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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

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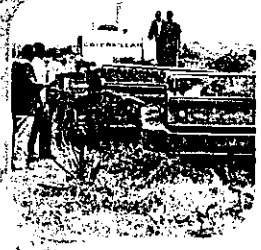
COMMON FUND FOR COMMODITIES
Project CFC/FIGHF/07

Product and market development of sisal and henequen


Multiplying Sisal by Meristematic Tissue Culture

Project completion report/Addendum A.5
Part Two: Tanzania

Tanzania, October 1998–September 2004

COMMON FUND FOR COMMODITIES

Product and market development of sisal and henequen

Project completion report, Addendum A.5—Part Two: Tanzania

Multiplying Sisal by Meristematic Tissue Culture

Tanzania
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.
General guidance and consolidation: Ms. Mayra. Sanchez Osuna, Project Manager.
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Common Fund for Commodities (CFC)
Stadhouderskade 55
1072 AB Amsterdam
P.O. Box 74656
1070 BR Amsterdam
The Netherlands
Telephone (++31 20) 575 4949
Fax (++31 20) 676 0231
e-mail: managing.director@common-fund.org
website: www.common-fund.org

United Nations Industrial Development Organization
Vienna International Centre
P.O. Box 300
A-1400 Vienna
Austria
Telephone (++43 1) 26026 3945
Fax (++43 1) 26026 6819
e-mail: M.Sanchez-Osuna@unido.org
website: www.unido.org

Food and Agriculture Organization of the United Nations
Commodities and Trade Division
Via delle Terme di Caracalla
00100 Rome
Italy
Telephone (++39 06) 5705 4201
Fax (++39 06) 5705 4495
e-mail: esc-registry@fao.org
website: www.fao.org

Project Completion Report

Sub-component A.5 Tanzania
"Multiplying Sisal by Meristematic Tissue Culture"

CONTENT

Abbreviations and acronyms

A.	Agave
ARI	Agricultural Research Institution
BAP	Benzyl Amino Purine
CFC	Common Fund for Commodities
CICY	Centro de Investigación Científica de Yucatán
H/Hyb.	Hybrid
IBA	Indole Butyric Acid
KEPHIS	Kenya Plant Health Inspectorate Service
KLS	Korogwe leave spot
ML	Mlola
MTC	Meristematic Tissue Culture
NCC	National Coordinating Committee
N.F.S.	Non-flowering sisal
PLTR	Purple leave tip roll
spp	Species
TC	Tissue culture
TSB	Tanzania Sisal Board
TZS	Tanzanian Shilling
UNIDO	United Nations Industrial Development Organization
WAU	Wageningen University of Agriculture (the Netherlands)
2,4-D	Dichlorophenyl Acetic Acid

I. Project sub-component summary

1. Title: Multiplying Sisal by Meristematic Tissue Culture

2. Location: Tanga (Tanzania) at the Agricultural Research Institute (ARI) Mlingano

3. Starting Date: October 1998

4. Completion Date: September 2004

5. Sub-component external financing – excluding counterpart contribution

Total subcomponent cost:	US$ 471,943
of which:	
CFC Financing:	US$ 237,446
Belgium Government:	US$ 81,088
UNIDO	US$ 153,409

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

II.1 Background and context

Sisal (*Agave Sisalana*) is a perennial monocarpic plant, which flowers once in its life, after which the plant dies. The best planting material are the bulbils produced at the flowering stage at 8-10 years of plant's age. The plant can also be propagated using suckers or rhizomes but their quality is low, fewer propagation materials can be obtained per unit area and it takes a long time to obtain the materials. The establishment of a bulbil nursery is one of the most costly undertakings in sisal cultivation for both the estate growers and the smallholders. Furthermore low replanting rate in the sisal industry have also contributed to inadequate poling plants to produce bulbils.

Indiscriminate planting of low quality materials has been a common phenomenon in most sisal estates especially in the period when the prices of fibre went down and production costs increased. This resulted in a considerable reduction in fibre yield per unit area (*Sisal Annual Report 1980*).

Production of sisal for pulp production has enhanced the need for mass propagation of good quality material in a short time to meet increasing demand. Taking all these factors into consideration, the application of modern biotechnologies was recommended to multiply the most promising cultivars by tissue culture.

In the past fifteen years the sisal industry has been desperately looking for a quick method of multiplying good quality planting material to restore productivity of the commercial cultivars. An initial micropropagation experiment of sisal by tissue culture was carried out in 1988 under the arrangement between the Tanzania Government (ARI Mlingano) and a private sisal estate (Amboni Ltd). The Wageningen University of Agriculture (WAU) (the Netherlands) and the Swiss Federal Research Station for Fruit Growing Viticulture assisted to propagate H.11648, and a total of 200 plants were brought back to Tanzania and planted in one of the Mjesani sisal estates which was owned by Ralli Estates. Out of the introduced plants 132 survived and were assessed in comparison with plants raised from ordinary bulbils. The comparison was performed in the second cut of the propagated plants, when the estate management was changed. The plants did not show any significant differences in terms of growth parameters and fibre yield and this initial trial proved that sisal could be propagated by tissue culture.

Micropropagation techniques have been reported to play a vital role in providing good quality planting material in henequen (M. Robert, *Micropropagation of Agaves*, 1992).

II.2 Objectives, outputs and targeted beneficiaries

The main objective of sub-component A.5 was to multiply promising sisal cultivars by MTC in order to promote the production of standard planting material, shorten the time needed by breeders to have planting material for commercial production and ultimately reduce the costs of planting material.

In particular the specific objectives of the sub-component were as follows:

- To multiply sisal bulbils by tissue culture and supply seedlings which are uniform and genetically pure to the sisal industry
- To speed up the breeding program of the crop through rapid mass propagation and evaluation
- To maintain a sisal gene bank by *in vitro* culture.

The expected output included the definition of a MTC technique for sisal and the production of a technical manual, for its application and production of new plants through the new system. The output was to be reached through the implementation of the following activities:

- Definition of a detailed program in consultation with other institutions carrying out MTC activities;
- Refurbishment of the laboratory and procurement of equipment for it; construction of a green house;
- Recruitment of technical advisory staff both local and international;
- Training of laboratory staff;
- Implementation of efficient methodologies for micropropagation of the agaves included in the project;
- Culturing new sisal plantlets under the guidance of experienced institutions;
- Raising plantlets in the green house;
- Transferring plants in open /closed nurseries to farmers' fields;
- Preparation of sisal research Business Plan;
- Preparation of final report and manual;
- Preservation of the sisal gene bank at ARI Mlingano and establishment of an *in vitro* bank.

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.

The project sub-component had the following strategy to achieve the expected output:

- Establishment of a well equipped tissue culture laboratory at ARI Mlingano and training of local personnel on basic tissue culture techniques;
- Implementation of efficient methodologies for micropropagation of selected agaves;
- Implementation of necessary activities to save the sisal gene bank at ARI Mlingano;
- Establishment of close links with sisal growers.

III. Implementation and results achieved

III.1 Establishment of an MTC laboratory

III.1.1 Designing and building the laboratory

The tissue culture (TC) laboratory is housed in the sisal pathology laboratory. The project international consultant, Dr, Manuel Robert from CICY, MEXICO, did the designing of the laboratory. The works done involved the laboratory and the green house (total renovation costs: TZS 12,332,134); they started in 1998 and finished in 2000 and included the following:

- MTC laboratory:
  Block walling, work bench sand and floor finishing
  Windows and doors
  Roofing and ceilings
  Plumbing and drainage system
  Fumigation
  Painting
  Electrical installation.

- Green house
  Reinforced columns
  Vertical wall extension
  Rescreeding floor;
  Construction of concrete benches
  Roof work;
  Frame for mosquito gauze.

The water system was also renovated by installation of water harvesting system, underground tank (50,000 litres) and overhead sim tank (5,000 litres). The two growth rooms and the storage room were equipped with shelves; and lockers in the laboratory and dressing room were provided. The generator was renovated; the lighting system in the growth rooms installed and air conditioners were purchased and fitted.

III.1.2 Equipping the laboratory

The laboratory was fully equipped by the project; the international consultant Dr. Manuel Robert in consultation with the local consultant Mr. Kennedy Mkumbo prepared a list of required equipment and reagents by . More equipment and reagents were procured throughout the project time. Annex 1 includes the list of laboratory equipment and reagents provided by the project. Consumables were also provided to the laboratory.

The project also procured office equipment and a car.

III.1.3 Training of laboratory staff

As the personnel at ARI Mlingano had no previous experience in biotechnology, training was provided to the laboratory staff. Initially it was planned that research officers and technicians should undergo three and two months training respectively. Unfortunately, this was not accomplished and training was as shown below:

Mr. Shabani Hamisi	4 weeks training at CICY Mexico (see Annex 5)
Ms. Beatrice Mlay	6 weeks training at ARI Mikocheni
Ms. Laddy Swai	3 weeks spilt programme training in South Africa
Ms. Anna Mhando	4 weeks training at ARI Mikocheni
Mr. Hassani Kiuluga	4 weeks training at ARI Mikocheni

Local and international consultants offered other informal on-the-job training during their periodic consultation missions to Mlingano, including a course on management of a tissue culture laboratory. Exchange visits also contributed to exchange experiences between ARI Mlingano and KEPHIS.

The following staff were offered exchange visit to KEPHIS:

Mr. Shabani Hamisi	3 days in 2000
Mrs. Beatrice Mlay	3 days in 2000 and 2 weeks in 2004
Mr. Hassani Kiuluga	2 weeks in 2004

A 1-day workshop on scientific and technical report writing was given in September 2002. During the workshop it was stressed how the information should be organized and presented in a CFC technical paper and how important it was to present the results achieved.

III.2 Experimental activities and establishment of a protocol

Experimental activities, carried out during the project period to define the most suitable tissue culture method for the agaves considered, are as follows:

- Management of contamination, including: maintenance of aseptic environment in MTC laboratory, surface sterilization of mother plants and explants to control exogenous contaminants, use of antibiotics to control endogenous contaminants;
- Cutting experiments;
- Nitrogen compound experiments;
- Growth regulators experiments;
- Choice of appropriate mother plants;
- Preconditioning of mother plants;
- Varying light conditions in the growth rooms;
- Use of different gelling materials to prepare induction, growth, multiplication and rooting media;
- Multiplication efficiency;
- Acclimatization of plantlets in the greenhouse and open nursery.

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 MTC 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. Preparation of mother plants
Mother plants for induction have mainly been sucker or nursery material raised from bulbils. Healthy plants of more than 30 cm high were collected. Suckers were collected from sisal fields no more than three years old and/or nursery material of no more than two years old.

Plants were collected directly from the field and there was no preconditioning period. However due to low initiation a precondition exercise was carried out after consultation with the international consultant and the experiments are still ongoing.

The plants were defoliated without injuring the growing point. After trimming the leaves, the plants were washed in plenty of tap water to remove all soil particles before they were washed with 2% soap detergent. The fibrous basal parts were removed and washed in sterile distilled water. The plants were then surface sterilized in 40% Jik for one hour then rinsed with sterile distilled water.

b. Preparation of explants
The mother plants were cut into longitudinal sections to expose the meristem, which was slowly traced by removing the primordial leaves. Only the upper part (0.5 cm) of the plant was taken for AGAVE Sisalana, AGAVE Hildana and H. 11648. The sections were then divided into small parts, which were placed into a 30ml induction media contained in baby jars and placed in the growth room. The cutting technique was continuously adjusted during the implementation of the sub-component; satisfactory results were finally achieved especially after the last visit to KEPHIS.

c. *Gelling material*
Gelling material used was agar at 8g /l in induction, at 7g/l in multiplication, at 6g/l in growth and at 10g/l in rooting. Occasionally in the growth media a mixture of gelrite and agar was used at 1.5g/l gelrite + 2.0g/l agar.

d. Hormones and carbon source
Concentration of hormones ranged between 10-15mg/l BAP and 0.025mg/l 2,4-D in induction and multiplication. For induction from 12.5mg/l to 15mg/l BAP was more effective. For multiplication10mg/l BAP was used. The rate of 2,4-D remained the same (0.025mg/l for both induction and multiplication). For growth the rate of BAP was 1mg/l. For rooting IBA was used instead of 2,4-D. The carbon source was icing sugar, sigma sucrose and common sugar at 30g/l.

e. Lighting conditions temperature and humidity
Induced cultures were put in growth rooms under 16-hour artificial light and 8-hour darkness. The temperature range was 25-28°C and the relative humidity range was 50-80%.

f. Shoot transfer
Developed shoots were grouped in bunches of two to three and put into multiplication media. Single shoots were also multiplied. These were put into the growth room with the same light regime of 16-hour artificial light and 8-hour of darkness. Very small shoots were put into growth media. Mature shoots were rooted in 10mg/l IBA for three weeks.

g. Acclimatization of shoots in green house
Rooted plantlets were transferred into polystyrene trays containing sterilized soil media and then placed in the green house for acclimatization. It has been observed that unrooted shoots could be planted directly into soil after dipping them in rooting media with 10mg IBA for 2-3 hours. After 75-90 days the plants were transferred to open nursery for acclimatization in the natural environment.

h. Nursery planting
Weaned plantlets were ready for nursery planting in 90 days. At Mlingano the H.11648 plants were planted side by side to normal bulbils in order to compare their performance.

III.2.2 Experiments conducted

A total of six experiments were conducted to control contamination, test the effect of growth regulators and nitrogen balances. With the exception of the experiments on control of contamination the results obtained so far from the hormone and nitrogen sources are not yet conclusive. The six experiments and the data collected are presented below.

1. Growth regulators
Six rates of cytokinin, BAP (0; 5; 10; 12.5; 15 and 17.5 mg/l) in combination with five rates of auxin 2,4–D (0; 0.01; 0.025; 0.05 and 0.075 mg/l) were designed to establish the effect of the combination of these hormones on shoot initiation in three cultivars of sisal. Each treatment consisted of 40 explants of each variety replicated twice in a complete randomized design. The trial was assessed for 12 weeks on shoot induction, number of shoot per explant and total shoot per treatment.

The results achieved are as presented below in Tables 1a and 1b. Many explants remained dormant and shooting rate was less than 40%. Shooting was random and could not be related to treatments. The experiment will be repeated in the future using four rates of BAP (0; 5; 10 and 15 mg/l) and four rates of 2,4–D (0; 0.01; 0.025 and 0.05 mg/l). Hybrid 11648 will be tested with five plants per treatment, replicated twice in a complete randomized design.

Table 1a. The effect of different rates of cytokinin (BAP) and auxin (2,4–D) on shoot production per explant in three cultivars of sisal –2002.

Auxin concentration (2,4-D) mg/l	Cytokinin concentration (BAP) mg/l								
	0			5			10		
	AS	AH	H	AS	AH	H	AS	AH	H
0	1.7	1	1	2	2	0	1	0	3.3
0.01	2	2	2.2	2.2	3.5	2	2.5	2	0
0.025	0	0	0	0	0	1.5	0	0	0
0.05	0	1	2	2	5	0	1	0	2
0.075	0	2	0	0	0	2	2	2	1

Table 1a (continued)

Auxin concentration (2,4-D) mg/l	Cytokinin concentration (BAP) mg/l								
	12.5			15			17.5		
	AS	AH	H	AS	AH	H	AS	AH	H
0	2	0	2	2.2	0	1.6	2.2	0	2
0.01	2.2	2	1.6	3	2	0	2	2.6	2.1
0.025	0	0	0	0	0	2	0	0	0
0.05	2	0	2	0	1	0	0	1	0
0.075	1	2	1	2.1	2.4	2	2	2	2

Where: AS: Agave Sisalana AH: Agave Hildana H: Hybrid 11648

Table 1b. The effect of different rates of cytokinin (BAP) and auxin (2,4–D) on explants with shoots 12 weeks after induction –2002.

Auxin concentration (2,4-D) mg/l	Cytokinin concentration (BAP) mg/l								
	0			5			10		
	AS	AH	H	AS	AH	H	AS	AH	H
0	3	1	1	2	1	0	1	0	3
0.01	2	1	5	9	2	5	2	1	0
0.025	0	0	0	0	0	2	0	0	0
0.05	0	1	1	2	4	0	1	0	1
0.075	0	1	0	0	0	10	1	1	1

Table 1b (continued)

Auxin concentration (2,4-D) mg/l	Cytokinin concentration (BAP) mg/l								
	12.5			15			17.5		
	AS	AH	H	AS	AH	H	AS	AH	H
0	1	0	4	1	0	3	4	0	3
0.01	4	1	3	1	4	0	3	5	6
0.025	0	0	0	0	0	4	0	0	0
0.05	3	0	2	0	1	0	0	1	0
0.075	1	2	1	6	5	6	1	1	2

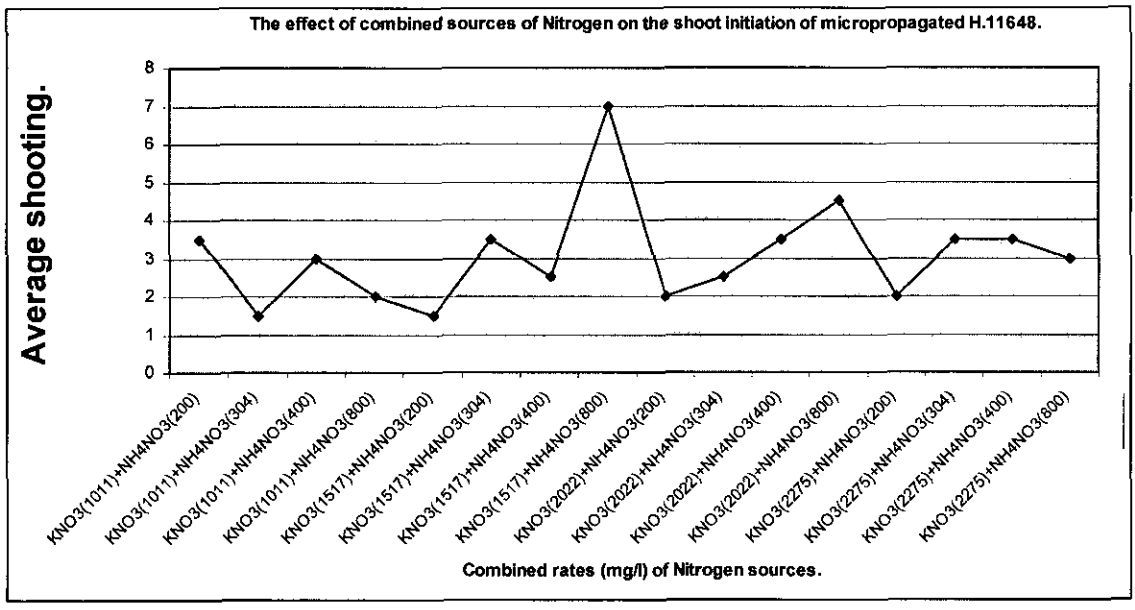
Where: AS: Agave Sisalana AH: Agave Hildana H: Hybrid 11648

2. Nitrogen balance experiment

The experiment was aimed at observing the effect of different concentrations of nitrogen sources on shooting. Four rates of Potassium Nitrate (KNO_3) (1,011; 1,517; 2,022 and 2,275 mg/) in combination with four rates of Ammonium Nitrate (NH_4NO_3) (200; 304; 400 and 800mg/l) were used. Forty explants of H 11648 were tested for each treatment and replicated twice.

Only 163 explants produced shoots and this was attributed to the problem of commercial sisal cultivars not responding to the henequen micropropagation protocol. However from the results it has been observed that KNO_3 (1,517mg/l) when combined with increasing rates of NH_4NO_3 (200, 304, 400 and 800mg/l) have an increasing shooting and 800mg/l is the maximum. A similar trend was observed when KNO_3 (2,022mg/l) was combined with the same rate of NH_4NO_3 (Figure 1).

Figure 1. The effect of combined sources of Nitrogen on the shoot induction of micropropagated H.11648



3. Experiments to control microbial contamination in tissue culture

Several chemicals were used at different concentrations to observe their effect on controlling fungal and bacterial contamination in cultures. Such chemicals include: Bayfidan, Benlate and Dettol which were used as surface sterillant and Oromex and Vitrobaf were used as inhibitors of microbial growth in the media, so they were incorporated in the media.

Dettol and fungicide experiment

A combination of Dettol and fungicides at different rates (Benlate 2.5g/l, Ridomil 2.5g/l, Bayfidan 10ml/l and Dettol 50.5ml/l) were used as surface sterillant for mother plants to control exogenous contamination. Exposure time of mother plants in fungicides and Dettol was set at 30 minutes, after which they were exposed in bleach (Jik 40%) for one hour. Explants were soaked into bleach (Jik 2%) for 5 minutes. Dettol at 5% was also

used to rescue contaminated shoots and also incorporated in the media with the objective of controlling endogenous contamination. The cultures were placed in growth room and assessed for microbial growth.

Preliminary observations indicate that the use of a combination of fungicide and Jik had a good control on most of the surface microorganisms. Supplementing with Dettol treatment for 30 minutes further decreased the surface contaminants for the first two weeks. After two weeks bacterial contamination reoccurred indicating that the chemicals utilized could not control endogenous contaminants. Another set back of Dettol was that none of the treated explants produced shoots. When used to rescue contaminated shoots Dettol was lethal as all the rescued shoots became bleached and died within 14 days.

Table 2. Effect of different chemicals to control microbial contamination on sisal explants.

Variety	Total explants induced	Treatment	CONTAMINATION ASSESSMENT								
			7 days			14 days			21 days		
			Total	%	source	Total	%	source	Total	%	source
AS	206	Ble(25),Ble(5)	28	14	F	18	9	B	nil	nil	-
AS	213	Ble(25),Ble(5)	8	4	B	nil	nil	-	8	3.7	B
AH	177	Bay(30),Ble(25, Ble(5)	9	5	B	nil	nil	-	nil	nil	-
AH	147	Bay(30),Ble(25, Ble(5)	7	4.7	B	nil	nil	-	nil	nil	-
AH	174	Bay(30),Ble(25, Ble(5)	4	2	F	nil	nil	-	nil	nil	-
AH	177	Bay(30),Ble(25, Ble(5)	44	25	B+F	71	40	B+F	23	13	B
AH	166	Ben+Bay(30) Ble(25),Ble(5)	12	7	B+F	37	22	B	5	3	B
H	150	Ben(30)Ble(25) ,Ble(5),Det(10)	29	19	B+F	8		B	nil	nil	-
AS	295	Ben(30)Ble25), Ble(5),Det(5)	47	16	B	32	11	B	30	10	B
AS	232	Ben(30),Ble(25) ,Ble(5),Det(20)	17	7	B	5	2	B	25	11	B
AH	116	Ben(30),Ble(25) Ble(5),Det(30)	nil	nil	nil	12	10.3	B	18	15.5	B
AH	205	Ben(30),Ble(25) ,Ble(5),Det(30)	nil	nil	nil	12	5.8	B	nil	nil	-
H	114	Ben(30),Ble(25), Ble(5),Det(30)	nil	nil	nil	10	8.7	B	nil	nil	-

Ben: Benlate 2.5g/l
Bay: Bayfidan10ml/l
Det: Dettol 5%
Ble: Bleach 40% and 2%
F: Fungus
B: Bacteria
( ): time in minutes
AS: Agave Sisalana
AH: Agave Hildana
H: Hybrid 11648

Oromex and Vitrobaf experiments

Experiments were established for testing the effectiveness of Oromex and Vitrobaf to control fungal and bacterial contamination. Different rates of Oromex were applied: 0; 20; 40 and 100 µl/l. Vitrobaf was tested using the following concentrations: 0; 114; 228 and 342 mg/l. Results are as presented in Tables 3a and 3b.

Preliminary results so far indicate that Oromex is effective against fungal contamination, but less effective on endogenic bacteria. Isolated cases of bacterial contamination started to appear in the baby food jars from the sixth day. The concentration of bacteria decreased as chemical concentration was raised.

Table 3a. Effect of Oromex on control of contamination

Replication	Treatment (µl/l)	Explants induced	Survivors	Contamination %	Source
1	0	40	2	95	B+F
1	20	40	0	100	B+F
1	40	40	37	7.5	B
1	100	40	36	10	B
2	0	40	38	5	B
2	20	40	38	5	B
2	40	40	39	2.5	B
2	100	40	39	2.5	B

F: Fungus
B: Bacteria

Table 3b. Effect of Vitrobaf on control of contamination

Replication	Treatment (µl/l)	Explants induced	Survivors	Contamination %	Source
1	0	40	3	92.5	B+F
1	114	40	1	97.5	B+F
1	228	40	36	10	B
1	342	40	37	7.5	B
2	0	40	37	7.5	B
2	114	40	39	2.5	B
2	228	40	40	0	
2	342	40	40	0	

F: Fungus
B: Bacteria

4. Cutting experiments

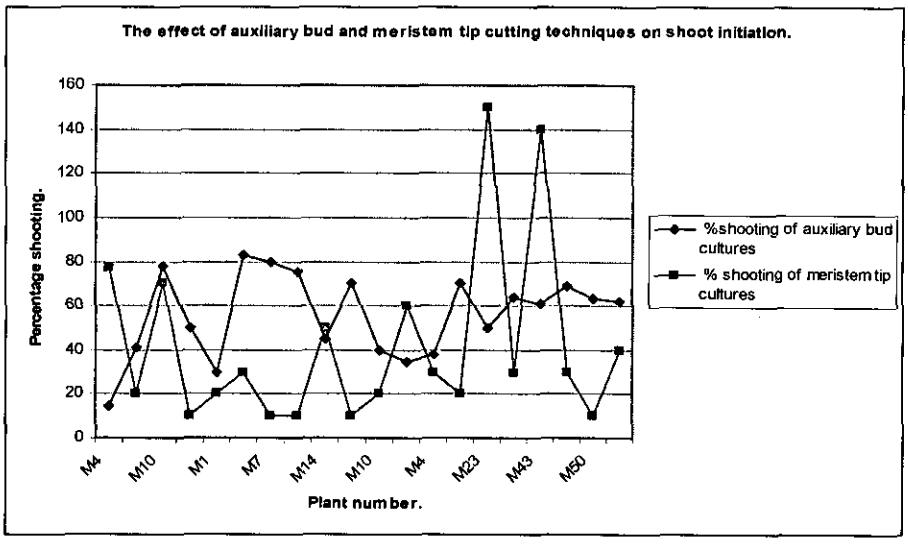
The commercial cultivars of Hybrid 11648, Agave Sisalana and Hildana have a very thin meristematic layer lying immediately beneath the leaf primordial. Mlingano MTC laboratory has faced low shooting during the first two years of project implementation. Probably this is due to the fact that culturing sisal commercial varieties from meristem tip is different from culturing henequen and adjustments were necessary.

A different cutting technique, where explants are extracted from both axillary buds and meristem tips, was tested on 20 clones of H.11648. Observations of shoot initiation have shown that axillary bud technique has a higher mean shooting percentage by 10% (Table 4) although the meristem tip cutting method recorded the maximum shoot initiation and the minimum as well (Figure 2). Similar results have been reported by KEPHIS during the experts exchange visit in 2004.

Table 4. Assessment of shooting using the axillary bud cutting technique vs. the meristem tip cutting technique.

Plant No	Axillary bud culture				Meristem tip culture			
	Explants	Total shoots	Average shoots/ explant	% shooting	Explants	Total shoots	Average shoots/ explant	% shooting
M4	8	5	1.3	14.4	8	4	0.5	76.9
M9	12	11	2.2	41	9	2	0.2	20
M10	14	16	1.5	78	11	8	0.7	70
M15	10	6	1.2	50	15	1	0.1	10
M1	11	4	1.3	30	11	2	0.2	20
M5	12	20	2	83	18	5	0.3	30
M7	15	21	1.8	80	12	1	0.1	10
M10	12	11	1.2	75	9	1	0.1	10
M14	11	5	1	45	11	5	0.5	50
M3	13	10	1.1	70	6	1	0.1	10
M10	10	8	2	40	11	2	0.2	20
M15	23	10	1.3	35	8	5	0.6	60
M4	13	13	2.6	38	15	4	0.3	30
M20	20	22	1.6	70	13	3	0.2	20
M23	8	6	1.5	50	8	12	1.5	150
M29	14	12	1.3	64	12	4	0.3	30
M43	18	13	1.2	61	5	7	1.4	140
M44	13	14	1.5	69	12	4	0.3	30
M50	11	11	1.6	63	19	1	0.1	10
M59	13	12	1.5	62	8	3	0.4	40
Mean	13.1	11.5	1.5	56	11.1	3.8	0.4	42

Figure 2. The effect of axillary bud and meristem tip cutting technique on shoot initiation.



5. Multiplication Efficiency

The observations collected indicate that shoots placed in multiplication phase seem to have different efficiency depending on how they were separated from the initial explant in induction. Single shoots took longer time to produce new auxiliary shoots and had fewer shoots, while bunches multiplied better.

From this experience a trial was set to assess the multiplication efficiency of the Hybrid 11648. Few selected shoots were placed in multiplication media in one, two, three, four and five bunches and assessed 4-8 weeks later. Observations are as shown in Table 5; more data needs to be collected to test the correlation between the number of shoots in multiplication media and the number of new shoots produced.

Table 5. Multiplication efficiency of selected clones of Hybrid 11648

Plant No	Date of subculture	No of shoots/bunch	No of new shoots	Efficiency
M35	16/2	1	1	1
M43	16/2	4	4	1
P24	17/2	4	33	8.25
P01	18/2	4	11	2.75
P12	12/3	3	6	2
P11	12/3	3	6	2
P32	12/3	2	5	2.5
M33	12/3	5	25	5
N12	15/3	2	10	5
N12	15/3	3	6	2
N20	15/3	2	22	11
Mean				**4.15**

III.3 Conservation of Mlingano gene bank

In the first half of the last century, the Sisal Experimental Station at Mlingano earned a reputation as the most important place in the world for studies on the breeding and cultivation of hard fibre producing agaves. Its most outstanding product was the famous hybrid H 11648, on which most of the cultivation in East Africa is based today.

The ARI station at Mlingano that took over the research, inherited the highest collection of sisal cultivars in the world. It included a collection of plants from Mexico and other parts of the world, as well as many hybrid lines produced over many years of genetic research, which were planted at three sites (Annex 2, A, B, and C) in the station and included 44 different Agave species (Annex 2, Table 1) and 135 hybrid lines, derived from the many crosses carried out over various decades (Annex 2, Table 2). The collection was established because all these materials were considered potentially important and worthwhile preserving for the future.

Many of the over 120 varieties were unfortunately lost over the years; some of the cultivars disappeared due to lack of propagation material like suckers or bulbils but

most of them because of abandonment. The plants were simply left to age, covered by weeds and plagued by disease. No collection of data was carried out to characterize the plants and nothing was done to preserve them. This situation was mainly due to lack of funds and, probably, to a low priority assigned to sisal research for some years.

In 2001 a new site (D) was established, using the rhizomes or bulbils collected from some of the plants (Annex 2, Table 3). Many of the accessions (27 species and 67 hybrids) had already been lost and only 85 lines were transplanted to this new site. Nine additional lines got lost over the past two years and another ten are in danger of disappearing since they are represented by only one or two individuals and have no new rhizomes. Furthermore, more plants are now in danger because they are affected by pests and disease. Once again, the lack of funds does not allow full maintenance of the collection.

The original objectives of the collection were to carefully preserve and evaluate all the planted material since the site presented the unique opportunity to compare, under the same environmental and cultivation conditions, the performance of many hybrids that had not been evaluated or for which the information had been lost.

In consultation with the international consultant Dr. Manuel Robert the following was suggested as a strategy to save the gene bank:

1. Plant the new site D;
2. Duplicate the collection at a different site, if Government regulations allow, the gene bank would be replicated in Kenya;
3. Select potential species and propagate them *in vitro*;
4. Plan for proper and efficient management practice;
5. Replant poling cultivars immediately using suckers;
6. *In vitro* preservation of endangered lines.

One cultivar, Agave Amaniensis, was successfully cultured *in vitro* and at the end of the project there were about 600 (value provided by the laboratory) plantlets being acclimatized in the green house. Eight potential cultivars have been selected for assessment for growth parameters. Assessment of the selected cultivars continued and to date H. 91 and Agave Amaniensis had taller plants with longer leaves when compared to the other varieties. Average leaf production however was low for Agave Amaniensis. Regarding leaf production, Agave Verschafeltii had high leaf number (11 leaves) followed by H. 91(8) and H.71 (8) and shown in Table 6 below.

Despite the fact that routine management of weeding and pest control are being reported, more funds should be provided to allow for implementation of the preservation strategy.

Table 6. Assessment of selected clones from the gene bank.

Variety	Mean Leaf No.	Leaf Length (cm)	Leaf Width (cm)	Plant Height (cm)
H. 11648	5	89.9	10.8	127.8
H. 8366	7	97.4	n.a.	134.0
Mlola1	4	77.8	9.0	106.6
H. No.91	8	112.6	12.1	155.6
H. 62025	7	107.7	10.25	139.5
A. Verschafeltii	11	80.9	9.0	121.2
H. 71	8	103.5	10.0	144.9
A. Amaniensis	2	119.9	11.5	153.8

III.4 Establishment of close links with sisal growers

The sisal growers have very close links to the MTC activities through visits, field days and training. Interested parties like the estate in Kilosa (Morogoro) have planted about 2,700 tissue-cultured plants at their estate. Another enterprise has also shown interest in tissue cultured Agave Sisalana.

III.5. Summary of results achieved

The laboratory, apart from the information included in the previous sections, provided no summary of results achieved. The laboratory drafted a business plan in June 2003, the document was supposed to be reviewed to include the production costs and the production plan for MTC sisal species. It is hoped that under the responsibility of TSB more commitment will be shown as the potential of MTC is very valuable for the future of the sisal industry.

III.6 Dissemination of results

Dissemination was done at the dissemination workshop in 2003 and at the final international dissemination workshop in 2004, during which a site visit to Mlingano was organized for all the participants. According to the laboratory managers, the farmers visited the laboratory itself, the greenhouse and the nursery frequently during project implementation. The Tanzanian Sisal Board should act as the link between the institution and the farmers.

IV. Lessons learned

IV.1 Development and operational lessons

The performance of the ARI Mlingano laboratory was evaluated as limited. Little commitment was shown by the management and by the staff of ARI Mlingano, despite the many efforts undertaken by the project stakeholders to improve the laboratory staff performance.

During project implementation different actions have been taken to resolve the issue, without any success especially because the promised change in the laboratory management never materialized. Lastly an exceptional National Coordinating Committee (NCC) Meeting was held on 15 July 2004 and it was decided to take away from ARI Mlingano the management of the MTC Laboratory. The responsibility of the laboratory was given to the Tanzania Sisal Board. It was also suggested to relocate the laboratory to a different institution, but the alternative was not feasible in the time scale given. The Director General of TSB was asked to set up a management structure that would turn round the laboratory in the shortest time possible.

The laboratory is fully equipped and staff is trained, so under an improved management it is foreseen that good results would be achieved for the benefit of the sisal industry.

IV.2 Problems encountered in implementation

According to the laboratory the following problems were encountered:

Training
The training program was insufficient taking into consideration that Mlingano staff had no practical experience at all in tissue culture or any biotechnological activity. The training could have been supplemented by more exchange visits to Kenya but these were also given very low priority. Short-term training in biotechnology laboratories would have also assisted Mlingano staff to solve the operational problems encountered during the project period.

Technical consultancy
The consultation period, especially for local consultancy, was insufficient. It is found that local consultancy was stopped, following the advice of the international consultant, a bit too early. Local consultancy was necessary to complement the efforts of the international consultant from CICY.

Low shoot initiation from induction and multiplication
Low shoot initiation has been a persistent set back at Mlingano laboratory. However, the experience gained with the cutting experiment proved that that A. Sisalana, A. Hildana and H. 11648 initiate better from axillary buds than from meristem tips. Also it has been learnt that plant tissues away from the meristem tip have higher concentration of microbial contaminants than the meristem tip. Partly, this is due to a long time stay in the soil, so this has posed a great challenge on the success of the shoot production using axillary buds.

Contamination
The problem of fungal contamination has been a great issue, especially at the outset of the project. However it was greatly reduced after utilization of different sterilization techniques, experience in seasonal collection of mother plants and identification of areas with low concentration of innoculums of fungal diseases.

Power
For the whole project time Mlingano has experienced irregular power supply due to rationing and faulty power line. The stand-by generator could not be run continuously due to limited supply of fuel. The problem of electricity supply to the institute has greatly affected supply of light in the growth rooms and frustrated efforts to culture more, hence the total output of the laboratory at Mlingano.

Quality of material produced
There has not been enough time to assess the quality of the material produced, as the plants are still at nursery stage. However, initial measurements of growth parameters, such as plant height, leaf number, and leaf length for the hybrid at Mlingano indicate the tissue cultured plants are growing better than the normal bulbils (Tables 7a and 7b).

Table 7a. Assessment of plantlets in open nursery at Mlingano (May 2004)

Type of planting material	Variety	Mean leaf number	Mean leaf length (cm)	Mean plant height (cm)
MTC plantlets	H 11648	8	37.3	38.2
Normal bulbils	H.11648	6	34.5	35.4
MTC plantlets	*A .Sisalana*	5.5	35.4	36.0

Table 7b. Assessment of plantlets in estate nursery at Kisangata (May 2004)

Type of planting material	Variety	Mean leaf number	Mean leaf length (cm)	Mean plant height (cm)
MTC plantlets	H 11648	6.0	14.8	12.3
MTC plantlets	*A. Sisalana*	5.0	15.6	9.4

V. Conclusions and recommendations

According to the experience gained in 4 years of operations, the best mother plants are from fields less than three years old or one year old nursery plants. Suckers from old fields mostly get phenolic oxidation. Observations indicate that the concentration of BAP are effective at rates between 10 -15 mg/l. The levels of 2,4-D used are 0.025mg/l for all stages except rooting, where 11mg/l IBA was used. Growth was better in media without hormones. The optimal initiating conditions for the explants was a light/darkness regime of 16/8-hour.

In terms of variations of each species or variety it can be concluded that the three agaves Hybrid 11648, Agave Sisalana and Agave Hildana behave differently in induction under the same conditions of light, temperature and humidity. Cubes were taken from the upper part only (meristem tip). Agave Sisalana outperformed the other two in the early trials but later on, after gaining experience in extracting the meristematic parts, H.11648 performed better than A. Sisalana and A. Hildana, with more than 50% initiated shoots. A. Hildana has been the poorest performer, but successful cubes produce 6-8 shoots per cube. In the three varieties shooting was between 4-12 weeks, while A. Amaniensis was the best with two parts shooting within eight weeks.

The project output included the definition of a meristematic tissue culture technique for sisal and production of a technical manual for its application and production of new plants through the new system. More time is required to carry out experiments to analyze the efficiency of the process, as the available information is not enough to come out with a technical manual/protocol for mass production of sisal varieties plants through tissue culture by Mlingano staff. The ongoing experiments need to be finalized and the results should be included in the manual/protocol (that should have been prepared by ARI Mlingano as one of the sub-component outputs).

The project published the Technical Paper No. 38 *"MANUAL FOR THE IN VITRO CULTURE OF AGAVES"* to disseminate the acquired experiences in this field.

More time is required to repeat the experiments so as to check the reproducibility of results that will lead to a reliable and well-defined tissue culture protocol. More financial support is needed to complete the process of defining the protocol, staff capability building, writing the technical manual and the technology transfer process.

Large-scale production is proposed to be an output of a technology transfer process after the procedure of micro propagating the local commercial cultivars is defined. The laboratory will be expected to produce enough biomass for establishing nurseries in estates depending on availability of funds from the industry and/or government

A draft business plan for sisal research in Tanzania, including the MTC laboratory activities has been prepared and distributed to relevant stakeholders for comments. When finalized and approved, the plan is expected to contribute to create the basis for massive MTC of agaves in Tanzania.

It is the opinion of the PEA that radical changes at the management level are required in order for the MTC laboratory at ARI, Mlingano, to deliver the same results as in Kenya by KEPHIS and subsequently to provide MTC plants to meet industry demand.

Annex 1. List of equipment and chemicals purchased during the project period

Item description	Category	Unit	Quantities procured over the project years							Total
			1998	1999	2000	2001	2002	2003	2004	
Office chair big size.	Furniture	pc		4						4
BAP	Lab chemic	g		25		50				75
IAA	Lab chemic	g				25				25
Indole3 butyric acid, IBA	Lab chemic	g		10						0
Pyridoxine	Lab chemic	g		5		20				25
P-Aminobenzoic acid	Lab chemic	g		100						100
Thiamine	Lab chemic	g		5		20				25
Toluodine	Lab chemic	g		100						100
Nicotinic acid	Lab chemic	g		100		200				300
Naphthalineacetic acid	Lab chemic	g		25						25
Glycine	Lab chemic	g		100		100				200
Glutamine	Lab chemic	g		25						25
Myo-inositol	Lab chemic	g		100		200			500	800
Gelrite	Lab chemic	g				2000				2000
Agar purified	Lab chemic	g		1000		2000				3000
Ammonium nitrate	Lab chemic	g		500		1000				1500
Ammonium chloride	Lab chemic	g		100						100
Ammonium sulfate	Lab chemic	g		100						100
Potassium nitrate	Lab chemic	g		1000		1000				2000
Potassium iodide	Lab chemic	g		1000						1000
Phytagel	Lab chemic	g		5000						5000
Calcium chloride dihydride	Lab chemic	g		500		1000				1500
Magnesium sulfate hepta dyhydride	Lab chemic	g				1000				1000
Potassium phosphate monobasic	Lab chemic	g		100		500		1000		1600
Potassium phosphate dibasic	Lab chemic	g		100						100
Ferrous sulfate hepta hydrate	Lab chemic	g				500				500
EDTA disodium salt	Lab chemic	g		100		100				200
Ethylene diamine tetraacetic acid.	Lab chemic	g		100						100
Manganese sulfate monohydrate	Lab chemic	g		100		200				300
Manganese chloride	Lab chemic	g		100						100
Zinc sulfate heptahydrate	Lab chemic	g		100		200				300
Boric acid	Lab chemic	g		100		500				600
Sodium molybdate dihydride	Lab chemic	g		100		200				300
Sodium phosphate monobasic	Lab chemic	g		250						250
Sodium phosphate dibasic	Lab chemic	g		100						100
Sodium chloride	Lab chemic	g		250						250
Sodium sulfate	Lab chemic	g		500						500
Sodium nitrate	Lab chemic	g		250						250
Magnesium chloride	Lab chemic	g		100						100
Cupric sulfate pentahydrate	Lab chemic	g		100		100				200
Cobalt chloride	Lab chemic	g		25		200				225
Potassium iodide	Lab chemic	g		100		300				400
Buffer solution pH 7	Lab chemic	ml		500		500				1000
Buffer solution pH 4	Lab chemic	ml		500		500				1000
Buffer solution pH 10	Lab chemic			500						500

Item description	Category	Unit	Quantities procured over the project years					Total
Ethanol	Lab chemic	Lt			60			360
6-Benzylaminopurine	Lab chemic					100		100
2,4-dichlorophenoxyacetic acid	Lab chemic		100			200		300
6-furfurylaminopurine	Lab chemic					100		100
Buffer pH 7 Fixanal for 500ml	Lab chemic	Ampils				6		6
Buffer for pH 4 Fixanal for 500ml	Lab chemic	Ampils				6		6
Agar-agar	Lab chemic	kg	5			50		55
ORO-MEX	Lab chemic	ml				250		250
Vitrobaf	Lab chemic	g	0			24		24
Sucrose	Lab chemic	kg	70					70
Icing sugar	Lab chemic	kg	70					70
Water filter phosphate catridge	Lab equip.	set				1		1
Water distillation unit	Lab equip.	set				1		1
Polystyrene seedling trays	Lab,tools	pc		102				102
Fibre board boxes	Lab,tools	box		1				1
Silica gel beads	Lab,tools	g		2000				2000
Silica gel beads	Lab,tools	g		3000				3000
Distillation unit model D4000	Lab,tools	unit		1				1
Bottle narrow mouth 60ml	Lab,tools	pc		10				10
Bottle narrow mouth 2000ml	Lab,tools	pc		5				5
Bottle narrow mouth 1000ml	Lab,tools	pc		5				5
Bottle narrow mouth 125ml	Lab,tools	pc		20				20
Bottle narrow mouth 2000ml	Lab,tools	pc		10				10
Bottle narrow mouth 500ml	Lab,tools	pc		20				20
Forceps blunt painted, 200mm	Lab. Tools	pc			4			4
Pre filter for horizontal laminar flow	Lab. Tools	pc			6			6
Luxmeter testo	Lab. Tools	pc			1			1
Cold lightsource	Lab. Tools	pc			1			1
Twin goose neck light guide	Lab. Tools	pc			1			1
Focussing attachment	Lab. Tools	pc			2			2
Polarisation filter	Lab. Tools	pc			2			2
Scapel holder no 2	Lab. Tools	pc			5			5
Scapel blades no 20	Lab. Tools	pc			100			100
Scapel model no 24	Lab. Tools	pc			100			100
Scapel blades no 22	Lab. Tools	pc			100			100
Scapel blades no 25	Lab. Tools	pc			100			100
UV germicidal lamp	Lab. Tools	pc			1			1
Replacement tubes for UV lamp	Lab. Tools	pc			2			2
Star desiccator, two sided mounting snap	Lab. Tools	pc			1			1
Ultra filtration filter	Lab. Tools	pc			250			250
Refillable calomel pH meter electrode	Lab. Tools	pc			1			1
Heating element for distiller model D4000	Lab. Tools	pc			2			2
pH meter porlarness 911 pH without electrode	Lab. Tools	pc			1			1
Measuring accessory set B	Lab. Tools	pc			1			1

Item description	Category	Unit	Quantities procured over the project years					Total
Pipetting aid	Lab. Tools				5			5
Kiwi knives	Lab. Tools	pc		24				24
Trolley service	Lab. Tools	pc		1				1
Aluminium foil	Lab. Tools	pc		50				50
Paper nabtools	pc		20				20	0
Gallon detergents	Lab. Tools	pc		5				5
Gallon Dettol	Lab. Tools	pc		5				5
Bleach	Lab. Tools	pc		10				10
Pre filter for horizontal laminar flow	Lab. Tools	pc				8		8
Gasket for verutoclave	Lab. Tools	pc				2		2
Scapel handles	Lab. Tools	pc				20		20
Bag high temperature	Lab. Tools	pc				500		500
Bag high temperature	Lab. Tools	pc				500		500
Scalpel blade	Lab. Tools	pc				300		300
Scalpel blade	Lab. Tools	pc				300		300
Forceps.	Lab. Tools	pc				20		20
Nalgene graduated cylinder	Lab. Tools	pc				5		5
Nalgene graduated cylinder	Lab. Tools	pc				5		5
Nalgene graduated cylinder	Lab. Tools	pc				5		5
Nalgene graduated cylinder	Lab. Tools	pc				5		5
Nalgene graduated cylinder	Lab. Tools	pc				2		2
Nalgene graduated cylinder	Lab. Tools	pc				3		3
Graduate cylinder with handles	Lab. Tools	pc				2		2
Graduate cylinder with handles	Lab. Tools	pc				2		2
Pipette	Lab. Tools	case				1		1
Pipette	Lab. Tools	case				1		1
Pipette	Lab. Tools	case				1		1
Pipette	Lab. Tools	case				1		1
Baby food jars closures	Lab. Tools	pc					1500	
Baby food jars	Lab. Tools	pc	1000			500	1500	3000
Culture vessels	Lab. Tools	pc					1000	1000
Fine tip lab markers	Lab. Tools	pc	50			50		100
Tape autoclaves indicators	Lab. Tools	pc				24		24
Magenta vessel similar to GA-7-3	Lab. Tools	pc	2000			400		2400
Magenta vessel similar to GA-7	Lab. Tools	pc	500			400		900
Dry bead sterilizer	Lab. Tools	pc		2		2		4
Dry bead sterilizer replacement beads	Lab. Tools	Pack				10		10
Laboratory tool set	Lab. Tools	pc				1		1
Digital thermometer	Lab. Tools	pc				2		2
Digital thermometer	Lab. Tools	pc				2		2
Digital 24hr timer controller	Lab. Tools	pc				2		2
Flask brush	Lab. Tools	pc				25		25
Bottle brush	Lab. Tools	case				8		8
Beaker brush	Lab. Tools	case				8		8
Sprayer c-15	Lab. Tools	pc				1		1
Cooling only window type AC	Lab. equip	pc	1					1
Cooling only window type AC	Lab. equip	pc	1					1
Cooling only window type AC	Lab. equip	pc	2					2
Pump head, MFLX easy-load,PSF/SS	Lab. equip	pc	1					1

Item description	Category	Unit	Quantities procured over the project years	Total
Tubing, MFLEX, TYGON LFL, #24,25	Lab.equip	pc	1	1
Drive, MFLEX, L/S, 10-600 rpm,115V	Lab.equip	pc	1	1
Timer, mini count down	Lab.equip	pc	1	1
Horizontal laminar flow cabinet	Lab.equip	pc	2	2
Replacement HEPA filters	Lab.equip	pc	2	2
Prefilters	Lab.equip	pc	6	6
High efficiency air purifier	Lab.equip	pc	1	1
Portable power transformer	Lab.equip	pc	1	1
One day controller	Lab.equip	pc	2	2
Express portable aluminium autoclave, electrical model	Lab.equip	pc	2	2
Stir hot plate, 50HZ240V	Lab.equip	pc	4	4
Balance, 6200GX.010 220V50HZ	Lab.equip	pc	1	1
Balance, anal multirange 220V50HZ	Lab.equip	pc	1	1
Pipette,W/O ejector, 0.5-10UL	Lab.equip	pc	1	1
Pipette,W/O ejector, 10-100UL	Lab.equip	pc	1	1
Pipette,W/O ejector, 20-200UL	Lab.equip	pc	1	1
Pipette, W/O ejector, 100-1000UL	Lab.equip	pc	1	1
pH C Meter, model 307	Lab.equip	pc	1	1
Meter W/probe. VWR MD8010220V	Lab.equip	pc	1	1
Electr., plast, gel filled 107/307	Lab.equip	pc	1	1
FRZR chest, G.P.,VWR,220V180F	Lab.equip	pc	1	1
Autoclave,CPP,220V9X18I N	Lab.equip	pc	1	1
Autoclave delux, 220V9X18IN	Lab.equip	pc	1	1
Pump115/230V 50/60HZ 8905A	Lab.equip	pc	1	1
Stemi 1000 W/stand &eye pcs 220V41800016	Lab.equip	pc	1	1
Hygrometer,DIG,Min/Max VWR	Lab.equip	pc	1	1
Hygrometer, DIG, W/Temp VWR	Lab.equip	pc	1	1
Conductivity meter MHH	Lab.equip	pc	1	1
Filter hold W/Rec 500/500ML	Lab.equip	pc	1	1
Calorimetre, Port W/Data Ret220V	Lab.equip	pc	1	1
Tadiran AC, indoor unit, TFE.515C	Lab.equip	pc	3	3
Tadiran AC, outdoor unit, TFE.515C	Lab.equip	pc	3	3
Tadran AC, indoor unit, TNL525c	Lab.equip	pc	1	1
Tadiran AC, outdoor unit,TNL525c	Lab.equip	pc	1	1

Item description	Category	Unit	Quantities procured over the project years	Total
Test Kit, Alkalinity M, CaCO3	Lab.tools	pc	1	1
Test kit, Ammonia low	Lab.tools	pc	1	1
Test kit, Ca hardness, CaCO3	Lab.tools	pc	1	1
Test kit, Chlorine	Lab.tools	pc	1	1
Test kit, Chlorine free, comb & TOT	Lab.tools	pc	1	1
Test kit, chromate	Lab.tools	pc	1	1
Test kit, chromium hexavalent	Lab.tools	pc	1	1
Test kit, cod low range(5-150)	Lab.tools	pc	1	1
Test kit cod medium range, mercury free	Lab.tools	pc	1	1
Test kit, Hydrazine	Lab.tools	pc	1	1
Test kit, Iron high	Lab.tools	pc	1	1
Test kit Nitrite low,	Lab.tools	pc	1	1
Test kit, P, Ortho. low, PO4	Lab.tools	pc	1	1
Glass beaker grad. 250ml	Lab.tools	pc	5	5
Glass beaker grad. 600ml	Lab.tools	pc	5	5
Glass beaker grad. 1000ml	Lab.tools	pc	1	1
Disposable pipette tips, yellow	Lab.tools	pc	1	1
Disposable pipette tips, blue	Lab.tools	pc	1	1
Petri dishes of glass 10 X 2	Lab.tools	pc	120	120
Volumetric flask, 100ml	Lab.tools	pc	6	6
Volumetric flask, 250ml	Lab.tools	pc	6	6
Volumetric flask,. 500ml	Lab.tools	pc	6	6
Volumetric flask, 1000ml	Lab.tools	pc	5	5
Washing bottle, 250ml	Lab.tools	pc	40	40
Magentic stirring bars,50 X7.5mm	Lab.tools	pc	3	3
Magentic stirring bars, 40 X7.5mm	Lab.tools	pc	3	3
Magentic stirring bars, 25 X6mm	Lab.tools	pc	3	3
Magentic stirring bars, 12 X7.5mm	Lab.tools	pc	3	3
Measuring cylinder, 50ml	Lab.tools	pc	5	5
Measuring cylinder, 100ml	Lab.tools	pc	5	5
Measuring cylinder, 250ml	Lab.tools	pc	5	5
Measuring cylinder, 500ml	Lab.tools	pc	5	5
Measuring cylinder, 1000ml	Lab.tools	pc	5	5
Beaker, 4000ml	Lab.tools	pc	4	4
Rack unwire white ACL20mm	Lab.tools	pc	1	1
Pipette cleaning sort E	Lab.tools	pc	1	1
Pipette, disposable non sterile3M	Lab.tools	pc	1	1
Sigma V8505Magenta vessels GA7	Lab.tools	pc	20	20
Sigma V8380Magenta vessels GA3	Lab.tools	pc	5	5
Sigma8630vessel for plant tissue culture	Lab.tools	pc	10	10
Sigma B8648 vessel B caps	Lab.tools	pc	10	10
Z13-058-3 amber bottles	Lab.tools	pc	1	1
Z11-851-6 plastic funnel	Lab.tools	pc	1	1

Item description	Category	Unit	Quantities procured over the project years				Total
Z14-274-3 angled funnel	Lab.tools	pc	1				1
MMM-810-34-12 Scotch Tape 3/4"	Lab.tools	pc	5				5
50-1679-02 Press sens tape	Lab.tools	pc	10				10
50-1730-06 Autoclave tape 3/4"	Lab.tools	pc	1				1
66-1002 Parafilm 2" X250'	Lab.tools	pc	2				0
66-1584-01Marking pens black	Lab.tools	pc	3				3
4110 Beaker brush black	Lab.tools	pc	12				12
1781-1 Flask brush	Lab.tools	pc	1				1
07-7700-01Flask brush white	Lab.tools	pc	1				1
29-9909-22 Knife blades ster #22	Lab.tools	pc	5				5
29-9905-23 Knife blades ster#23	Lab.tools	pc	5				5
29-9800-03 scalpel handle sz3	Lab.tools	pc	10				10
29-9800-04 scalpel handle sz4	Lab.tools	pc	10				10
76-7700-40 spatula S/S wood handle	Lab.tools	pc	5				5
04-1800-03 weigh tray mcro SWD	Lab.tools	pc	500				500
04-1800-06 weigh tray micro SWD	Lab.tools	pc	500				500
04-1800-09 weigh tray large	Lab.tools	pc	500				500
Micro spatula S/S 4S7	Lab.tools	pc	2				2
76-7666 micro spatula 6X1/2x1/8	Lab.tools	pc	5				5
36-6000-44 Forceps CP200	Lab.tools	pc	10				10
36-6000-48 Forceps CP250	Lab.tools	pc	10				10
77-8630-02 Mag stir Bar oct	Lab.tools	pc	3				3
Baby food jars	Lab.tools	pc	1000			500	1500
Vessel for growing phase	Lab.tools	pc				1000	1000
Lids for vessels	Lab.tools	pc	1000			1000	2000
Battery N 70	Lab. Equip	pc		1			1
PC monitor	Lab. Equip	pc			1		1
Computer Gateway	Lab. Equip	set	1				1
Printer, Laserjet-6L	Lab. Equip	pc	1				1
Hewlet Packard scanner	Lab. Equip	pc	1				1
UPS smart 700	Lab. Equip	pc	1				1
Photocopier Canon, NP6317 F134402	Lab. Equip	pc	1				1
Camera Canon PC1007, No 183412748	Lab. Equip	pc		1			1
Camera battery charger	Lab. Equip	pc	1				1
Sterioscope microscope	Lab. Equip	pc	1				1
TOYOTA-land cruiser-Prado	vehicle	pc	1				1

Annex 2. Status of the ARI Mlingano germplasm collection

Table 1. Agave species in the collection sites A, B, C and D at ARI Mlingano

Name of the Species	SITE			
	A	B	C	D
A. americana Aurea				
A. americana marginata Aurea 679				
A. americana Ex Nairobi				
A. americana x A. amaniensis				
A. ameniensis				
A. amaniesis variegated				
A. angustifolia				
A. angustifolia variegated				
A. attenuata				
A. bergerii				
A.cantala				
A. cantala Maguey				
A. fourtunae				
A. franzosnii				
A. furcrea gigantea				
A. furcrea Ex- West Africa				
A. furcrea cubensis				
A. Ghiesbreghtii				
A. heterocantha				
A. horrida				
A. lespenassei				
A. lespinassei Ex- Thika				
A. lespinassei x A. cantala				
A. miradorensis				
A. muilmanii				
A. nirvana				
A. sartorii				
A. sisalana				
A. spectabils				
A. verschafeltii				
A. wercklei				
A. xylonacantha				
A. zapupe				
Bubu Ex- Kulasi				
Bunchy top sisal Ex- Lambo				
Dwarf sisal Ex- Moshi				
Iradiated sisal no.2015				
Irradiated sisal no.3011				
Non-flowering sisal No. 32 Ex Thika				
Non-flowering sisal No. 7 Balam				
Sanservieria cylindrica				
Sanservieria intermedia				
Sanservieria ehrenbergii				
Sisal type collected by Grundy				

Note: The accessions at sites A, B and C are based on the species registered in the original maps of the sites.

Table 2. Accessions of Agave hybrids at ARI Mlingano

Hybrid number	SITE A	SITE B	SITE C		SITE D
Mlola 1					
1073					
1078					
1079					
Mlola 11					
B 114					
11646					
11648					
11664					
11674					
11699					
1252					
B 126					
1291					
13					
1300					
136					
138					
B 173					
B 182					
1871					
B 202					
B 208					
22					
254					
259					
B 26					
GA 27					
HA 38					
Mlola 487					
586					
MX 599					
62-7					
62-71					
62-75					
62-9					
6410					
68120					
69-3					
69-36					
7006					
7007					
7010					
7012					
71					
7045					
7111					
7111 Ex Balam					
713					
714					
716					
725					
7161					

Hybrid number	SITE A	SITE B	SITE C		SITE D
759					
Mlola 76					
7736					
817					
8366					
859					
874					
91					
93					

Table 2 (continued). Accesions of Agave Hybrids at ARI Mlingano

59 series

Hybrid number	SITE A	SITE B	SITE C	SITE D
100				
105				
11				
110				
125				
126				
127				
130				
138				
139				
14				
15				
158				
16				
20				
24				
27				
28				
29				
3				
32				
38				
4				
41				
42				
43				
45				
47				
5				
6				
64				
66				
69				
70				
71				
75				
84				

Table 2 (continued). Accessions of Agave hybrids at ARI Mlingano

61 series

Hybrid number	SITE A	SITE B	SITE C		SITE D
1					
2					
3					
5					
6					
7					
8					
9					
10					

65 series

Hybrid number	SITE 'A'	SITE 'B'	SITE 'C'		SITE 'D'
1					
10					
101					
127					
13					
133					
15					
22					
25					
30					
33					
35					
44					
50					
54					
58					
59					
6					
60					
62					
68					
77					
8					
92					
95					
96					
97					

Note: The accessions at sites A, B and C are based on the original maps of the sites.

Table 3. Germplasm collection at ARI Mlingano (October 2003)

Where: LOST IN SERIOUS DANGER REDUCED NUMBER OF PLANTS

Plot No.	Variety	Number of plants				Number of suckers	Pest/disease
		2001	2002	Feb-03	Oct-03	Oct-03	
1	Hybrid. No. 8366	14	14	15	15	13	KLS, Chlorosis
2	Hybrid No. 59/3	1	1	6	5	2	Chlorosis
3	Hybrid No. 71	15	15	17	15	14	KLS, Chlorosis
4	Hybrid. No. 62/71	7	6	9	9	8	KLS, Chlorosis
5	Hybrid. No. 1073	2	2	2	2	4	KLS
6	Hybrid. No. 22	12	12	12	12	10	KLS
7	Hybrid. No. 59/100	10	10	8	6	9	KLS, Chlorosis
8	Hybrid. No. 65127	12	12	11	10	13	KLS
9	Hybrid. No.11648	14	14	13	14	5	KLS, Chlorosis
10	Hybrid. No.59/27	8	8	7	7	8	Chlorosis
11	Hybrid. No.59/105	2	2	2	2	0	KLS
12	Hybrid No. 59/126	1	1	Lost			
13	Hybrid. No. 1871	8	7	9	6	17	KLS
14	N.F.S. No. 32 Ex Thika	8	8	8	8	4	Chlorosis
15	Hybrid No. 11646	2	2	2	2	2	KLS, Chlorosis
16	Mlola No. 487	10	10	10	10	16	KLS, Chlorosis
17	*Agave amaniensis x A. americana*	1	0	Lost			
18	*Agave spectabilis*	11	11	10	10	16	KLS
19	*Agave cantala*	13	13	14	13	20	Chlorosis
20	*Agave angustifolia*	3	3	3	3	3	KLS, Chlorosis
21	Hybrid No. 1300	13	13	13	13	16	KLS, Chlorosis
22	*A. amaniensis variegeted*	8	7	5	5	0	KLS
23	*Agave lespinassei*	3	3	3	3	1	Chlorosis
24	Hybrid No. 65/33	9	9	9	9	2	KLS, Chlorosis
25	Hybrid No. 6410	11	11	10	10	6	Chlorosis
26	Irradiated sisal No 2015	8	9	9	9	12	Chlorosis
27	N.F.S. 7 Balam	3	3	3	3	5	
28	Mlola No. 76	11	11	12	11	8	KLS
29	Hybrid No. 93	10	9	10	9	3	Chlorosis
30	*Fucraea gigantea*	5	4	4	4	0	
31	Hybrid No. 1079	1	0	Lost			
32	Hybrid No. 61/7	15	15	15	15	6	Chlorosis
33	Bubu Ex kulasi	1	1	1	1	0	
34	*Agave amaniensis*	14	14	13	13	9	Banding, Chlorosis, Scales, Weevil attack.
35	Hybrid ML No. 65101	7	7	6	6	0	KLS, Chlorosis
36	Hybrid No. 817	2	3	3	3	3	Chlorosis
37	*Agave sisalana*	7	7	4	4	0	PLTR
38	Mlola No. 1	12	12	12	12	4	Chlorosis
39	Hybrid No. 7012	2	2	2	2	12	
40	Hybrid No. 65077	4	3	2	2	0	KLS, Chlorosis

Plot No.	Variety	Number of plants				Number of suckers	Pest/disease
		2001	2002	Feb-03	Oct-03	Oct-03	
41	Hybrid No. 68120	1	1	1	1	0	Chlorosis
42	Hybrid No. 65133	4	4	4	4	8	KLS
43	Hybrid No. 65/8	1	1	1	1	1	KLS
44	Hybrid No. 1291	3	3	2	2	0	KLS
45	Hybrid No. 65035	3	3	2	2	0	KLS
46	Hybrid No. 61/9	5	5	5	5	8	KLS
47	Hybrid MI No. 11	1	0	Lost			
48	Hybrid No. 65022	4	4	4	4	0	KLS, Chlorosis
49	Hybrid No. 65095	4	5	4	4	4	KLS
50	Hybrid No. 7045	5	5	5	5	0	KLS
51	Hybrid No. G.A. 27	5	4	4	3	8	Chlorosis
52	Hybrid No. 65068	8	8	6	5	5	Chlorosis
53	Hybrid No. 91	4	4	3	3	0	KLS
	Hybrid No. 61/10	1	1	5	5	14	KLS
	Hybrid No. 62/71 Ex 7227	1	0	1	Lost		
	Hybrid No. 61/1	1	0				
	Hybrid No. 874	1	0				
	Hybrid No. 62/71	1	0				
54	Hybrid No. 59/6	13	13	13	12	9	Chlorosis
55	Hybrid No. 65025	11	10	10	10	4	KLS, scales
56	Hybrid No. 65015	7	7	7	7	8	KLS
57	*Agave vershafeltii*	12	7	12	10	4	KLS
58	Hybrid No. 725	3	12	3	3	0	
59	Hybrid No. 61/5	5	5	4	5	1	KLS
	Hybrid No. 254	5	5	7	5	5	KLS
60	Hybrid No. 716	8	8	8	8	0	KLS
61	Hybrid No. 65/58	1	1	1	1	0	
62	No. 3011	3	3	5	3	4	KLS
	Hybrid No. 6513	6	6	3	6	5	KLS, Chlorosis
	Irradiated Sisal	2	2	2	2	0	KLS, Chlorosis
63	Hybrid No. 59/4	12	1	12	12	7	KLS
64	Hybrid No. 759	3	4	3	3	0	KLS, Chlorosis
65	Hybrid No. B. 173	10	10	10	10	7	KLS
66	Hybrid No. B. 126	11	11	11	11	8	KLS, Chlorosis
67	Hybrid No. 62/75	15	7	13	13	33	KLS, Chlorosis
68	*Sanservieria ehrnbergii*	14	6	23	19	4	
69	Hybrid No. 59/20	7	7	6	5	4	KLS, banding
70	Hybrid No. 59/43	12	12	11	11	7	Chlorosis
71	Hybrid No. 65059	7	7	7	7	5	KLS
72	Hybrid No. 859	11	10	9	9	0	KLS, Chlorosis
73	*Agave lespinassei x Agave cantala*	7	7	6	6	5	KLS
	Hybrid No. 61/10	7	7	7	7	9	KLS, Chlorosis
74	Hybrid No. 7261	6	6	6	6	1	KLS, Chlorosis
75	Hybrid No. 65096	3	3	3	3	2	KLS
	Hybrid No. 65030	7	7	6	6	3	KLS, Chlorosis

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

The data presented in the tables 1 and 2 below has been taken from monthly/quarterly reports prepare by ARI Mlingano
(Lack of consistency in some of the data provided is due to quality of the original information.)

Table 1. Year 2003

	Up to March 2003		*Up to June 2003*		*In August 2003*		
Accession	**Growth**	**Multipli-cation**	**Growth**	**Multipli-cation**	**Growth**	**Multipli-cation**	**Rooting/ Green-house**
A. Sisalana	630	300	556	568	469	511	1,320
A. Hildana	150	140	241	195	341	180	70
Hybrid 11648	740	600	797	1,192	148	337	120
A. Amaniensis	80	0	0	0	1850	40	0
Amaniensis ex Mlingano	0	0	80	0	108	40	0
Total	**1,600**	**1,040**	**1,674**	**1,955**	**2,916**	**1,108**	**1,510**

Table 1. Year 2003 (continued)

Accession	*October – November – December 2003*								
	Growth			**Multiplication**			**Greenhouse**		
	Oct	**Nov**	**Dec**	**Oct**	**Nov**	**Dec**	**Oct**	**Nov**	**Dec**
A. Sisalana	49	415	51	125	280	214	398	868	512
A. Hildana	0	25	0	3	30	0	230	626	500
Hybrid 11648	93	654	649	237	200	100	100	900	600
A. Amaniensis	710	1652	0	525	504	0	0	0	0
Amaniensis ex Mlingano	0	0	0	0	0	0	0	0	0
Total	**852**	**2,986**	**700**	**890**	**1,014**	**314**	**728**	**2,394**	**1,612**

Table 2. Year 2004

January-February

	January 2004					*February 2004*				
Accesion	**Induc-tion**	**Growth**	**Multi-plica-tion**	**Green-house**	**Nur-sery**	**Induc -tion**	**Growth**	**Multi-plica-tion**	**Green-house**	**Nur-sery**
A. Sisalana	2,167	112	412	400	100	550	360	164	400	0
A. Hildana	49	31	0	495	0	0	0	0	490	0
Hybrid 11648	141	112	146	380	480	2,400	28	228	380	0
Sisal ex-Amani	0	365	471	254	0	0	200	400	630	0
Total	**2,357**	**620**	**1,029**	**1,529**	**580**	**2,950**	**588**	**792**	**1,900**	**0**

March – April

	March 2004					*April 2004*				
Accesion	**Induc-tion**	**Growth**	**Multi-plica-tion**	**Green-house**	**Nur-sery**	**Induc-tion**	**Growth**	**Multi-plica-tion**	**Green-house (to date)**	**Nur-sery**
A. Sisalana	0	0	0	1,018	373	0	0	0	1,917	0
A. Hildana	0	0	0	313	177	0	0	0	312	0
Hybrid 11648	3,974	0	0	1,920	0	3,263	0	1,362	1,515	0
Sisal ex-Amani	0	0	0	630	0	0	0	0	630	0
Total	**3,974**	**0**	**0**	**3,881**	**550**	**3,263**	**0**	**1,362**	**4,374**	**0**

May - June

	May 2004					*June 2004*				
Accesion	**Induc-tion**	**Growth**	**Multi-plica-tion**	**Green-house (to date)**	**Nur-sery**	**Induc-tion**	**Growth**	**Multi-plica-tion**	**Green-house (to date)**	**Nur-sery**
A. Sisalana	0	0	0	1,917	0	0	0	0	1,917	0
A. Hildana	0	0	0	312	0	0	0	0	312	0
Hybrid 11648	3,456	0	1,280	1,515	0	5,686	1,355	4,428	1,515	0
Sisal ex-Amani	0	0	0	630	0	0	0	0	630	0
Total	**3,456**	**0**	**1,280**	**4,374**	**0**	**5,686**	**1,355**	**4,428**	**4,374**	**0**

Table 3. Plants in Mlingano and Kisangata nurseries as of June 2004

Accession	Nursery	
	Mlingano	**Kisangata**
A. Sisalana	2299	1280
A. Hildana	394	0
Hybrid 11648	1351	1500
Sisal ex Amani	0	0
Total	**4044**	**2780**

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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Telephone: (+43-1) 26026-0, Fax: (+43-1) 26926-69
E-mail: unido@unido.org, Internet: http://www.unido.org