Dr. Otte George Dangatuk

MOLECULAR GENETICS OF AFRICAN *FAIDHERBIA ALBIDA* (DEL.) A. CHEV.

A RESEARCH PROPOSAL PRESENTED TO THE AFRICAN FORESTRY RESEARCH NETWORK (AFORNET), THE AFRICAN ACADEMY OF SCIENCES, P. O. BOX 14917 NAIROBI, KENYA.

INVESTIGATORS:
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EXECUTIVE SUMMARY

Faitherbia albida formely Acacia albida, is an important agro-silvo-pastoral tree species in the Sahelian Africa. It retains its leaves during dry season and sheds them off during wet season. And by so doing, and being a leguminous tree, it enriches the soil, provides micro-climate favourable for crops growth and provides green fodder rich in carbohydrates and protein during dry season for the livestock. It provides timber, fuelwood, medicine and food in the arid and semi-arid lands (ASALs). Owing to the excessive recent drought which has led to desertification, human agricultural activities, refugee problem in the ASALs, the germplasm of this important resource/commodity in the ASALs has been severely threatened to the extent that Food and agriculture Organization (FAO) Panel of Experts on Forest Genetic Resources included it among the endangered species and gave it a priority status for conservation and study to help reverse the situation.

F albida which was once widely distributed in west, east and southern Africa is now found in small scattered populations in few areas of East Africa compared to west Africa. In Tanzania such populations have been docu,munted to occur in Mwembe while in Kenya, they are found in the dry northern part of the country at Kainuk and Tot. Pockets of trees have also been reported to occur along some rivers and the shores of lake Victoria. It is not known exactly whether there are populations *of F. albida* in Uganda and it could be interesting to find out it they exist in Uganda and whether they are also faced with extinction. To our knowledge, the eastern African F albida, although equally endangered like others elsewhere in Africa, has not received any conservation efforts like their counterparts in west and southern Africa. This is not fair because recent reports indicate that eastern Africa is the probable centre of origin of this species and hence theoretically, this region should contain the highest level of genetic diversity of this species. This therefore emphasizes the reason why planned ex-situ and in-situ conservation of this species should take careful consideration to this region.

The amount of genetic diversity within and among the different populations of F. albida found in East Africa is unknown at present. Meaningful conservation of this species would require perfect knowledge of its genetic

structure and mating system, so that populations that are rapidly losing genetic variability and those with unique genes or allele combinations are given priority status. Similarly, populations that tend to be genetically uniform can be conserved through representative samples. The knowledge of genetic structure and mating system of F. albida, coupled with a reconnaissance survey which maps the areas where it is presently found in east Africa, the type of ethno-botanical uses and the danger (s) each of these populations is undergoing would provide useful information necessary to design a meaningful conservation strategy for this species.

The objectives of this proposal are: to conduct a survey to detect the areas where F. albida occur, document the ethno-botanical uses in different regions of east Africa and provide information regarding the potential and/real dangers to its existence. The second major objective is to use molecular and provenance trials to accurately determine the genetic variation and differentiation within and among populations of f. albida in East Africa.

This research is expected to supplement the efforts of FAO, the International Plant genetic Resources Institute (IPGRI) , Kenya Forestry research Institute (KEFRI) and International Centre for Research in Agroforestry (ICRAF) concerned with conservation of tree genetic resources. It is inline with the World Convention on Conservation of plant bio-diversity article passed in the Rio de Janeiro summit. More importantly, it is expected to arrest the loss of bio-diversity in ASALs and to conserve the genetic diversity in F. albida which is an important tree species in dry environments. Maintenance of F. albida in ASALs provide the pastoralists with fodder for their livestock, fuel wood and it improves soil fertility and give them hope. At its conclusion this research is expected also to have collected germplasm of this species which can be shared with interested parties such as KEFRI and ICRAF. And shall draw distribution map of this species in East Africa and propose conservation strategies that would prevent loss of genetic diversity in this important species.

1.0 INTRODUCTION

Faidherbia albida syn. *Acacia albida* is a leguminous woody species belonging to the Acacieae tribe of the mimosoideae sub-family. The species is widely distributed throughout the arid and semi arid (ASAL) zones of Africa. In Eastern Africa, it is found in Tanzania, Kenya, Uganda, Somalia, Sudan and Ethiopia (Brenan, 1959; Wickens, 1969). *F. albida* is an important agro-silvo-pastoral species in the Sahelian Africa, whose unique phenology of retaining leaves during the dry season and shedding them during the wet season provides a microclimate favourable for crops growth, while providing green fodder rich in protein and carbohydrates in the dry season for livestock. It enriches the soil and also used for timber, medicines and food (Wickens, 1969). In Sahelian Africa, drought and human activities have endangered the genetic resources of this species prompting its inclusion among the endangered plant species and therefore given priority status for conservation and study by FAO Panel of Experts on Forest Genetics Resources (FAO, 1977).

The East African populations are the least understood and so far no conservation initiatives are in place for them. Little is known about the distribution and ethno-botanical uses of F. albida in East Africa. The breeding biology, genetic diversity and mating systems information which are necessary to support any meaningful germplasm conservation efforts for this species are poorly understood, especially in the context of this species in East Africa. The East African region has scattered populations of *F. albida* around Tot and Kainuk in Kenya and Mwembe in Tanzania. Other scattered populations have been reported along the coastal part of Kenya around Lamu, along river courses and lakes (Dangasuk, Unpublished data). The existence of f. albida populations in Uganda is not exactly known. These scattered populations are getting wiped out to give room for cultivation. There is real danger that some of these rich genetic diversity which comprise important germplasm for this species shall be lost forever if immediate action is not taken to conserve them. Small populations such as these scattered f. albida populations in east Africa are known to suffer negative effects of inbreeding and random genetic drift (Falconer, 1960; Laikre, 1999) which normally lead to reduced fitness and may end up in extermination. Owing to their small population size, it is feared that the F. albida populations in East africa are experiencing random gnetic drift and inbreeding effects. It would be interesting to find out if the diminishing of the F. albida in East Africa has come about as a consequence of these factors or human activities of both. Surprisingly, small populations may also contain high genetic diversity as has been reported for *Alleum aaseae* (Smith and Pham, 1996) which results from out-crossing mating system coupled with perennial life cycle like that of *F. albida.* It is not known whether this is true for F. albida in east Africa. If this is true then it would provide a strong case for conserving the species in this region.

F. albida, being a long-lived perennial treeit forms an important ecological component of the ASALs. Owing to their unique adaptation in these arid environments, where there is less plant species diversity, their moderate growth and reproductive rates and being a riverine species, they are quite susceptible to human activities like most desert plants (Martinez-Palacious et al, 1999). It is probably for these reasons that F. albida is facing the greatest challenge for existence in the East african ASALs and it would be important to document and estimate amount of genetic diversity they contain because some of these populations occupy specific agro-ecological zones. which may make them important material for initial breeding, and germplasm conservation.

Although *F. albida* has been included among the endangered species by FAO (1977) and recommended for germplasm conservation, at present there are no conservation initiatives for the east African populations. Such efforts require a clear understanding of the reproductive biology, mating systems and the amount of genetic diversity present in the species. Large populations in Africa have been categorized as West African, East African and Southern African populations based on ecological adaptations (Joly et al 1992, Vandenbelt, 1992 and Dangasuk et al 1997). The West and Southern African populations have been extensively studied and their genetic diversity well characterized (Joly, 1991; Joly et al 1992) and it is easier to initiate breeding program and conservation measures for them. Genetic diversity studies in *F. albida* have been undertaken using provenance evaluation in the field (Vandenbelt, 1991; Dangasuk 1999), and isozyme genetic loci (Joly et al 1992; Dangasuk, 1999). In most cases these have provided base line information regarding the amount of genetic diversity and probable mating systems of *F. albida*. However, recent data indicated that although provenance evaluation and isozyme genetic loci have been useful in studying inter-provenance variations, they have not been quite useful in elucidating intra-provenance genetic variability (Dangasuk, 1999). Field evaluation has often suffered the confounding environmental factors while isozyme loci are few and most of them were found to be less polymorphic for analyzing individual provenance. The new breed of molecular genetic markers such as restriction fragment length polymorphism (RFLP) (Chalmers et al., 1992); Random amplified ploymorphic DNA (RAPD) (Williams et al., 1990); amplified fragment length polymorphism (AFLP) and microsatellites (Hughes and Queller et al., 1993), have opened new ways of assessing genetic diversity in many plant species including *F. albida*. RAPD is preferred for this study due to its simplicity and the fact that it does not require the use of isotopes. RAPD have been used to assess genetic variation in many plant species and microorganism including *Hordeum bulbosum* (Gudu, 1993). Lilium martagon (Persson et al., 1998) Prunus (Casas et al., 1999), chestnut (Li and Midmore, 1999), Moringer (Maluvu et al., 1999), blacklocust (Major et al., 1999) microorganism (Williams, et al., 1990) and can be used successfully for the same purpose in *F. albida*. A combination of provenance evaluation in the field, isozyme analysis and molecular RAPD marker analysis would help estimate inter and intra provenance genetic variation among the East African *F. albida* and would assist not only conservation efforts but breeding and improvement of this species. Isozyme and RAPD analyses would provide a closer genetic examination of the east african f. albida than would be possible from morphological traits alone. We are particularly interested in the level of differentiation among populations and the level of genetic diversity within populations to provide vital information that would assist us decide which populations might require a higher conservation priority. Moreover, this information in conjunction with ecological, ethno-botanical and demographic data is necessary for planning a comprehensive conservation strategy for this species. For example, two or more populations containing all the allozymes in the East African populations may be recommended and preferred than trying to conserve the whole diverse populations with similar alleles. Similarly, large populations with intermediate allele frequencies may be given preference to reduce the probability of losing some alleles. The provision of the present distribution range of F. albida in East Africa is particularly important, given their rapid disappearance where they were few years ago, they may no longer be there. This information would also provide the geographic distance between the scattered population of F. albida and may be usedto decide

whether their is possibility of gene flow among them and hence the maintenance of broad genetic base even if the populations are small. Initial genetic studies in West African F. albida population by Jolly et al (1992) and few populations from across Africa by Dangasuk (1999) have indicated that for these populations, most of genetic diversity is contained within and less among populations. For such genetic populations, conservation of representative sample populations is one possibility to capture genetic diversity. However, if the East African f. albida, are found to contain most of genetic diversity among the scattered populations, then they will pose a difficult conservation challenge because each population would be considered as an independent evolutionary unit meriting attention. And given the long life cycle coupled with out-crossing nature of f. albida, relatively large independent breeding populations would be maintained to adequately represent the high amounts of genetic present in each population.

We are also aware of problems of basing conservation measures entirely on allelic diversity , because it tends to ignore quantitative inheritance and genotype X environment interaction as pointed out by Baur and Schid (1996) and Kunin and Lawton (19996). This is why it is necessary to carryout provenance tests and survey the diversity as it exists under different environments of east africa. The conservation of F. albida in-situ and ex-situ is envisaged based on the principles of modern conservation (Jana, 1999; Pagiola et al., 1997). There is therefore need to initiate regional *F. albida* conservation project in East Africa with the following objectives:

1. To survey and report the current status of the species in Kenya, Uganda and Tanzania.
2. To study the existing genetic diversity within and among the disjunct populations in the region.
3. To initiate conservation and domestication measures.

1.1 Expected output

(i) Data on distribution and ethnobotany uses of the East African *F. albida* shall be provided. This data is useful for conservation efforts (in situ and ex situ). The data on distribution of the E. African *F. albida* will also report on the amount of threat (danger) to *F. albida* genetic resources in East Africa.

(ii) Data on flowering and reproductive biology under different agroecological zones shall be made available. This data is useful for seed collection efforts.

(iii) Germplasm seed collection shall be done on the in situ populations and this shall complement the FAO efforts to conserve *F. albida*.

(iv) Fine description of genetic diversity available within and among populations of *F. albida* shall be made using provenances tests, isozyme genetic loci and RAPD molecular DNA markers. The relationships among the East African *F. albida* shall be inferred based on estimates of

genetic distance among different populations. This is useful for germplasm collection and conservation.

(v) Finally, in the provenance evaluation tests that shall be carried out away from the seed source would help determine the agro-ecological range of the different *F. albida* populations. This information is useful for breeding and introductions of this species to different agroecological systems.

2.0 MATERIALS AND METHODS

2.1 Survey of the status of *F. albida* in East Africa

Literature review to gather important existing information about the species in general and the East African populations in particular. And also suspected areas of occurrence of the species will be marked out from the literature. Reconnaissance visits by roads to areas listed as locations of the species. In areas where the species is located details of the location such as; name of location, altitude, longitude and latitude will be recorded. Detail description of the location will be done with respect to natural physical features such as rivers, mountains and general state of the terrain, as well as artificial features such as roads, towns, villages etc. Weather record for each location will be consulted to give data for rainfall and temperature. Other tree species and vegetation associated with *F. albida* will be documented. Pattern of distribution of trees in each population (scattered or gregarious), size of each population, age distribution of the trees within each population will be recorded. Threats to the population in each location, such as lack of regeneration due to grazing by wild or domestic animals, agricultural activities (human interference), anthropological activities, etc. will be recorded.

2.1.1 Soil survey

Soil samples will be collected from selected auger sites from every population and analyzed in the laboratory for soil chemical and physical properties.

2.1.2 Within population tree survey

The number of trees in each population will be counted and their heights and diameters measured.

2.2 Genetic diversity

2.2.1 Sample collection for genetic diversity studies

For these studies plus trees will be marked from within each populations of *F. albida* in East Africa for selection and breeding purposes and samples of seeds, stem cuttings and leaves will be collected from the population at random to determine the genetic diversity. From each population about 1 – 10% of the trees will be randomly surveyed depending on population size.

2.2.1 Morphological variation

Genetic variability in seed characteristics will be evaluated based on differences in seed length, width, thickness and weight, as described by Dangasuk et al. (1997)

2.2.2 Provenance evaluations in different sites

Seedlings will be raised in various nurseries in East Africa for both nursery provenance evaluations and field trials in different sites. For example Kainuk, Tot and Lamu in Kenya; Kotido in Uganda and Mwembe in Tanzania. In each nursery in every site, 100 seeds per provenance will be nipped manually and each seed sown in a polythene bag measuring 5.5 x 18cm filled with soil. Four months old potted seedlings will be transplanted into the field in a randomized complete block design (RCBD) with repeated observations. Monthly assessment of the trees will be started one month after transplanting until the twelfth month after which assessment will be done on yearly basis.

2.2.3 Molecular and biochemical techniques.

Isozyme and Polymerase Chain Reaction (PCR) based methods will be adopted as fast methods to study the genetic diversity and structure within and among the species populations.

2.2.3.1 Isozyme analysis

Sixty clean seeds per provenance will be sterilized with hydrogen peroxide (30%) for 30 min., rinsed with distilled water, nipped to permit water absorption and germinated at a controlled temperature of 30°C for 48 hours in a Growth Cabinet. The germinants with tap roots of >1.5cm will be washed in double distilled water and homogenized in extraction buffer: Tris-HCl pH 7.8 {0.1M tris (base), 0.01M potassium Chloride, 0.01M Magnesium Chloride, 0.001M EDTA Sodium salt, 5% PVP-40, 20% PVpP, 1% BSA and 1% Mercaptoethanol}. Discontinuous starch gel electrophoresis will follow the procedures of Murphy et al., (1996) and Wendel and Weeden (1989). Electrode buffer: Tris Citrate pH 7.0 {0.125M tris (base) and 1.0M Citric acid} and Gel buffer: EDTA-HCl-Tris pH 7.0 {0.0014M EDTA, 0.05M L-Histidine and 0.1M tris (base)}.

2.2.3.2 RAPD DNA analysis

DNA Isolation from Plants

1. Leaf tissue will be ground into a fine powder in liquid nitrogen with a mortar and pestle. Care being taken during grinding to avoid shattering the mortar due to extreme cold.
2. β-mercaptoethanol (β-ME) be added to 2 x CTAB extraction buffer (*2 x CTAB = 10g CTAB [hexadectyltrimethylammonium bromide] composed of: 140-ml 5M Sodium chloride; 25-ml 2M Tris-HCl, pH 8.0; 20-ml 0.5M EDTA)*, to a final concentration of 0.2%. Heat the CTAB plus βME solution in a 60°C water bath for 5min.
3. 500µl of the buffer plus βME will be aliquot into a 1.5-ml microcentrifuge tube. And an equivalent of 100mg of the fine nitrogen powdered tissue added and placed in a 60°C water bath for 45min.
4. 500µl of CI (*CI=Chloroform-Isoamyl Alcohol=A solution of chloroform and isoamyl alcohol in the ratio of 24:1*) will be added and the tube closed. Extraction will be done by gently inverting the tube for 10 min.
5. Then centrifuged for 5min at 7,000rpm in a microcentrifuge at room temperature.
6. The upper (aqueous) phase will be transferred to a fresh tube using a wide bore pipette tip and re-extracted with CI. Again centrifuged as in step 5 and the aqueous phase transferred to a fresh tube.

7. 1ml absolute ethanol will be added and the DNA allowed precipitating at –20°C for at least 30min. (*Note: precipitating overnight substantially increases the yield*).

8. Then centrifuged for min at 7,000rpm to pellet the DNA.

9. Decant the ethanol and briefly dry the pellet in a vacuum centrifuge.

10. The Pellet is then re-dissolved in 100µl of 1xTE (*10xTE[Tris-EDTA] is composed of: 0.01M Tris-HCl, pH 7.5; 0.001M EDTA*) and 10µl of 3M Sodium acetate and 2.5 volumes of 95% ethanol added. Then precipitated at -20°C for 30min.

11. It will be centrifuged for 5min at 7,000rpm. Then the ethanol decanted and 1ml of 70% ethanol added to wash the pellet. Then re-centrifuged for min at 7,000rpm. Two 70% ethanol washes may be necessary to remove traces of CTAB or chloroform.

12. The pellet is then dried in a vacuum centrifuge until all visible traces of ethanol are gone. Do not over dry the pellet. Again the pellet is re-dissolved in 200µl of 1xTE.

13. The concentration and purity of the sample will be checked in a spectrophotometer before taking readings at 260nm and 280nm. An optical density of 1 at 260nm corresponds to a double-stranded DNA concentration of approximately 50µg/ml. The ratio of the readings at 260nm/280nm should be approximately 1.8; lower readings indicate contamination with protein and/or phenol.

2.3 Double stranded DNA amplification

This is the most common type of amplification; it can produce DNA products for many uses. The idea is to produce a large amount of double-stranded DNA copied from a particular gene region. The following solutions will be assembled prior to amplification:

Table 1. Levels above which various solvents inhibit PCR

Solvent	Inhibitory level
Ethanol	10%
Urea	1.5M

DMSO	1%
Formamide	10%
SDS	0.01%

1. DNA template DNA
2. 10 x *Taq* polymerase buffer (composed of: 10mM Tris-HCl pH 8.0, 1.5mM MgCl2, 50mM KCl, 0.01% NP-40, 0.01% Triton x 100).
3. 10 x dNTPs (dATP 2.0mM, dGTP 2.0mM, dCTP 2.0mM, dTTP 2.0mM). These are the raw materials for DNA synthesis, and are usually used at 200µM. The 10x solution is thus 2mM for each dNTP.
4. Oligonucleotide primers, diluted to 10µM.
5. $MgCl_2$ in water (usually 150mM).
6. *Taq* polymerase (kept cold).
7. Distilled and sterilized water.

Basic Reaction. For 25µl reactions

1. Mix the following in a microcentrifuge tube: 2.5µl 10x Taq buffer, 2.5µl 8mM dNTPs (i.e. 2mM each of dATP, dGTP, dCTP, dTTP), 1.2µl each of two primers (10µM stock solutions), 0.5-1.0 U *Taq* polymerase. Add ddH_2O to make 24µl per reaction. The order in which these are added is not important so long as the enzyme comes last. The enzyme solution, which is in a heavy glycerol-stabilizing buffer, should be well mixed and should be kept on ice or in the freezer until needed.
2. About 1µl template DNA (in ddH_2O or 0.1xTE) is added. Then any additives such as extra Mg^{++}, DMSO, etc are added.
3. 1 drop of mineral oil (common pure drug store variety) is added to prevent evaporation of the sample. If condensation forms on top of the PCR tube during cycles the reaction may not work. The spinned in microcentriguge for 5 sec. Note: larger reactions need more oil).
4. Place tube in thermal cycler and start run.
5. PCR of 25 to 35 cycles will be performed using the following temperature profile:

-Denaturation 96°C for 15 sec. (a longer initial time is usually desirable).

-Primer annealing 55°C for 30 seconds.

-Primer extension 72°C for 1.5 minutes.

2.4 Required Nuclear Ribosomal Gene Primers:

The following primers will be used:

18e-5' CTGGTTGATCCTGCCAGT

18j-5' GCCTGCGGCTAATTTGACTCAACACGGG

18d-5' CACACCGCCCGTCGCTCCTACCGATTG

28y-5' CTATCCGGGATTCCCCTAGTAACGGCGAGT

28ee-5' ATCCGCTAAGGAGTGTGTAACAACTCACC

28v-5' AAGGTAGCCAAATGCCTCATC

18h-3' AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA

5.8c-3' TTGCGTTCAAAGACTCGATGGTTCA

28z-3' AGACTCCTTGGTCCGTGTTTCAAGAC

28x-3' GTGAATTCTGCTTCATCAATGTAGGAAGAGCC

28jj-3' AGTAGGGTAAAACTAACCT

2.5 Electrophoresis.

Amplified products will be separated by electrophoresis in 2.5% agarose gels in gel buffers of TBE or TAE and visualized by ethidium bromide staining. The sizes will be compared with *hindIII* or *AvaI/BglII* digested λ bacteriophage DNA which will be included in each gel. Electrophoresis will stop when the dye front has reached the end of the gel.

3.0 REQUIRED INPUTS

3.1 Moi University contribution

-Salaries of investigators,
-Laboratory space,
-House the project

3.2 Project inputs

-Vehicle (4x4 wheel drive Toyota land cruiser)
-PCR machine (Thermal cycler)
-Power packs (4)
-Chemicals
-Polaroid photographic systems
-UV light system

-Agarose gel apparatus
-Computer, printer plus statistical programmes
-Gel tanks
-Centrifuge ultra-speed (vertical or fixed angle rotor 2 – 5ml tubes)
-Tissue homogenizer
-Goggles UV protective
-Spectrometer
-microfuge tubes

4.0 WORKPLAN

January – March 2000

-Purchase of vehicle

-Literature review on F. albida

-Reconnaissance surveys

-Order and purchase of equipment and chemicals

April – June 2000

-Detail population sampling

-Establishment of nursery sites

July – September 2000

-Fresh leaves collection for DNA analysis

-DNA isolation

-PCR – RAPD procedure

October – December 2000

-Seed collection

-Within population tree survey (height and diameter measurements)

-Soil sample collection and analysis

-Morphological analysis of seed characteristics

-Sowing of seeds in the nurseries

-Isozyme analysis

January – March 2001

-Provenance assessment at the nurseries

-Nursery maintenance

-Preparation for provenance field trials (ploughing, fencing, design lay out, digging of holes)

April – June 2001

-Transplanting of potted seedlings into the field

-Statistical analysis of tree survey data, soil survey data, DNA analysis data, and isozyme data analysis.

-First report preparation

July – September 2001

Final report preparation

5.0 BUDGET

5.1 equipments

Items	Qty	US$
-PCR machine (Thermal cycler)	1	3,000.00
-Power packs (4)	4	10,000.00
-Polaroid photographic systems	1set	1,500.00
-UV light system	1	2,000.00
-Agarose gel apparatus	1set	600.00
-Computer, printer plus statistical programmes	1set	4,000.00
-Gel tanks and moulds	4	1,000.00
-Centrifuge ultra-speed (vertical or fixed angle rotor 2 – 5ml tubes)	1	3,000.00
-Tissue homogenizer	1	300.00
-Goggles UV protective	1case	100.00
-Spectrometer	1	200.00
-microfuge tubes	3pkt	200.00
Sub-Total		25,900.00

5.2 Chemicals and consumables

-Chemicals for DNA analysis	5,652.26
-Chemicals for Isozyme analysis	5,200.00
Sub – Total	10,852.26

5.3 Vehicle (4x4 wheel drive)

Toyota land cruiser (one)	15,000.00
-Maintenance (mechanical)	5,000.00
-Insurance/Road lincence	1,000.00
-Fuel/engine oil/lubricant etc	10,000.00
Sub-Total	36,000.00

5.4 Research Assistant incentives

Particulars	Rate	US$
(a) Number of persons	4	
(b) Incentive per person per month	50US$	

© Duration of Data collection.....18 months
Incentive total cost (axbxc).....3,600.00

5.5 Cost of causal labour

Particulars.....Rate.....US$
(a) Number of persons.....8
(b) Incentive per person per month.....30US$
(c) Duration of data collection.....18 months
Incentive total cost (axbxc)..... .4,320.00

5.6 Honorarium Scientists

Particulars.....Rate.....US$
(a) Number of scientist.....3
(b) Honorarium/scientist/year.....3000.00
(c) Total Duration of Scientific Activities.....2 years
Total honorarium.....18,000.00

5.7 Local and International Travels for Scientists (excluding air-tickets)

Particular.....Rate.....US$
(a) Number of Scientist traveling 3
(b) AAS rate.....75.00 US$/day
(d) Number of days/year.....15
Total travel cost (axbxc).....3,375.00

5.8 Communications

(E:mail, Fax, Telephone, Postage etc) at 150.00US$/month for 24 months.....3,600.00

5.9 Literature

(Photocopying, purchase of articles from CAB international, journals etc).....2,000.00

5.10 Contigencies (10% of total budget).....10,764.70
5.11 Grand Total.....118,411.70

29/10/1999

Dear Prof. Gudu,

I hope you are doing fine, despite the heavy workload. I believe you received the manuscript sent as an attachment and might probably be working on it now.

Find here the AFORNET proposal as an attachment.

According to Dr. Iba Kone if there are students involved in the project, they should apply for a separate grant from AAS (the junior scientist research grant) but not be included in the main proposal. Consequently, I have left out the item for student from the budget.