and in vitro growth

Suckers with a circumference, ranging from 20cm to 30cm with no visual signs of pathogenic infections (without reddish-brown colouration at the base of the plants or other physical deformities) were selected from the field with the help of growers. Once in the laboratory, leaves were carefully stripped off using a knife/surgical blade, starting at the base of the bole (stem) while ensuring that the top part of the crown, where much of the meristem tissues for shoot induction are concentrated, was not damaged. This was achieved by making a final cut 4 mm (above the point of attachment to the bole) and 2cm above the crown. Such prepared plants were scrubbed using a soft brush under running tap water to remove soil debris and dead plant tissues. Then they were soaked in water containing a few drops of Tween 20 (20 Polyoxyethylene; 20 sorbitan monolaurate supplied from BDH laboratories), for 30 minutes after which explants were surface sterilized under a running laminar air flow with 40% commercial Jik for 20 minutes, followed by 3-4 rinses with distilled

sterile water. After that cubes measuring 1×1 cm were made from top-most layer, rinsed again in 2% Jik and finally placed upright in the initiation media.

Figure 3. Surface sterilization, cutting to cubes and inoculated cubes in growth chamber

c. Growth conditions

Induction studies are carried out under conditions of: 24 hours light regime, four days total darkness followed by 16/8 hours (day/night) regime, or 12/12 hours (day/ night).

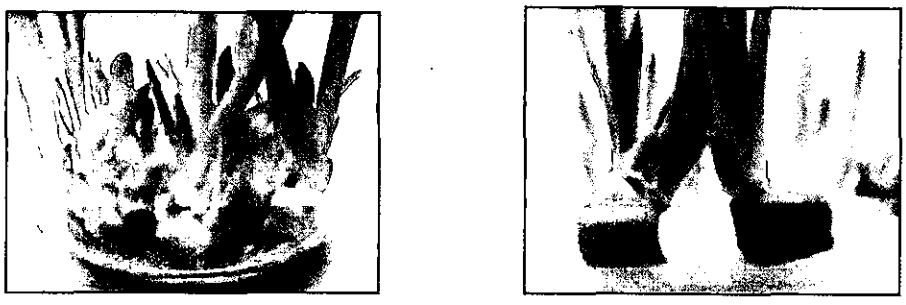
All cultures for growth or multiplication were maintained under 16/8 hours (day/ night) regime. Data collected included number of shoots after seven days , three weeks, six weeks; contaminants, and the number of new shoots on materials under multiplication medium after four weeks.

III.2.2 Experiments Conducted

1. Induction studies

Induction of cubes with 24-hour daylight conditions was unsuccessful in regeneration of auxiliary shoots from 1x1 cm cubes of 1300, Hybrid 11648 and Agave Sisalana cultured in modified medium containing: 0; 5; 10; 12.5 or 15 mg/l BAP combined with 0.025 mgl^{-1} of 2,4-D, even after three months of culture, though cubes turned completely green within 10 days of placement into the induction media. Placement of cubes for four days under total darkness followed by 16/8 hr (day/night) regime was not effective in enhancing shoot emergence of plants of genotype 1300.

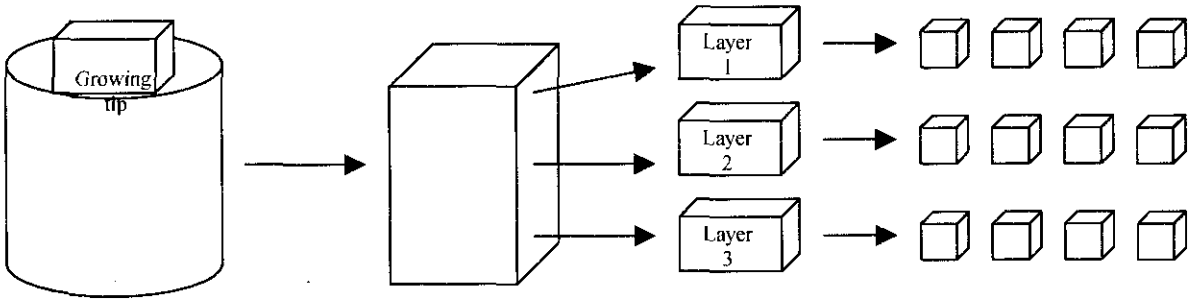
Figure 4. Shoot emergence from cubes.



2. Effect of age and proximity of meristematic layer on emergence of auxiliary shoots from sisal cubes

Experiments were carried out to determine whether the meristematic layer of the mother plants influenced shoot formation from cubes. Cubes were made from three layers of the crown as illustrated below (Figure 1).

Figure 5. Illustration of the three levels of cubes used in studying the effect of meristematic layer on the emergence of shoots.



The top layer was denoted as M1, middle level as M2, and third level as M3. For every level 30 cubes were used per treatment for all the genotypes. The emergence of new shoots was recorded after two weeks, three weeks, and four weeks. Table 1 shows the data collected (number of auxiliary shoots) from cubes sourced from M1, M2 and M3.

Table 1. Effect of the cutting depth on emergence of shoots from cubes.

Genotype	Average number of shoots recorded per cube											
	1 Week			2 Weeks			3 Weeks			4 Weeks		
	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3
Teita selection	2	0	0	2.1	0	0	4.8	0	0	21	9.2	1.2
H 11648	1.1	0	0	1.5	0	0	1.8	0	0	2.5	1.3	0
1300	1.5	0	0	1.5	0	0	1.8	0	0	2.1	1.8	1
A. Hildana	1.1	0	0	1.8	0	0	2.1	0	0	2.5	0	0

Emergence of shoots was first recorded in the top most layer (level M1) for all the genotypes. Proliferation was higher in the Teita selection. Shoots in the second and in the third layers only started being recorded after four weeks.

Growth regulators, BAP and auxins had a positive effect in shoot regeneration from cubes. BAP at 10-15 $mgl,^{-1}$ enhanced shoot proliferation in Agave Sisalana, accession 1300, Hybrid 11648, Agave Hildana and Teita selection (Table 2.)

Table 2. Effect of growth regulators on emergence of new shoots.

Genotype	Average number of shoots after three weeks of induction at different BAP concentration					Average number of shoots after six weeks of induction at different BAP concentration				
	0 mg/l	5 mg/l	10 mg/l	12.5 mg/l	15 mg/l	0 mg/l	5 mg/l	10 mg/l	12.5 mg/l	15 mg/l
Teita selection	0	0	1	2	8	0	1	7	20	30
1300	0	0	0	0	0	0	1	10	15	10
H 11648	0	0	1	1	1	0	2	2	4	4
A. Sisalana	0	0	1	1	1	0	2	3	5	3
A. Hildana	0	0	1	1	1	0	2	3	4	4

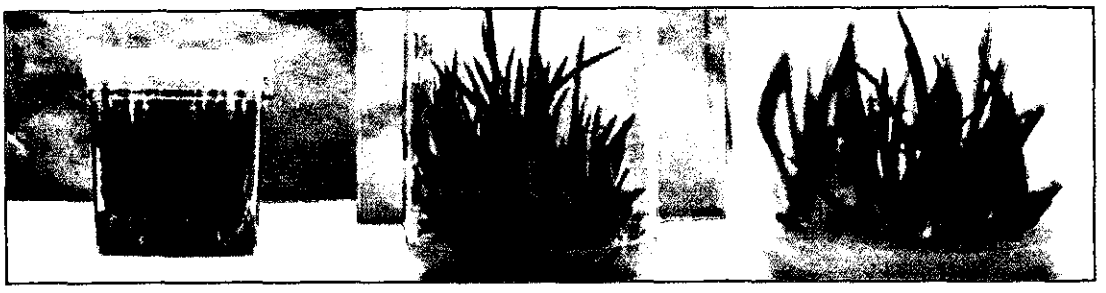
3. In vitro studies using natural light

Experiments were carried out to analyze the effect of natural light on shoot emergence from cubes. For Teita hybrid, out of 30 cubes initiated in medium containing 15 mg/l of BAP, 16 cubes produced at least one shoot after three days and an average of six shoots within three weeks. The remaining cubes produced an average of three shoots within the 21 days. Some cubes had as many as 50 shoots after 21 days. 50% of cubes from genotype 1300 produced one shoot per cube in three days whereas Hybrid 11648, Agave Sisalana and Agave Hildana produced one shoot per cube after seven to ten days. No more shoots were produced after 28 more days and only 10% of the cubes remained dormant. This showed that natural light could be used to initiate sisal in absence of electricity.

4. Multiplication medium studies

In order to increase the biomass for shoot multiplication cubes producing single shoots were carefully nipped to remove the entire shoot at the base level after which cubes were transferred into medium containing 10 or 12.5 mg/l of BAP combined with 0.025 mg/l of 2,4-D. All plants produced five or more shoots within four weeks, irrespective of genotype. Further shoot proliferation achieved by transfer of individual shoots (of approximately 5 cm height) into medium containing 10 or 12.5 mg/l of BAP combined with 0.025 mg/l of 2,4-D, resulted in regeneration of 4-5 auxiliary shoots at the base of the inoculated shoot within four weeks. Agave Sisalana, Hybrid 11648 and Agave Hildana produced an average of five, well formed and strong shoots, while variety 1300 produced a cluster of shoots which were pale green and thin. However, when these clusters were placed in growth medium containing 0.05mg/l gibberellic acid, they elongated, became stronger and also attained a dark green colour.

Figure 5. Proliferation of shoots of sisal propagated at Plant Quarantine Station.



5. Studies on decontamination of shoots

Four replicates of shoots of selected clones of sisal genotypes Agave Sisalana, Teita selection, Agave Hildana, Hybrid 11648, and 1300 were rinsed in disinfectants as listed below and cultured in fresh medium without rinsing in distilled sterile water. The treatments were:

- 10 minute rinse in 10% Jik (Commercial bleach containing 3.5% Sodium hypochlorite);
- 10 minute rinse in 50% dilution of commercial disinfectant Dettol;
- 10 minute rinse in 10% dilution of commercial laboratory grade hydrogen peroxide (commercial formulation containing 3.5% active ingredient of peroxide ions);
- 10 minute rinse in 25% dilution of commercial laboratory grade hydrogen peroxide;
- 10 minute rinse in 50% dilution of commercial laboratory grade hydrogen peroxide;

Contaminations were recorded weekly up to six weeks. Table 3 below shows the results.

In another experiments, Oromex was used as sterilant in the control of fungal and bacterial contaminants. Oromex was used at various concentrations namely: 0; 20µlL-1; 40µlL-1 and 100µlL-1. Oromex at levels 20µlL-1; 40µlL-1 and 100µlL-1 effectively slowed contaminations in all cultures studied; however contamination started showing later, indicating that the disinfectant was effective for surface contaminants and not for internal contaminations.

Table 3. Effect of surface sterilization on contamination of tissue cultured sisal plants after one week (Wk1) and after two weeks (Wk2)

Genotype	Concentrations	Wk1	Wk2	Remarks
Teita selection	10% NaOCl	-	-	Plants scotched by NaOCl
	25 % Dettol	X^2	X^1	Plants scotched by Dettol
	10 %Hydrogen peroxide	X^1	X^2	
	25% Hydrogen peroxide	X^1	X^1	Caused scotching of plants, which later recovered.
	50% Hydrogen peroxide	-	-	Caused scotching of plants, which later recovered.
H 11648	10% NaoCl	-	-	
	25% Dettol	X^2	X^1	
	10% Hydrogen peroxide	X^1	X^2	
	25% Hydrogen peroxide	X^1	X^1	
	50% Hydrogen peroxide	-	-	
1300	10% NaOCl	-	-	
	25% Dettol	X^2	X^2	
	10% Hydrogen peroxide	-	-	
	25% Hydrogen peroxide	-	X^1	
	50% Hydrogen peroxide	-	-	
H 11648	10% NaOCl	-	-	
	25% Dettol	X^2	X^1	
	10% Hydrogen peroxide	X^2	X^1	
	25% Hydrogen peroxide	X^1	X^1	
	50% Hydrogen peroxide	-	-	
A. Sisalana	10% NaOCl	-	-	
	25% Dettol	X^2	X^2	
	10% Hydrogen peroxide	X^2	X^1	
	25% Hydrogen peroxide	X^1	X^1	
	50% Hydrogen peroxide	-	-	

X^1 denotes fungus,
X^2 denotes bacterial contamination, and
- denotes no contamination.

6. In vitro bulking of sisal biomass, production, and field performance of sisal generated from tissue culture

Tissue culture protocol for micro-propagation of sisal was refined. Individual shots cultured on modified medium containing 12.5 mgl^{-1} of BAP combined with 0.0025 mgl^{-1} 2,4-D were able to generate three to four new shoots for all sisal genotypes studied. The production achieved in the year 2003-2004 was over 54,000 plants (Annex 2). Figure 6 below shows the subdivision of shoots to transfer to bulking/ multiplication media.

Figure 6. Sisal multiplication process.

7. Acclimatization studies

Acclimatization is the transfer of plants from the growth room atmosphere to the outside atmosphere in soil under greenhouse conditions. This is carried out in a glasshouse and the plants are planted in trays with holes (about 4cm) filled with soil. Acclimatization hardens off the plants after they had been growing in controlled conditions in the laboratory and prepares them for establishment in the open field. Direct transfer of un-rooted (height of 5cm), tissue-cultured plants resulted in low take of 30%. But when the un-rooted shoots were over 7cm tall, 95% of them survived. If initial plants taken to green house were un-rooted, 6-8 weeks in the glasshouse were required, but if initial plants were rooted *in vitro* this period was reduced to four weeks. Figure 7 shows fully acclimatized plants ready for field transfer.

Figure 7. Acclimatized tissue culture plants in polystyrene trays ready for field planting.

8. Performance of tissue culture plants in the field

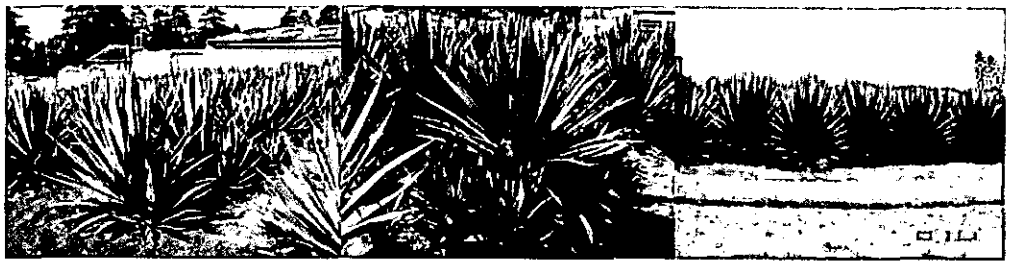
In collaboration with Teita estates, a field trial comparing traditional bulbils and tissue culture derived plants was planted in the year 2002. The parameters measured were: plant height, number of leaves, height of growing tip, and length of longest leaf.

Using 3-month average data the tissue culture and plants raised from bulbils were compared. The data was analyzed and is presented in Annex 3. Tissue culture plants were better performers and outgrew bulbil-derived plants. (Annex 3). Figure 8 shows tissue culture plants at different stages of growth.

Due to competition arising from close spacing at the nursery, Teita Estates transferred the plants to production fields late February 2004. Because of unprecedented drought conditions prevailing at the time not much growth was recorded on the plants between March and July 2004. However, the plants data on new leaves is being recorded on a monthly basis.

Figure 8a. Sorting tissue culture plants, field planting of tissue culture plants and bulbils and comparative growths of tissue culture and bulbil plants at Teita estates.

Figure 8b. Sisal Tissue Culture Plot at Plant Quarantine Station, Muguga.



III.3 Establishment of close links with sisal growers

The sisal growers have shown great interest in the MTC activities and are willing to test in their estates the tissue cultured plants. As mentioned some plants were transferred to Teita Estates (Annex 2) and in order to gain more insight into performance, the laboratory continued to transfer more material to the fields. The collaboration with Teita Estates has been very instrumental for the success and dissemination of MTC. This estate provided elite material that is paving the way to large-scale micro propagated plantations with the planting trials.

The table below shows the number of sisal plants transferred to Teita Estates in the year 2004. Plantings at Alphega (Nakuru) were planned for December 2004.

Table 3. Plants transferred to Teita Estates.

Code	Accession	Transfers March 2004	Transfers October 2004
1	H 11648	1152	1400
2	Teita selection	1536	1500
3	1200	764	1000
4	A. Hildana	887	960
5	1300	582	510
6	A. Sisalana	803	630

Crop planted in the month of March 2004 was done on blocks of tissue of tissue culture plants interchanged with blocks of bulbils. However, as from October an attempt has been made to completely randomize the plantings, to take care of variations in soil fertility etc. An example of the adopted scheme is shown below. Each plot within a block was planted with seven lines at a spacing of 35x35cm.

Figure 9. Completely randomized planting at Teita Estates

Block 1	
Hildana bulbils	11648 Tissue culture
Hildana Tissue culture	Hildana bulbils

Block 2	
11648 Tissue culture	11648 Tissue culture
Hildana bulbils	Hildana Tissue culture

Block 3	
11648 Tissue culture	Hildana Tissue culture
11648 Tissue culture	Hildana bulbils

Block 4	
11648 Tissue culture	Hildana bulbils
Hildana Tissue culture	11648 Tissue culture

III.4 Summary of results achieved

1. Sisal multiplication through tissue culture was achieved for sisal genotypes for all sisal genotypes initiated at KEPHIS. The Teita selection and 1300 were the most prolific. However all the other genotypes were able to produce a minimum of four shoots from cubes initiated in tissue culture.

2. Tissue culture generated plants at the moment cost about KES 5 (US$ 0.062) from induction to pre-nursery (including electricity, water, labour, maintenance and chemicals/reagents). This cost will be reduced with time as staff benefit from experience.

3. The age of the mother plant has a significant influence on quality, quantity and the speed of emergence of auxiliary shoots. Younger plants produced shoots earlier than old mother plants.

4. Tissue culture plants performed much better than bulbils in the pre-nurseries, where both were panted at a spacing of 35x35cm. Follow-up evaluation of the

performance of these plants up to maturity in the production fields will certainly demonstrate the superiority of tissue culture plants. Performance of plants derived from suckers from tissue culture plants and those from bulbils and from suckers from old fields will be also be compared in the future to come up with conclusive results and recommendations.

III.5 Dissemination of results

Information on the sub-component results was continuously disseminated through the Kenya Sisal Board (KSB). The interest of the growers was constantly very high and sisal estates were keen to evaluate the performance of the MTC plants against the performance of the bulbils.

It is expected that KSB, the private sisal producers and the Kenyan Government will continue supporting the laboratory in the future to put into practice the experience gained and to fully utilize the skills developed by the laboratory staff to contribute to its sustainability and to provide the sisal estates with optimal planting material.

IV. Lessons learned

IV.1 Development lessons

The performance of the KEPHIS laboratory in Kenya was better than that of the ARI Mlingano laboratory in Tanzania, despite the numerous drawbacks (like the loss of large quantities of green material during the renovation works) and the fact that less financial support was provided by the project to KEPHIS than to ARI Mlingano. Nevertheless during project implementation the laboratory could not prove to be able to delivery MTC plants in quantities sufficient to meet the demand of the sisal estates in Kenya. It is foreseen that this will be possible in the future, if the Government will keep supporting the laboratory through the KSB and the estates establish partnership agreements with KEPHIS.

The laboratory staff would have benefited from training in scientific and technical reports writing, as the quality of the reports delivered, including the information provided to prepare this report, does not adequately reflect the quality and quantity of the work done.

IV.2 Operational lessons

The international expert developed a very good relationship with the laboratory staff, and the guidance provided to the technicians and to the laboratory manager through formal and hands-on training proved to be effective.

More coordination with and/or the selection of a better contractor would have avoided the contamination occurred during the renovation works.

V. Conclusions and Recommendations

1. The sisal tissue culture as an effective, efficient means of checking the production of leaf yields of all sisal genotypes has been demonstrated by the preliminary trials. However it is necessary that the tissue culture plants be followed up throughout the productive life span of the crop. This will help answer questions like whether the performance can be sustained, how the tissue culture plants perform under pest pressure in the field, and how much suckers from tissue culture plants from pre- nurseries and multiplication fields can contribute to build-up of biomass in order to reduce production costs.

2. The follow-up of field trials and collection of other plants from the fields was slowed down by transport constraints; timely supervision of operations should be allowed.

3. The financial support of the Kenyan Government, through the KSB is vital to allow the laboratory's delivery of MTC plants to the industry; KSB role is crucial to maintain close cooperation with the estates.

Annex 1. List of equipment and chemicals/reagents purchased by the project

Table 1. Equipment

No.	Item Description	Unit of Issue	Quantity	Cost in Euros
1	Water distillation unit + phosphate Cartridge S/No 108756041 (GFL)	SET	1	2115
2	Microwave oven 20L S/No 502327005211	Unit	1	. 108.50
3	UV lamp, germicidal VL – 215G 2x15W	Unit	1	164
4	Replacement tubes for 215G lamps	Unit	2	15.80
5	Scalpel holders, stainless steel size 4	Unit	20	162.40
6	Sterile scalpel blades size 24	PKT	84	441
7	9L sterilizing autoclavable bags 300x500mm	Case of 750	3	105.60
8	3L sterilizing autoclavable bags 255x400mm	Case of 1000	2	74
9	Tape autoclave 1'' x 500'' /RL	Roll	18	99
10	EC 215 Multirange conductivity meter S/No KO13356	Unit	1	442
11	Recorder temperature RH E-9376-01	Unit	2	1170
12	Batteries for above (4pcs)	Pkt	2	13
13	Light meter E-40400-20 S/No. Q073671	Unit	1	131
14	Micropipette tips 2-200 uL (1000/cs)	Case	3	20.10
15	Micropipette tips 5-3000 uL (100/cs)	Case	2	17.90
16	Micropipette tips	Case	1	7.35
17	A)Sony Digital camera model DSC-P12, 2 batteries, software, cables, Carrying bag, 2 memory sticks (12M & 164M), adapter and manual	No	1	526.31
18	Vertical autoclave model KSG 40/60- 1-E	No.	1	10,790
19	Seed trays	No.	400	
19	Horizontal laminar flow hood complete with tubular stand, gas tap and UV germicidal lamp	No.	1	30,800.00
20	Split air conditioner 18000 BTU	No.	1	894.74
21	Window Air conditioner	No.	1	HK$ 2,483.14
22	Inste clave	No.	1	1200
23	Analytical balance with internal calibration		1	1410
24	Digital bench top pH meter			770
25	Spare electrodes for pH meter	No.	1	190
26	Dry bead sterilizer 'HP QUARTZ'		1	95
27	Dry beads for above sterilizer	PACK	10	200
28	Vacuum dessicator	No.	1	88
29	Dessicator 24-hole plate	No..	1	54
30	Thermometer with temperature probe	No.	3	174
31	Programmable plug in time switch	No.	4	100
32	Magenta GA7 vessels	No.	2000	4500
33	Polypropylene covers for above vessels	No.	1000	
34	Standard straight forceps 8''	No.	20	162
35	Adjustable volume pipette (500-5,000 µl)	No.	1	64
36	Adjustable volume pipette (100-1,000 µl)	No.	2	230
37	Adjustable volume pipette (20-200 µl)	No.	2	230
38	Adjustable volume pipette (5-50 µl)	No.	2	230
39	Micropipette tips 2-200µl	Pcs	1000	7.5
40	Timers	Pcs	2	157.9

Table 2. Chemicals/Reagents

2001

No.	Item Description	Unit of Issue	Quantity	Cost In Euros
	Inositol	Gms	700	420
	Thiamine-HCl	Gms	100	85
	Magnesium sulphate	Gms	500	69
	Calcium chloride	gms	500	68.50
	Potassium nitrate	gms	1.5	147.20
	Potassium phosphate	Gms	500	77
	Ascorbic acid	Gms	100	25
	Agar	kg	2	240
	Nicotinic acid	Gms	100	45
	Pyridoxine-HCl	Gms	100	65
	Glycine	Gms	500	54
	Ammonium nitrate	kg	1.5	195
	Zinc sulphate	Gms	500	46.50
	Copper sulphate	gms	500	98.70
	Pottasium iodide	gms	500	100
	Boric acid	gms	500	35
	Sodium molybdate	gms	100	93.50
	Iron sulphate	kg	1	144.25
	Sodium EDTA	gms	500	90
	Manganese sulphate	gms	500	139
	Calcium pantothenic acid	gms	100	23
	Ethanol	litres	10	236
	Styroseal	litres	50	158.50
	Kicstart	litres	20	48
	Peat moss	bail	5	30

2002

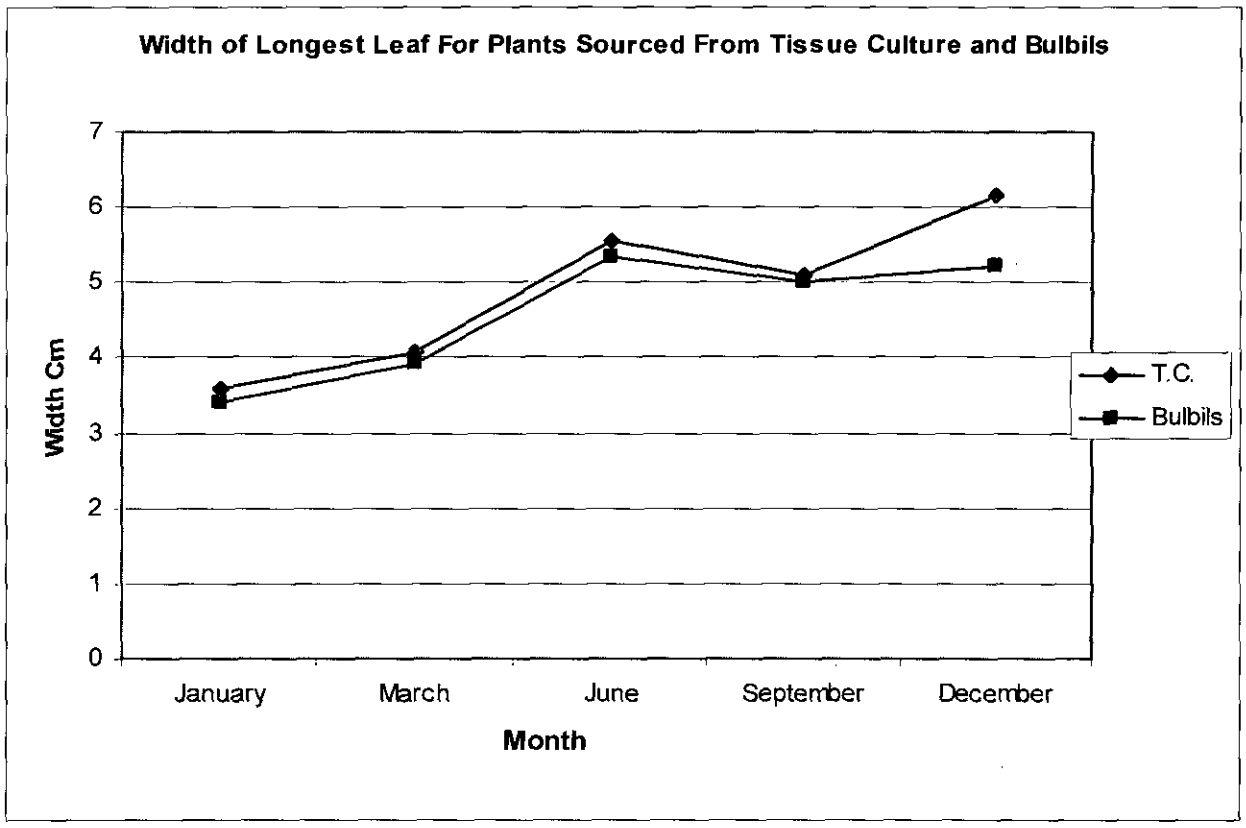
No.	Item Description	Unit of Issue	Quantity	Cost In Euros
1.	Inositol	Gms	500	140
2.	Thiamine-HCl	Gms	25	45
3.	Magnesium sulphate	Gms	500	36
4.	Calcium chloride	Kg	2	50
5.	Potassium nitrate	Kg	2	70
6.	Benzyl Ammino Purine	Gms	28	196
7.	Potassium phosphate	Gms	500	18.90
8.	Ascorbic acid	Gms	100	16.5
9.	Sucrose	Kg	50	41.30
10.	Agar	Kg	14	1,120
11.	Phytagel	Gms	500	52.50
12.	pH buffer 4 tablets	Tabs	30	14.20
13.	Nicotinic acid	Gms	200	92.10
14.	Pyridoxine-HCl	Gms	25	35.40
15.	Glycine	Gms	100	30
16.	Cobalt chloride	Gms	100	40
17.	Ammonium nitrate	Kg	2.5	100
18.	Giberellic acid	Gms	5	100
19.	Zinc sulphate	Gms	500	18
20.	Ethanol	lts	100	1298

2004				
No.	Item Description	Unit of Issue	Quantity	Cost In Euros
1.	Inositol	Gms	200	150.20
2.	Manganese sulphate	Gms	500	42.50
3.	Calcium chloride	Kg	2	59
4.	Potassium nitrate	Kg	2	42.50
5.	Benzyl Ammino Purine	Gms	15	124
6.	Tween 20	Mls	500	53
7.	Sodium hydroxide	Kg	2	42
8.	Boric acid	Kg	2	78
9.	Ferrous sulphate	Kg	1	92.40
10.	Sodium EDTA	Kg	1	65
11.	Hydrochloric acid	Litres	10	38
12.	Ethanol	Litres	82.5	594
13	Ammonium nitrate	Kg	2	120
14.	pH buffer 4 tablets	Tabs	50	30
15.	pH buffer 7 tablets	Tabs	50	30
16.	Agar	kg	50	
17.	Vitrobaf	gms		
18.	Oromex	mls	200	
16.	Pyridoxine-HCl	Gms	10	35.40

Annex 2. Comparison of the performance of tissue culture derived plants and traditional bulbils (Agave Sisalana) at Teita estates

Figure 1. Average leaf width (cm) of longest leaf of tissue cultured (TC) plants vs. bulbil plants

	January	March	June	September	December
TC	3.58	4.07	5.56	5.10	6.16
Bulbils	3.41	3.94	5.34	5.01	5.22



Note: Plants were transferred to production fields (Teita estates) in mid February 2004. The estate in consultation with KSB and KEPHIS are monitoring the crop performance. Data being recorded include rate of sucker development, number of new leaves, pest/disease incidences. Harvest data, fibre quality is also to be recorded.

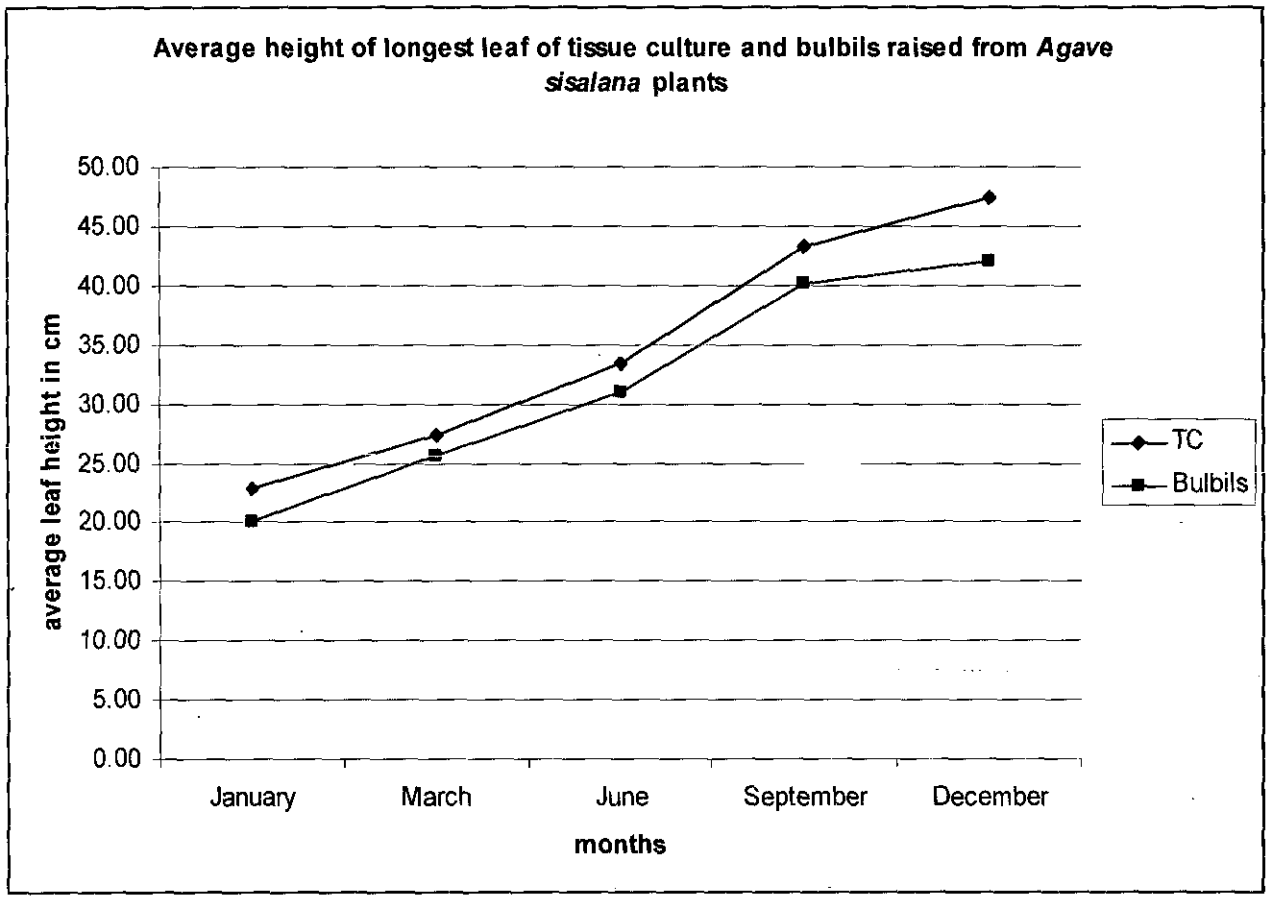
Table 1. Leaf width (cm) of longest leaf of tissue cultured (TC) plants vs. bulbil plants (Jan- Dec 2004, Teita estates)

Plant No	Tissue culture					Bulbils				
	Jan.	March	June	Sept.	Dec.	Jan.	March	June	Sept.	Dec.
1	4.00	4.50	5.70	5.90	4.5	3.50	4.00	5.80	6.00	5.5
2	4.50	4.90	5.80	6.00	6.5	4.00	4.50	5.50	6.00	6.5
3	4.00	4.00	5.10	5.20	4.5	4.00	4.30	6.00	4.00	5
4	4.00	4.20	5.60	5.80	4.5	3.00	3.90	4.80	5.00	4
5	2.00	2.20	4.00	5.20	4.5	4.00	4.50	5.50	3.00	4
6	3.50	4.30	5.90	5.90	4	3.00	4.00	5.30	4.00	5
7	4.50	4.50	3.80	3.70	5	2.50	2.50	4.80	5.00	6
8	3.50	3.70	4.00	4.60	4.8	3.00	3.50	5.60	5.00	5
9	3.30	3.90	4.00	4.80	5.5	4.00	4.20	5.50	6.00	6
10	3.60	3.70	5.00	5.30	6	2.80	4.00	5.00	6.00	5.2
11	3.80	3.90	4.90	5.00	5	3.20	4.00	4.90	4.00	3
12	3.40	3.70	5.10	5.00	6	4.00	4.30	5.40	5.00	4.5
13	3.40	3.50	5.90	6.40	5	3.10	4.00	5.60	5.00	5
14	3.70	5.00	6.20	6.40	5	4.10	4.50	4.90	4.00	6.5
15	4.40	4.50	6.10	6.20	5	3.00	4.30	5.80	4.00	5.5
16	3.70	4.00	5.70	5.80	5	4.00	4.20	5.80	4.50	5
17	3.80	4.00	5.50	5.50	6.6	4.00	4.10	4.40	4.50	5.5
18	3.50	3.60	4.80	4.70	6	1.80	2.50	5.20	5.00	5.3
19	3.00	3.50	5.00	5.00	6	4.00	4.10	5.80	5.00	6.6
20	4.00	4.80	7.00	6.70	6	4.00	5.00	4.90	5.00	5.5
21	3.50	4.50	5.20	5.20	4.2	3.50	4.20	4.60	4.00	6
22	4.50	4.60	5.60	6.00	5	3.00	3.80	5.90	4.00	2
23	4.00	4.10	6.00	5.70	5	4.00	4.30	5.34	6.00	5.5
24	3.00	4.00	5.50	5.30	5.2	2.50	2.60	4.90	5.00	6.8
25	4.00	4.30	6.60	6.20	6	3.00	4.00	4.70	4.00	6
26	4.00	3.80	5.80	5.50	6.5	3.50	2.80	6.00	5.00	5.5
27	3.00	3.30	5.70	5.90	4	3.50	4.30	6.00	6.00	6
28	3.60	3.90	5.70	6.00	5	4.00	4.30	5.90	6.00	5.5
29	4.50	5.00	6.90	6.50	6.5	3.50	4.40	5.40	6.00	6
30	4.10	4.50	6.20	6.10	5	3.30	4.30	6.20	6.00	6
31	3.70	3.90	5.30	4.70	4.3	4.50	4.50	4.30	4.00	5
32	3.90	4.30	6.50	6.50	5.5	3.30	4.00	5.10	5.00	6
33	3.70	4.30	6.30	6.00	5	3.90	4.00	5.90	4.00	5
34	3.10	3.90	6.40	5.60	5.5	4.00	3.90	5.90	5.00	5.5
35	3.20	4.00	5.70	6.00	5.5	4.60	4.60	5.90	5.00	5.5
36	3.50	4.70	6.40	6.50	6.5	4.10	4.30	5.20	6.00	4
37	1.40	4.80	6.30	6.50	5	4.20	4.00	5.70	5.20	5.5
38	3.20	3.50	5.90	5.50	5	4.00	4.30	5.50	5.50	4.5
39	4.50	4.20	5.60	5.00	5.5	3.50	4.40	4.50	5.50	4.5
40	3.50	4.00	5.60	5.70	6	2.50	3.30	5.30	5.50	4
41	3.50	4.20	5.60	5.20	5.8	3.00	4.00	5.60	6.00	5
42	3.50	3.70	5.30	5.00	4.3	3.50	4.00	5.50	5.50	6
43	4.00	3.90	5.60	4.90	5	3.00	3.70	5.30	5.00	5
44	3.00	4.00	5.70	4.80	5	2.00	3.90	5.70	6.00	4.5
45	3.50	3.50	5.20	5.30	58	3.50	4.00	5.80	5.00	3
46	3.00	3.40	4.80	4.10	6	3.50	4.20	5.50	5.00	5

Plant No	Tissue culture					Bulbils				
	Jan.	March	June	Sept.	Dec.	Jan.	March	June	Sept.	Dec.
47	3.00	3.90	5.20	5.50	5.2	3.50	4.20	5.20	3.50	5
48	3.50	4.10	5.40	4.90	4.5	2.80	3.60	4.00	5.00	5
49	3.50	4.90	6.10	6.50	5.7	2.80	2.90	5.60	5.00	5
50	3.80	4.00	5.70	5.20	4	4.00	3.90	5.30	5.00	5.5
51	3.30	4.00	5.50	5.80	4.8	2.30	3.50	5.00	6.00	6
52	3.00	4.20	5.30	5.20	5.5	2.50	3.00	5.10	5.00	6
53	4.30	4.50	6.50	6.00	3.8	3.00	4.00	4.80	5.00	5
54	3.20	3.50	5.00	4.80	5	2.70	3.50	5.60	5.00	5
55	2.90	3.50	4.50	4.00	5.5	4.00	3.60	5.50	5.00	5
56	4.00	4.50	6.00	6.00	5.7	4.20	4.60	5.00	5.10	6
57	3.70	4.10	5.50	5.00	6	2.90	3.00	5.30	5.00	6
Average	**3.58**	**4.07**	**5.56**	**5.50**	**6.16**	**3.41**	**3.94**	**5.34**	**5.01**	**5.22**

Figure 2. Average height of longest leaf of tissue culture (T.C.) plants vs. bulbils plants.

	January	March	June	September	December
TC	22.79	27.37	33.31	43.22	47.37
Bulbils	20.03	25.56	31.04	40.11	42.07



Note: Plants were transferred to production fields (Teita estates) in mid February 2004. The estate in consultation with KSB and KEPHIS are monitoring the crop performance. Data being recorded include rate of sucker development, number of new leaves, pest/disease incidences. Harvest data, fibre quality is also to be recorded.

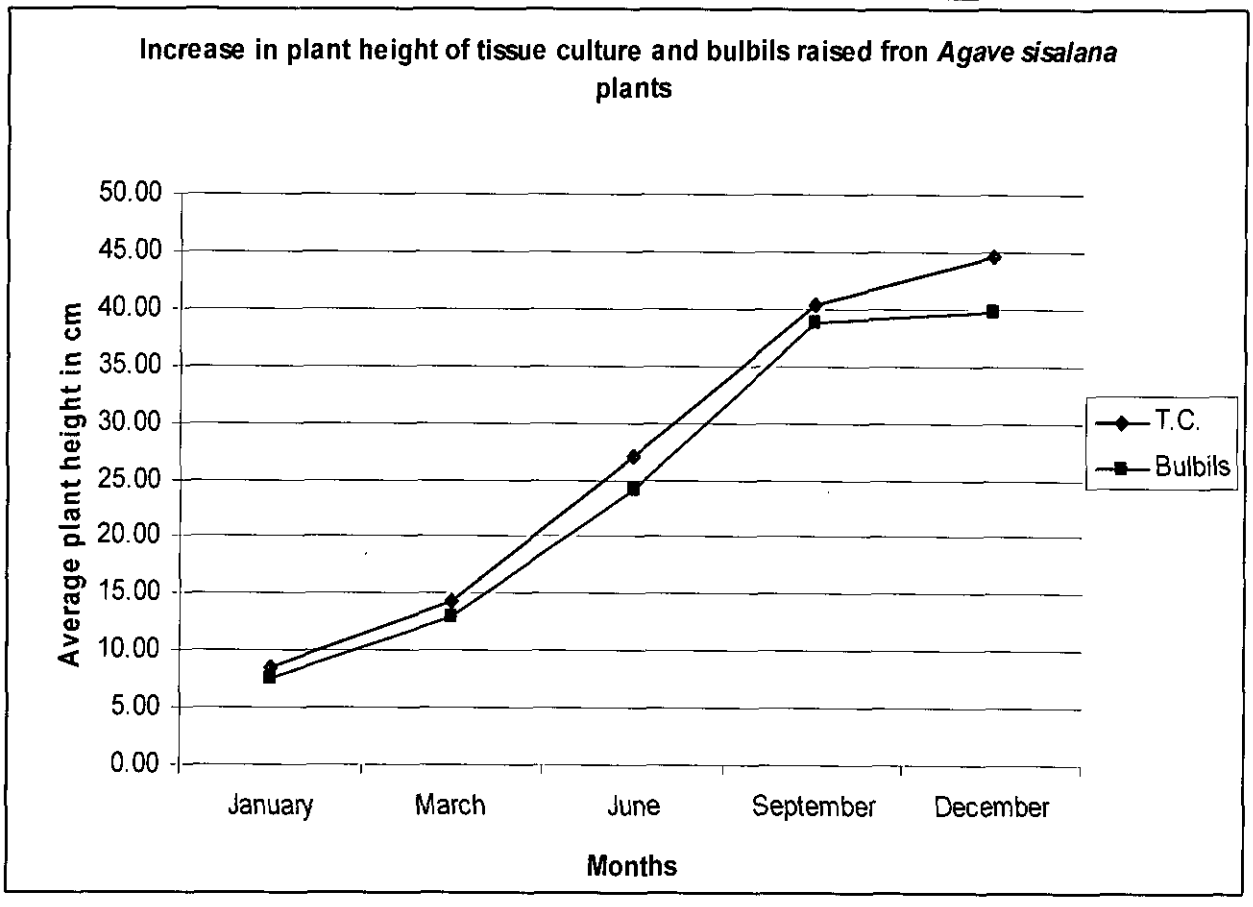
Table 2. Height (in cm) of longest leaf of tissue culture (TC) plants vs. bulbils plants. (Jan- Dec 2004, Teita estates).

Plant No	Tissue culture					Bulbils				
	Jan.	March	June	Sept.	Dec.	Jan.	March	June	Sept.	Dec.
1	28.50	28.00	37.50	57.50	50	23.00	27.00	33.80	46.00	45
2	31.50	31.00	39.50	57.50	58	26.50	29.00	35.60	49.00	54
3	31.50	31.80	31.80	48.00	48.5	25.00	28.00	34.90	37.00	28
4	26.00	26.50	32.50	34.00	40	18.00	21.00	23.00	30.00	25
5	6.00	15.50	28.50	33.00	40	23.00	29.00	30.00	25.00	40
6	26.00	32.50	36.00	45.00	44	20.50	26.00	26.50	25.00	35
7	32.50	35.00	29.00	32.00	46	11.00	11.50	29.70	40.00	39
8	22.50	23.00	23.50	27.50	34	16.50	24.50	32.50	44.00	41
9	25.80	26.00	28.00	43.00	53	25.10	26.00	31.20	48.00	44
10	23.50	27.00	33.70	46.00	52	12.00	28.00	33.00	45.00	47
11	27.60	28.00	27.70	37.00	18	17.80	30.00	32.50	33.00	36
12	19.00	25.00	27.50	40.00	36	22.50	29.50	32.80	40.00	43
13	29.50	32.00	32.90	57.00	43	19.20	30.50	38.80	49.00	40
14	28.00	30.50	39.00	57.00	60	24.10	32.00	31.10	48.00	48
15	30.00	30.50	37.70	56.00	60	21.00	29.00	36.00	35.00	31
16	29.50	29.20	37.70	45.00	51	26.00	30.30	32.40	36.00	37
17	25.50	26.50	38.80	44.00	40	23.50	27.50	29.00	40.00	56
18	24.50	27.00	29.00	35.00	45	12.20	19.50	29.20	32.00	52
19	26.00	28.50	31.00	39.00	54	22.40	26.00	37.70	30.00	49
20	23.00	32.00	39.50	42.00	56	30.00	33.50	30.00	53.00	45
21	26.50	27.00	33.50	44.00	52	21.00	28.50	28.70	37.00	35
22	26.00	31.00	39.20	54.00	20	20.00	23.00	34.30	34.00	42
23	23.50	26.00	35.50	54.00	52	24.00	28.00	31.03	47.00	47
24	16.00	22.00	26.00	30.00	58	9.00	11.00	28.50	39.00	46
25	28.00	31.00	41.00	53.00	50	19.00	25.00	26.50	36.00	52
26	26.50	29.00	30.80	57.00	60	14.00	19.00	35.00	48.00	55
27	12.50	24.50	30.70	48.00	59	25.50	29.00	33.70	50.00	28
28	21.50	27.50	33.20	53.00	42	29.00	29.00	33.00	45.00	40
29	25.90	34.00	30.90	52.00	56	22.00	28.00	29.50	45.00	65
30	22.40	28.30	39.60	41.00	50	23.50	27.00	38.50	35.00	43
31	24.50	26.50	34.50	35.00	47	24.30	29.50	29.50	28.00	24
32	24.20	30.00	38.00	40.00	57	18.50	24.50	30.00	39.00	45
33	16.50	31.50	38.50	40.00	43	18.50	26.00	36.00	45.00	45
34	23.60	27.00	33.00	39.00	51	24.60	29.00	35.50	37.00	38
35	25.80	26.00	34.50	40.00	53	22.90	28.50	34.30	43.00	48
36	21.50	33.50	40.20	43.00	41	21.50	27.00	33.90	40.00	53
37	14.50	31.00	37.30	41.00	56	22.50	23.00	33.70	36.00	39
38	19.70	23.00	31.00	36.00	45	26.00	29.00	32.60	42.00	44
39	25.00	30.00	33.50	48.00	42	21.00	30.50	26.00	41.00	47
40	22.00	29.00	34.60	54.00	36	14.00	23.00	33.80	42.00	40
41	21.00	25.50	31.80	48.00	36	24.00	28.50	29.50	50.00	50
42	16.00	20.00	27.00	42.00	56	19.00	24.50	30.70	39.00	30
43	22.50	23.50	34.00	34.00	51	17.50	24.50	26.00	42.00	43
44	14.50	22.50	29.00	29.00	36.5	15.00	23.00	31.70	44.00	51
45	20.00	25.00	35.30	43.00	36	23.00	26.50	30.00	41.00	48
46	15.00	22.00	28.50	53.00	45	20.00	26.50	30.50	45.00	55

Plant No	Tissue culture					Bulbils				
	Jan.	March	June	Sept.	Dec.	Jan.	March	June	Sept.	Dec.
47	19.40	22.00	28.30	42.00	37	20.20	27.50	26.00	33.00	40
48	25.60	26.50	35.10	48.00	52	15.30	23.50	20.00	40.00	35
49	21.10	31.00	35.00	41.00	49	10.10	14.50	34.00	40.00	35
50	20.60	25.00	31.50	32.00	53	22.50	23.00	20.20	39.00	28
51	21.90	29.00	36.60	32.00	51	11.50	21.50	27.80	50.00	31
52	21.10	24.50	29.00	50.00	60	12.00	18.50	31.50	50.00	46
53	21.50	29.50	37.40	34.00	38	19.20	28.00	27.40	41.00	24.5
54	18.50	25.00	30.30	55.00	50	16.10	23.50	29.00	36.00	36.5
55	12.00	19.00	25.00	26.00	41	20.30	25.00	32.60	31.00	43
56	23.80	29.00	36.60	38.00	42	19.90	24.00	26.90	35.00	41
57	21.80	28.00	31.80	39.00	68	15.90	19.00	32.00	36.00	50
Average	**22.79**	**27.37**	**33.31**	**43.22**	**47.37**	**20.03**	**25.56**	**31.04**	**40.11**	**42.07**

Figure 3. Average increase in plant height (in cm.) of tissue culture (TC) plants vs. bulbils plants.

	January	March	June	September	December
T.C.	8.46	14.35	27.06	40.32	44.57
Bulbils	7.46	13.03	24.20	38.77	39.80



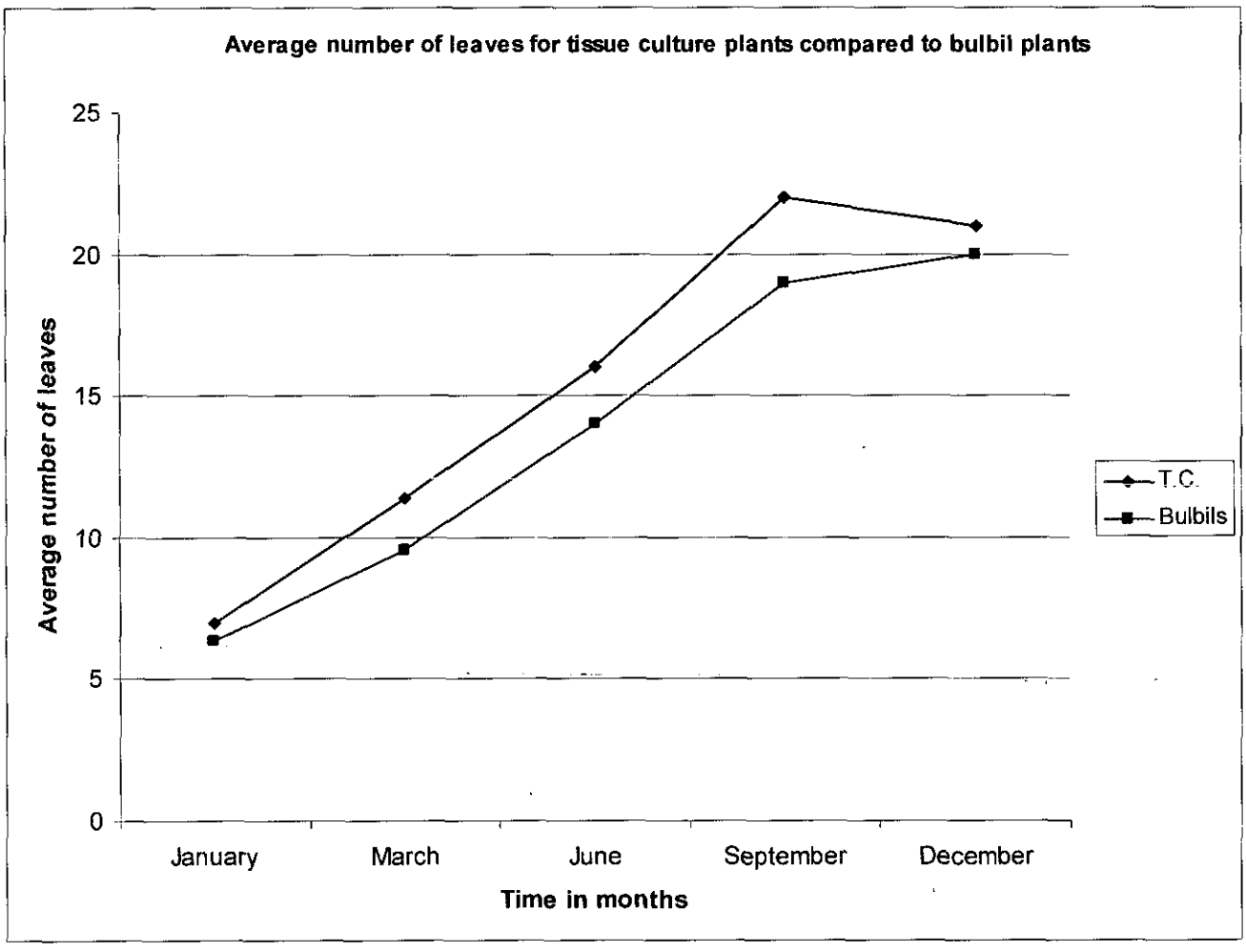
Note: Plants were transferred to production fields (Teita estates) in mid February 2004. The estate in consultation with KSB and KEPHIS are monitoring the crop performance. Data being recorded include rate of sucker development, number of new leaves, pest/disease incidences. Harvest data; fibre quality is also to be recorded.

Table 3. Increase in plant height (in cm) of tissue culture (TC) plants vs. bulbils plants. (Jan- Dec 2004, Teita estates).

Plant No	Tissue culture					Bulbils				
	Jan.	March	June	Sept.	Dec.	Jan.	March	June	Sept.	Dec.
1	4.00	18.00	30.60	53.00	47	4.00	12.00	30.00	48.00	61
2	9.00	16.00	20.00	53.00	55	12.50	20.00	32.20	47.00	50
3	2.50	18.00	25.00	47.00	44	8.00	15.50	32.10	35.00	25
4	12.00	14.50	28.50	35.00	38	6.00	9.00	21.00	25.00	22
5	3.00	9.00	21.00	35.00	35.5	8.00	12.50	25.00	29.00	39
6	12.00	21.00	29.00	40.00	42	4.00	14.00	24.00	27.00	28
7	15.00	20.00	28.50	28.00	43	6.50	10.50	19.50	40.00	37
8	12.00	8.50	23.00	27.50	36	8.00	9.50	27.00	44.00	36
9	9.50	11.50	24.00	43.00	26	12.50	14.50	21.50	48.00	42
10	10.00	12.50	29.50	42.00	50	11.90	11.50	22.50	46.00	41
11	9.20	16.00	20.00	37.00	17	10.50	14.50	23.00	33.00	39
12	8.50	15.50	19.50	38.00	35	7.00	14.50	20.00	40.00	44
13	11.00	17.00	30.00	56.00	40	10.00	11.50	27.00	49.00	39
14	9.50	18.50	36.50	40.00	55	5.00	14.50	22.50	30.00	47
15	13.50	18.50	37.50	51.00	50	6.50	14.00	29.50	31.00	20
16	12.80	14.50	33.00	43.00	47	8.50	14.50	25.00	36.00	34
17	9.50	14.50	30.00	43.00	35	7.50	15.50	16.50	45.00	54
18	10.10	10.00	25.00	39.00	50	4.00	7.00	20.00	35.00	44
19	13.40	10.00	26.00	40.00	53	10.50	15.50	26.00	36.00	47
20	9.00	18.50	32.20	44.00	52	12.00	22.50	26.00	47.00	43
21	5.00	14.50	26.00	41.00	50	6.00	15.00	22.00	38.00	33
22	8.00	17.50	36.90	54.00	19	10.00	10.00	30.00	31.00	41
23	9.50	13.00	23.50	47.00	51	8.50	16.00	24.40	43.00	46
24	5.00	11.00	20.00	28.00	57	4.00	5.50	22.00	36.00	45
25	11.00	16.00	32.90	47.00	49	4.00	15.50	16.00	34.00	51
26	8.50	18.50	30.00	54.00	61	10.00	11.50	28.00	42.00	55
27	10.00	12.50	24.50	43.50	56	8.00	13.50	27.00	48.00	17
28	8.90	13.50	25.00	52.00	41	13.10	18.50	30.50	48.00	39
29	15.00	22.00	33.10	45.00	55	11.30	13.50	22.60	53.00	64
30	8.00	14.00	29.00	39.00	51	8.20	16.50	31.60	35.00	41
31	4.00	11.00	22.00	30.00	46	7.00	12.50	19.00	28.00	12
32	9.50	20.00	33.50	38.00	55	2.00	13.00	27.50	39.00	42
33	2.50	16.00	31.00	40.00	40	8.00	14.00	30.80	40.00	41
34	10.50	16.00	26.00	46.00	50	8.50	13.50	30.00	36.00	44
35	13.00	14.50	30.00	35.00	51	9.50	17.50	29.50	40.00	44
36	11.00	18.50	29.00	43.00	40	9.00	16.50	29.00	37.00	52
37	2.50	12.50	30.70	36.00	55	5.00	15.00	30.00	34.00	38
38	4.50	12.00	22.50	35.00	44	10.50	14.00	27.00	37.00	43.5
39	9.00	16.50	28.00	32.00	29	5.00	13.50	16.50	39.00	45
40	11.60	16.50	32.00	42.00	35	8.00	9.00	26.00	39.00	42
41	8.50	13.50	31.00	46.00	34	4.00	13.50	22.00	52.00	49
42	4.00	9.00	20.50	47.00	59	5.00	8.50	22.00	40.00	22
43	12.00	13.00	29.50	39.00	47	11.00	13.50	19.00	39.00	40
44	5.00	14.50	17.50	30.00	31	8.00	15.00	25.00	39.00	45
45	5.50	13.50	26.50	40.00	34	8.50	12.50	24.00	37.00	45

Plant No	Tissue culture					Bulbils				
	Jan.	March	June	Sept.	Dec.	Jan.	March	June	Sept.	Dec.
46	5.00	10.50	18.00	24.00	44	6.00	10.50	22.00	45.00	49
47	6.50	10.50	24.50	38.00	36	6.60	11.00	19.50	37.00	39
48	5.50	9.50	27.00	40.00	50	3.00	10.00	12.40	39.00	31
49	8.50	18.50	23.00	36.00	48	6.00	8.00	28.30	40.00	36
50	7.90	10.00	23.50	39.00	52	9.50	15.00	20.00	31.00	26
51	10.50	16.00	30.00	42.00	46	4.50	13.50	18.50	40.00	30
52	6.50	14.00	27.50	37.00	49	3.50	8.00	25.50	32.00	44
53	5.00	13.50	30.00	38.00	36	6.00	13.00	19.00	39.00	24
54	7.10	10.50	25.50	41.00	47	2.90	10.50	24.50	38.00	34
55	4.00	6.50	16.00	31.00	37	7.50	11.00	27.00	37.00	41
56	12.00	14.00	31.00	34.00	40	8.50	12.50	16.00	41.00	40
57	6.50	13.00	27.00	34.00	65	6.00	9.50	25.00	36.00	46
Average	**8.46**	**14.35**	**27.06**	**40.32**	**44.57**	**7.46**	**13.03**	**24.20**	**38.77**	**39.80**

Figure 4. Average number of leaves of tissue culture (TC) plants vs. bulbils plants.



Note: Plants were transferred to production fields (Teita Estates) in mid February 2004. The estate in consultation with KSB and KEPHIS are monitoring the crop performance. Data being recorded include rate of sucker development, number of new leaves, pest/disease incidences. Harvest data, fibre quality is also to be recorded.

Table 4. Number of leaves of tissue culture (TC) plants vs. bulbils plants (Jan- Dec 2004, Teita Estates).

Plant No	Tissue Culture					Bulbils				
	Jan.	March	June	Sept.	Dec.	Jan.	March	June	Sept.	Dec.
1	10	15	22	29	21	7	12	18	19	22
2	10	15	22	29	30	8	12	16	22	26
3	7	12	14	23	25	8	12	16	19	12
4	7	12	18	21	21	6	8	11	14	8
5	5	8	13	19	14	6	9	13	16	22
6	8	11	16	17	18	8	10	15	18	12
7	8	12	15	17	19	3	2	5	9	17
8	5	10	14	19	17	5	7	17	18	15
9	6	10	16	23	27	7	12	13	22	16
10	8	12	18	20	21	2	9	12	21	27
11	8	11	16	20	9	6	9	16	20	14
12	9	11	16	19	14	8	12	12	20	22
13	7	11	16	26	22	6	9	16	21	15
14	8	13	19	26	36	7	12	17	21	19
15	8	14	20	26	24	6	8	17	22	16
16	8	14	20	23	21	8	12	15	20	17
17	7	11	18	20	22	7	10	15	17	27
18	6	10	15	18	20	5	6	8	12	19
19	5	10	14	19	23	7	8	13	15	23
20	10	16	23	26	19	11	16	15	28	20
21	7	10	15	19	23	7	10	14	19	15
22	8	14	19	29	6	6	10	15	18	21
23	7	12	17	20	25	9	13	14	24	33
24	7	10	13	15	25	2	3	14	18	21
25	8	14	20	29	22	7	9	15	22	20
26	7	8	12	26	30	4	5	6	11	27
27	6	10	14	20	25	7	12	17	21	12
28	7	11	16	27	16	8	12	17	28	19
29	9	15	21	24	25	8	13	15	21	33
30	7	12	17	20	25	7	10	15	18	15
31	8	10	13	25	21	7	11	10	18	7
32	8	12	18	22	24	5	7	10	12	21
33	6	12	17	21	18	7	11	14	18	22
34	5	11	15	18	20	7	10	19	21	17
35	8	14	19	21	29	9	14	19	21	19
36	7	13	20	23	15	8	13	19	21	25
37	3	13	18	23	27	8	13	20	22	19
38	5	10	15	21	16	8	12	14	21	21
39	8	13	19	22	17	6	9	15	20	23
40	7	10	15	26	6	4	6	14	19	20
41	8	13	19	26	18	6	10	11	19	23
42	6	9	12	25	27	5	8	12	24	15
43	5	10	13	16	19	5	8	10	17	21
44	6	8	11	13	12	5	7	16	21	23
45	7	12	17	20	14	8	11	14	19	23
46	5	6	9	21	28	7	10	13	17	27

Plant No	Tissue Culture					Bulbils				
	Jan.	March	June	Sept.	Dec.	Jan.	March	June	Sept.	Dec.
47	7	12	16	24	14	6	9	11	21	23
48	7	10	14	26	20	6	8	6	14	18
49	9	14	19	22	23	2	4	17	20	18
50	5	9	13	13	26	8	12	16	19	10
51	6	10	15	16	26	10	12	8	20	16
52	5	8	13	25	29	3	6	12	25	24
53	7	12	18	19	16	5	8	10	14	8
54	6	10	14	19	20	5	7	14	15	19
55	5	9	12	15	20	6	10	19	20	20
56	8	12	18	25	18	7	13	7	31	22
57	8	12	18	22	31	3	5	9	11	24
Average	**7**	**11**	**16**	**22**	**21**	**6**	**18**	**14**	**19**	**20**

Annex 3. Different Stages of *in vitro* multiplication of sisal by tissue culture

Year 2003

	January – March 2003			April – June 2003		
Accession	**Growth**	**Multiplica-tion**	**Rooting/ Acclimati-zation**	**Growth**	**Multiplica-tion**	**Rooting/ Acclimati-zation**
A. Sisalana	100	1,130	1,152	83	812	407
Hybrid 11648	158	1,370	1007	120	1130	412
1300	170	304	252	160	464	188
Teita selection	1,606	956	245	1,780	1,080	70
Blue Sisal	0	0	0	60	210	0
Mlola 487	20	35	0	0	15	42
1200	149	540	210	120	798	30
Mwatate selection	0	140	140	340	280	12
A. Hildana	45	380	112	34	562	150
Total	**2,248**	**4855**	**3,118**	**2699**	**5,351**	**1,311**

Year 2003 (continued)

	July – September 2003			October – December 2003		
Accession	**Growth**	**Multiplica-tion**	**Rooting/ Acclimati-zation**	**Growth**	**Multiplica-tion**	**Rooting/ Acclimati-zation**
A. Sisalana	36	898	766	120	488	1,173
Hybrid 11648	25	654	1,122	198	1,298	1,534
1300	172	226	678	50	139	860
Teita selection	230	873	698	407	4,280	400
Blue Sisal	30	600	120	65	640	142
Mlola 487	10	20	60	10	165	150
1200	130	213	910	283	2,284	940
Mwatate selection	200	420	200	53	198	234
A. Hildana	106	252	915	79	490	1065
Total	**941**	**4,266**	**5,469**	**1,265**	**9,982**	**6,498**

Different Stages of *in vitro* multiplication of sisal by tissue culture (continued)

Year 2004

	January – March 2004			
Accession	**Initiation**	**Growth**	**Multiplication**	**Rooting / Acclimatization**
A. Sisalana	140	252	987	514
Hybrid 11648	1,130	2,262	2,276	602
1300	47	209	392	51
Teita selection	0	2,605	3,878	3,353
Blue sisal	0	691	81	1,066
Mlola 487	0	113	360	0
1200	0	1,523	3,367	1,514
Mwatate selection	0	0	0	0
A. Hildana	49	70	836	216
Total	**1,366**	**7,725**	**12,177**	**7,316**

	April – June 2004			
Accession	**Initiation**	**Growth**	**Multiplication**	**Rooting / Acclimatization**
A. Sisalana	316	118	219	307
Hybrid 11648	180	1,228	2,086	2,058
1300	0	481	519	2,341
Teita selection	0	768	2,848	1,155
Blue sisal	0	691	8	1,066
Mlola 487	0	113	360	0
1200	0	1,523	3,367	1,514
Mwatate selection	0	0	0	0
A. Hildana	0	123	690	1,906
Total	**496**	**5,185**	**7,383**	**8,916**

	July –September 2004			
Accession	**Initiation**	**Growth**	**Multiplication**	**Rooting / Acclimatization**
A. Sisalana	0	112	471	434
Hybrid 11648	0	2,300	2,084	1,910
1300	0	368	266	1,255
Teita selection	0	2,886	3,562	2,606
Blue sisal	0	531	885	1,245
Mlola 487	0	169	280	242
1200	0	7,178	2,350	4,470
Mwatate selection	0	649	120	237
A. Hildana	0	1,393	649	1,338
Total	**0**	**15,586**	**10,667**	**11,737**

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