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Publication date:
2007

Document version
Publisher's PDF, also known as Version of record

FOREST & LANDSCAPE

SAFRUIT

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FOREST & LANDSCAPE WORKING PAPERS 21 / 2007

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Publisher
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Series-title and no.
Forest & Landscape Working Papers no. 21-2007 published on www.sl.life.ku.dk

ISBN
ISBN 978-87-7903-328-3

DTP
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Citation
Sanou, H., Korbo, A., Tougani, A., Rabiou, A., Kambou. S., Ouedraogo, M., Diallo B.O., Parkouda C., Ræbild, A. & Jensen. J.S. 2007. Protocol for establishment of trials with Baobab and Tamarind within the SAFRUIT project. Forest & Landscape Working Papers no. 21-2007. Forest & Landscape Denmark.

Preface

This working paper includes protocols for establishment of breeding seedling orchards for Baobab and Tamarind and a provenance experiment with Baobab. The seeds have been collected from January 2006 to March 2007. The seeds are sown in nurseries in Burkina Faso (CNSF - Centre National de Semencés Forestieres and INERA - l'Environnement et des Recherches Agricoles), Mali (IER – Institute Economique et Rurale) and Niger (INRAN - Institut National de Recherches Agronomiques du Niger). The seed orchards will be established on locations in Mali, Niger and Burkina Faso. It is the aim to obtain a better foundation for future genetic management by selecting specific provenances, and identifying and selecting specific individual trees for future breeding and deployment in West Africa. The purpose of the project is also to study the gene ecological variance especially in relation to the threatening desertification and climate change in the region. The provenance trials will specifically assist in the latter objective in the short term. The Baobab trials should be used to identify specific good mother trees for leaf production (vitamin – C) and on a long term scale, the trials can hopefully provide knowledge about genetic variation in fruit quality and production.

The project is carried out in the frame of the EU - INCO project SAFRUIT (Sahelian Fruit Trees, contract no. 015465) including partners from West Africa and Europe.

Contents

1. Protocol 1: Establishing a baobab seed orchard

The objective is to establish a seed orchard with provenances originating from the countries participating in the SAFRUIT project. Other objectives are gene conservation, research objectives and to serve as a base for forest tree breeding. The seed orchard includes 12 provenances, 6 from Mali, 3 from Niger and 3 from Burkina Faso. Each provenance will be represented by 15 different mother trees. In total there will be approximately 180 families. The seedling seed orchard should include many local plants. At least 33% should be from the local country. The other provenances should comprise maximum 67%.

One of the purposes of the trial is to capture genetic variation. As 10 trees include $1 - 1/2N = 95\%$ of the additive variation, a number of 180 contains a huge variation with more than 99.9% of the existing additive variation of the selected populations. There is no need to include more trees.

To estimate progeny breeding values it will be acceptable to include approximately 24 plants from each family/tree. To produce the 24 plants it will be necessary to sow 30 seeds per family. If fewer than 24 plants are available but still more than 6, it is acceptable to include the family in the trial. It is suggested to sow more seed than the estimated need of plants, as a restricted germination rate can be expected. There are several ways to do this, e.g. sow more families (if 10 are needed, sow 15 etc.) or sow 2-5 seed per bag and remove redundant plants at an early stage.

In the nursery, the seeds shall be sown in plastic pots and placed in a randomized block design with 3 blocks. Each family will be represented by 10 pots per block. The pots shall be placed together in groups of 2x5. The family shall be placed randomly in each block. This means that one should avoid placing families with different neighbours and positions in each block. It is very important that the plants are clearly labelled, so their identity can never be confused.

Correct pre-treatment gives a better germination rate. Best results are obtained by treatment (1 hour in concentrated sulphuric acid, and 24 hours soaked in water (message by Kambou 2007). Scarification is also a good tool to obtain fast and equal germination. Scarifications should follow the sulphuric-acid treatment to avoid fungi infections. One should be sure there is sufficient space between plots for managing and doing measurements on the plants.

The nursery trial should be placed homogeneously place in the nursery to ensure equal conditions. Irrigation and nutrients should be added equally to all plants.

1.1 Measurements

The plants shall be measured for the first time approx. one month after germination. Measurements include:

- Germination rate
- Time for germination
- Height,
- Root collar diameter
- Number and size of leaves
- Survival

(This can be optional, if a 2 year nursery trial with all families are established, or if the plants are measured after establishing the field trial).

1.2 The field trial (seedling seed orchard)

Plants will be planted in the field trial during September-October 2007 or at the start of the rainy season 2008. The plants can be planted as soon as sites and plants are ready. A suitable area for the trial shall be identified in advance, so site preparation can be done, if necessary. The area should preferably be identified several months before.

Site preparation should be carried out according to good trial practice. The site should be placed on a location with a minimum distance of 500 meters to the nearest baobab tree. The large distance is necessary because it is a seed orchard that should be protected from pollination from outside.

The whole planting procedure should be described in a logbook where all details are noted, who did what, when and where? All deviations from plans must be described.

From each family 24 healthy plants should be selected for the field trial; weak and undersized plants should not be selected. If necessary, plants should be hardened off 1-2 weeks before transporting to the site (water). The seedlings should be well watered before transport. Avoid wind damage during transportation by erecting appropriate screens on the truck.

The plants shall be transported to the trial site in a flow which is suitable for the planters. It is very important that the plants are not left in the sun.
Plants should be protected sufficiently while waiting to be planted (by keeping them in shade and preventing them from drying out.

Suggested design:

- All plants per family used in the field trial should be counted.
- We suggest that many blocks should be used (>= 10). The more blocks – the easier it will be to make a random distribution and the greater the

degree of freedom for the analysis. For example the number of blocks can equal the average number of plants in the trial. If the number of plants in each family is the same (i.e. 24 for each family, then 24 blocks can be made. If the number of plants is lower, then the number of blocks can be reduced.

- The plants shall be planted in a randomized block design – and if there are sufficient plants - with 24 blocks and 180 plants pr. block. This means that each family is represented once in each block. It is important that the plants will be placed randomly within blocks.
- The plants should be distributed to all the blocks, so each block should have approximately the same number of plants. The placement of the family number in blocks should be done during the planning phase. An example is shown in Appendix B. It is easily done in a spreadsheet on the computer. If this model is followed, the placement of plants will be close to random and errors will be minimized. In this way, the "blocks" can be packed in the nursery, and this will make the planting procedure much easier.
- Single tree plots should be used. It is robust and can tolerate errors in the design.
- Each plant shall be distinctly labelled so there will be no confusion.
- The field trial should be planted block-wise. A plant from each block will be randomly picked and planted. When all plants in block 1 are planted – then continue with block 2. The blocks will then be planted continuously from one end of the trial to the other end.
- The distance should be 7 meters between the rows and 2 meters between plants (there should be a possibility for thinning in early age. However in general, the sizing of the trial is flexible and can be varied).
- The planting should ideally start from the corner in south-west, then line wise planting from south to north as in a normal coordinate system.
- Immediately after planting the position of the trees should be recorded on a map. This should be done quickly and before any labels disappearing from the plants. The trials should be properly marked with poles to show the borders between blocks and the surrounding vegetation.

The description of the site should include a general site description plus maps. Annex A below presents outline for site description. The following maps are needed:

- a location map showing the site's relative position to a town or other well known characteristic landmarks; the scale would be from 1:100,000 to 1:1,000,000 with GPS coordinates.
- a map showing in detail how to get access to the trial, usually in a scale of 1:50,000-1:100,000.
- a detailed map of the layout of the trial with blocks and the seed lots marked, in appropriate scale, is essential. This map should clearly show compass direction as well as location of each plant, planting space and date of planting, a description of the demarcation used for blocks and plots, border lines, as well as surrounding vegetation and landscape features. An

accurate sketch should also include position X,Y of each tree. This can be done on a computer (drawn in a computer spreadsheet e.g. Excel).

- Finally an environmental map showing the layout of blocks in relation to environmental variation would be useful. This could include either contours, or a three-dimensional drawing (optional).

Protect the area against animals if necessary.

1.3 Post planting maintenance

Necessary weeding should be carried out as often and as long as necessary. Weeding should be done in the whole trial (or at least block wise).

PHOTO: MORTEN ALBAN KNUDSEN

These Baobab trees are mainly used for collecting fruit. Almost all parts of the tree can be used, making it a truly multipurpose species: Fruits contain an energy- and vitamin-C-rich pulp often used to make refreshing and nutritive beverages. Seed can be eaten fresh, dried or roasted, or be used to extract oil. Seed can also be fermented to produce a protein rich condiment for food preparation.

2. Protocol 2: Establishment of nursery trial of baobab with all local provenances (represented by single tree origins)

The overall objective is to study provenance variation of baobab in the early stages of growth. The aim is to provide improved knowledge of family and provenance genetic variation, and provide plans for gene resource management. This is primarily a nursery trial, but the plants can be transferred to field trials as well.

Trial 1: 2 year study in the nursery of all available progenies from Mali (6). The number of provenances and families per provenance is optional (flexible). This part relates to the Mali part of the NuTree project (Baobab variation on various levels)

Trial 2: To investigate provenance variation of baobabs throughout its area of distribution. International collection of 30 provenances (Even though this is an objective of the SAFRUIT project, these trials are optional for all partners.

Partners should have the options to utilize data from all available progenies, and data should be produced fast and efficiently for publication. This material will only be studied in the nursery. There are too few plants per family for selection and this will only be a genetic variation study (family components, provenance components). The design is made to study adaptation to stress in the nursery and basic genetic components.

The nursery trial is composed of two parts: A family part based on progenies of Malian provenances, and a provenance trial based on provenance only. There are two possibilities

1) The two parts should be placed close together in the nursery (in this way it is not necessary to include Malian provenances in the provenance trial).

2) Include some of the Mali provenances in the provenance trial and the trials can be placed independently of each other. This requires a few more plants in the provenance trial.

2.1 Families

There are estimated 240 families. 16 seeds should be sown of each family and placed in a randomized block design with many blocks. E.g. 16 blocks or 8 blocks with 2 replications within each block. This will be voluminous with 16 x 240 plants at maximum, equal to 3840 plants.

2.2 Provenances

Suggested design for provenance trials: There are 24 provenances and enough seed should be sown to produce 100-120 plants. Therefore 2880 (3600 if 6 Mali provenances are included) plants will be needed.

Preparing the provenance seed lot: The provenance collection should be established from a seed mix of at least 20 individual baobab mother trees, and at best a mixture from all the provenances collected. Therefore only a few seeds should be used from each single tree seed lot. Only a total of 120 plants are needed.

Some of the provenances (Benin and Soudan) are provenance collections, and the seeds should be picked directly from these seed lots.

Table 1. Provenances available

Mali1	Burkina1	Togo	Tanzania 1	Soudan 2 p
Mali2	Burkina2	Benin1 p	Tanzania 2	Soudan 3 p
Mali3	Burkina 3	Benin 2 p	Tanzania 3	Malawi 1
Mali4	Niger 1	Benin 3 p	Tanzania 4	Malawi 2
Mali5	Niger 2	Senegal	Tanzania 5	Kenya
Mali6	Niger 3	Mozambique	Soudan 1 p	Tanzania 6

Be careful to keep information of provenance identity.

The plants should be established in a randomized block design with 4 blocks. In each block there will be 30 plants per provenance in one plot. The plots are placed randomly within each block. Each plot should be planted in a system of 5 columns x 6 rows.

6	12	18	24	**30**
5	11	17	23	29
4	10	16	22	28
3	9	15	21	27
2	8	14	20	26
1	7	13	19	25

Labelling: Minimum the first and the last plant should be labelled:

Comments: If the trial is going to be followed in the nursery only, a fewer number 40-60 trees can also be used per provenance. This is because of the expected small environmental variation. For field trials however, a larger number 80-120 trees per provenance are normally recommended. This is due to neighbour effects, and to allow thinning and precise estimation of dieback frequency in the trials.

2.3 Nursery preparations

The plastic pots should be arranged in such a way so that mistakes are avoided and that measurements can be done easily. There should be easy access to measure height and diameter etc. of each plant.

The trial should be placed under conditions which are similar for the whole trial (or at least within each block).

The plants should be treated uniformly (irrigation, nutrients).

A principle drawing of the trial: blocks, plot should be drawn (use a spread-sheet – example e.g. on the computer to make the drawings.

Optional
Provenance trials: after germination has finished, the trial can be reorganized (by taking away the pots without plants). Keep 20 or 24 plants per plot and re-organize the trial. The trial becomes smaller and easier to measure.

2.4 Measurements

First year: is basically a starting year.

- Measurement of height after the growth season,
- Diameter,
- Survival rate
- Number of leaves after full development of leaves

Second year: Suggested measurements:

- Diameter is measured 2-6 times during the season
- Height measured 2 times
- Start of leaf production recorded
- Time of leaf drop recorded
- Number of branches measured (ramification)
- Number of leaves counted
- Survival rate after year 2
- Insects – attack by insects
- Leaf length measured
- Petiole length, width measured
- Vitamin A - assessment

Measurements of nutrients can be done (Appendix D in this protocol). An individual protocol will be made later.

After two seasons the plants have to be finally measured. At this stage it can be decided whether to stop the trial or transfer it to the field.

2.5 Field trial (optional)

The plants should be transferred to field trial, for long term observations. The number of plants can be reduced to 80 by taking away the poorest plants. The design is a randomized block design with 4 blocks and 20 plants per plot (provenance)). The field trial should be established in an 3x3 meter design. 9 square meters per plant and approximately 2.3 ha should be needed for a field trial. For documentation of the trial: the field trial description should be followed.

2.6 Stress treatment in the nursery

Stress can be applied through various watering regimes, nutrient, salt, ethylene glucol etc. It is outside the scope of this paper to make a specific protocol for this. However this should require a larger number of plants than recommended earlier in this paper.

PHOTO: MORTEN ALBAN KNUDSEN

This Baobab is mainly used for its leaves. The leaves are especially rich in vitamin A and are appreciated as a sort of spinach in local dishes. The branches are cut back every year ensuring a new supply of leaves. Fibres from the bark are used to make cords, which are used for making rope and string. Most of the plant has uses in traditional medicine, which to some extent gives rise to a local and international trade with Baobab tree products.

3. Protocol 3: Establishing of a Tamarind seed orchard

The objective is to establish a seed orchard with provenances originating from the countries participating in the SAFRUIT project. Other objectives are gene conservation, research objective and a base for forest tree breeding. The seed orchard includes 12 provenances of which 4 are from Mali, 4 from Niger and 4 from Burkina Faso. Each provenance will be represented by 15 different mother trees. In total there will be 180 families. Tree families in the field should be selected in such a way, that they are not neighbours.

The seeds shall be sown in plastic pots. To estimate progeny breeding values it is recommended to include 24 plants from each family/tree. To produce the 24 plants it will be necessary to sow more seeds per Family (one method is to sow two seeds in each pot, or to do pre-germination before planting). 24 plants is a minimum because selection should be done on a statistical sound basis. Around 12-14 would be an absolute minimum.

The plants should be placed in a randomized block design. It would be most practical to keep a number of 3 blocks in the nursery, each with 10 replicates from each family in the block. The families should be placed randomly in each block. The pots shall be placed together in groups for practical reasons. The identity of all the plants shall be ensured by labelling the plants. Space for managing the measurements on the plants must be considered also. (In principle a higher number of blocks can always be used – this would be reasonable if a large part of the research takes place in the nursery). The block design assures that all plants will have an equal chance to be exceptionally favoured or stressed by external environmental factors.

The nursery trial should be placed on a uniform place in the nursery to ensure equal conditions. Irrigation and nutrients should be added equally.

3.1 Measurements

The plants must be measured for the first time a few months after germination.

Measurements includes

- Germination rate
- Time for germination
- Height,
- Root collar
- Diameter.
- Survival rate

3.2 The field trial (seedling seed orchard)

Plants should be planted in the field trial in autumn 2008 (after the first growth season), or kept in the nursery until the next rainy season. A suitable area for the trial shall be identified in advance, so site preparations can be done in advance, if necessary.

The plants shall be transported to the trial site in a successive flow so the plants do not have to stand in the field for a longer time. It is very important that the potted plants are not left in the sun.

From each family, it would be preferable to select 24 of the most vital plants for the field trial. If less than 24 plants and more than 6 are available, then it is still acceptable to include the family in the trial.

The plants shall be planted in a randomized block design with 24 blocks and 180 families/block. This means that each family is represented once in each block. It is important that the plants will be placed randomly within blocks. This can be secured by mixing the pots around. For practical reason, the number of blocks can be reduced to 12 blocks with twice as many families per block. The families should be placed randomly within the block to minimize co-ancestry.

Each plant shall be distinctly labelled in such a way so there will be no confusion about the identity.

There shall be 5 meters between the rows and 2 meters between plants. The trial shall be planted continuously beginning with row 1, row 2 etc. until all 180 plants from block 1 are used. Then continue with block 2 etc – until all blocks are planted.

- The plants should be distributed to the blocks, so each block should have the same number of plants. The placement of the family number should be done in a block. An example is shown in Appendix B. It is easily done in a spreadsheet on the computer. If this model is followed, the placement will be close to random and errors will be minimized. By this way, the plants can be prepared and packed "blockwise" in the nursery "packs", and this will make the planting procedure much easier.
- Single tree plot design should be used. This is robust and can tolerate errors in the design (and lack of thinning).
- Each plant shall be carefully labelled in such a way so as to allow no confusion.
- The field trial should be planted block-wise. A plant from each "pack" (block) will be randomly picked (from the pack) and planted. When all plants are planted in block 1, then continue with block 2. The blocks will then be planted continuously from one end of the trial to the other end.
- There should be 7 meters between the rows and 2 meters between plants (this is of course flexible according to the area etc.).

- The planting should ideally start from the corner in south-west, then line wise planting from south to north as in a normal coordinate system.
- Immediately after planting the position of the trees should be recorded on a map. This should be done as soon as possible and before any labels disappear from the plants. The trials should be properly marked with poles to show the borders between blocks and the surrounding vegetation.

The description of the site should include a general site description plus maps. Annex A presents suggestions for site description. The following maps are needed:

- a location map showing the site's relative position to a town or other well known characteristic landmarks; the scale would be from 1:100,000 to 1:1,000,000 with GPS coordinates.
- a map showing in detail how to get access to the trial, usually in a scale of 1:50,000-1:100,000.
- a detailed map of the layout of the trial with blocks and the seed lots marked, in appropriate scale, is essential. This map should clearly show compass direction as well as location of each plant, planting space and date of planting, a description of the demarcation used for blocks and plots, border lines, as well as surrounding vegetation and landscape features. An accurate sketch should also include position X,Y of each tree. This can be done on a computer (drawn in a computer spreadsheet, e.g. Excel). The position should be recorded. All trials should move from south-west corner to north-east (as in a normal coordinate system).
- Finally an environmental map showing the layout of blocks in relation to environmental variation would be useful. This could include either contours, or a three-dimensional drawing (optional).

Protect the area against animals if necessary.

Appendix A

SAFRUIT PROJECT NETWORK: PROCEDURES AND DESIGN IN TRIAL STABLISHMENT

FIELD TRIALS • SITE DESCRIPTION

The site description data is collected at the beginning of the trial establishment and should include the following: (the * marked field should be filled as a minimum)

Species:*_____

LOCATION

Name of the site*: _____

Country*: _____

Province*: _____

District:* _____

Latitude* (degrees and minutes): _____

Longitude* (degrees and minutes): _____

Altitude* (m above sea level): _____

Managing office/institution*: _____

Owner*: _____

Distance to nearest office responsible for management (km): _____

Distance to nearest villages/towns (km)*: _____

Number of inhabitants in the nearest villages/towns: _____

Type of area (e.g. research station, managed forest, etc.)*: _____

_____

Add map(s) (see section above).

CLIMATE

Nearest weather station:

Name of the station: _____

Latitude (degrees and minutes): _____

Longitude (degrees and minutes): _____

Altitude (m a.s.l.): _____

Climatic data[1]	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Year*
Rainfall (mm)													*
Rainfall (mm) Max													
Rainfall (mm) Min													
Temp mean (ºC)													*
Temp. mean max.2 (ºC)													
Temp. mean min.3 (ºC)													

1 Period of observations: _____ (specify years)

2 Average of daily maximum temperatures

3 Average of daily minimum temperatures

Rainy season:

Number/type of seasons* (normally one? One rainy season):

one: _____ two: _____ even: _____ irregulars: _____

Period(s): _____ (specify months)

No. of intermediate days proximate to growing season: _____

No. of wet days in growing season: _____

Dry months (number month per year of < 50 mm rain)*:_____

Prevailing wind (direction, period, speed)*: _____

TOPOGRAPHY*

__ Flat/gentle (0-8 %) __ Intermediate (9-30 %) __ Steep (>30 %) < /P>

SOIL

Please tick the following soil description (as good as possible)*

Soil texture		Soil depth		Soil drainage/ Waterlogging		Gravel content, topsoil	
1. Light/sandy	☐	1. Shallow (< 50 cm)	☐	1. Well drained	☐	1. None (< 15 %)	☐
2. Medium/Loamy	☐	2. Deep (50-100 cm)	☐	2. Seasonal	☐	2. Gravelly (15-35 %)	☐
3. Heavy/clayey	☐	3. Very deep (> 100 cni)	☐	3. Permanent	☐	3. Stony (> 35 %)	☐
Organic matter content		**Reaction (pH)**		**Soil salinity**		**Ground water**	
1. Poor (< 2 % DM)	☐	1. Acid (pH < 6.5)	☐	1. None <4 ds/m	☐	1 1 - Shallow (< 50 cm)	☐
2. Medium (2-5 % DM)	☐	2. Neutral (6.5-7.5)	☐	2. Moderate 4-8 ds/m	☐	12. Deep (50 - 150 cm)	☐
3. Rich (> 5 %)	☐	3. Alkaline (pH> 7.5)	☐	3. High >8 ds/m	☐	13. Very deep(>150 cm)	☐

VEGETATION

Natural (original) vegetation type*: _____

_____

Dominant natural (original) genera/species*: _____

_____

Land use history: _____

_____

_____

_____

ADDITIONAL INFORMATION

Apparent major problems of site (pests, diseases, etc.): _____

_____

_____

Economics data, viz farm gate prices for fodder, fuel wood, small size timber, extractives, and other minor produce :

_____

_____

_____

_____

REPORT AUTHORS – DATE:

_____

Appendix B

Plot design. This actual design for Tamarind is a planned as a single tree design. Because of the few plants available for the whole trial, it was decided to include all families – even if the numbers are very few. The reason is to maximize genetic diversity and utilize the plant material. They do not contribute to the research.

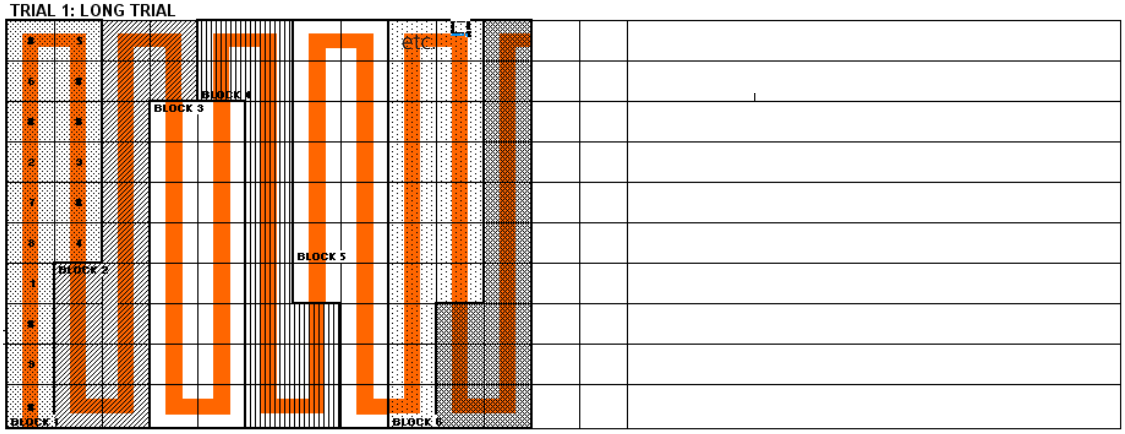
		Avail.	Blocks										Used	Waste
Pays	**Provenance**	**plants**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**		
Niger	Maradi	47	3	3	3	3	3	3	3	3	3	3	30	17
Niger	Maradi	41	3	3	3	3	3	3	3	3	3	3	30	11
…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
Mali	Zambougou	30	3	3	3	3	3	3	3	3	3	3	30	0
Burkina	Liptougou	27	3	3	3	3	3	3	3	2	2	2	27	0
Mali	Sam	26	2	2	2	2	3	3	3	3	3	3	26	0
Mali	Zambougou	24	3	3	3	3	2	2	2	2	2	2	24	0
Niger	Maradi	24	2	2	2	2	3	3	3	3	2	2	24	0
Burkina	Liptougou	23	3	2	2	2	2	2	2	2	3	3	23	0
Niger	Maradi	23	2	3	3	3	2	2	2	2	2	2	23	0
Niger	Maradi	22	2	2	2	2	3	3	2	2	2	2	22	0
…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
Niger	Parc W	7	1	1	1	1	0	0	1	0	1	1	7	0
Niger	Torodi	7	1	1	1	1	0	0	0	1	1	1	7	0
Niger	Torodi	7	1	1	1	1	1	1	1	0	0	0	7	0
Burkina	Noberé	4	1							1	1	1	4	0
Mali	Komodiguili	4		1	1	1	1						4	0
Mali	Sam	4						1	1	1	1		4	0
Niger	Parc W	4	1	1	1							1	4	0
Niger	Torodi	4				1	1	1	1				4	0
Burkina	Liptougou	3	1										1	2
Burkina	Liptougou	3		1	1	1							3	0
Burkina	Liptougou	2					1	1					2	0
Mali	Sam	2							1	1			2	0
Mali	Zambougou	2									1	1	2	0
Niger	Parc W	2	1	1									2	0
Niger	Parc W	2	1	1									2	0
Burkina	Noberé	1			1								1	0
Burkina	Noberé	1										1	1	0
Burkina	Peni toussiama	1					1						1	0
Mali	Komodiguili	1									1		1	0
Mali	Komodiguili	1								1			1	0
Mali	Komodiguili	1								1			1	0
Mali	Sam	1									1		1	0
Niger	Torodi	1										1	1	0
Burkina	Liptougou	0											960	
Total per block			**96**	**96**	**96**	**96**	**96**	**96**	**96**	**96**	**96**	**96**	**960**	

Appendix C

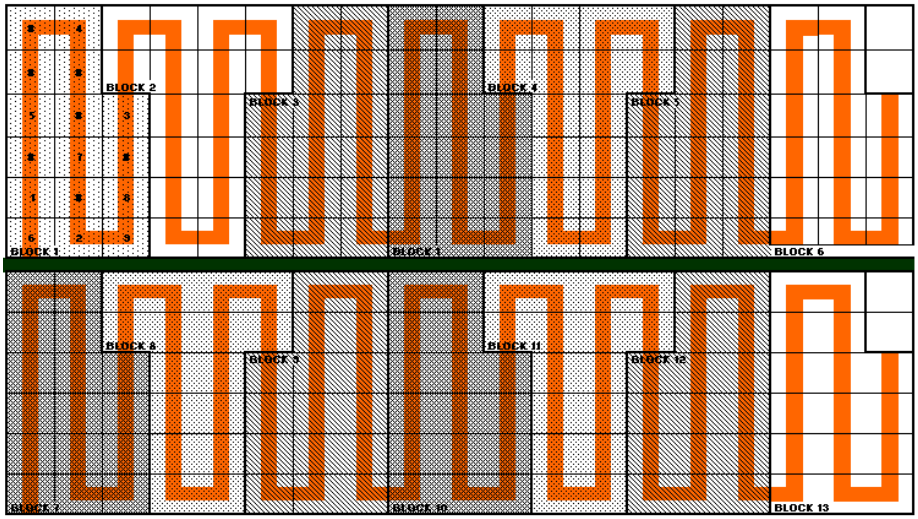
Single tree plot. Principles of establishment (planting) of seed orchard by a random design. Trial 1: used in long designs or designs with a large number of blocks (>15). Trial 2: Same principle, but the trials are divided in larger sections. The single blocks are more compact and the design is favorable when 1) smaller number of blocks are relevant, 2) if the area forms an approximately square form 3) If there is a high environmental variation. Planting happens from left to right. This is a case example with 16 single tree plots per mother tree. The number of trees per block can vary. When all plants in one block is planted, a new block will be started in the next coming plot. There can be more plants from the same family in the blocks, but they will still be randomly placed in the trial.

(This principle can also be applied for larger plots with several trees per plot (e.g. provenance trials).

TRIAL 1: LONG TRIAL



TRIAL 2: DESIGN IN A NORMAL SQUQRE



Appendix D

Protocol for leaf collection for provitamin A.
Charles Parkouda, DTA, Burkina Faso

Protocole d'échantillonnage des feuilles en pépinière

Paramètres à analyser

A déterminer ??? Nous compléterons si nécessaire

Quantité

A déterminer en fonction des paramètres que vous aurez retenus.

Matériels

Mortier et pilon en porcelaine ou en bois, étuves, sachets opaques, sécateur

Méthodologie

- ☞ Cueillir les feuilles de chaque pot de pépinière (descendant)
- ☞ Peser les feuilles de chaque pot de pépinière (descendant)
- ☞ Faire sécher les feuilles de chaque pot dans une étude réglée à 50 °C jusqu'à poids constant
- ☞Peser les feuilles séchées de chaque pot de pépinière après séjour dans l'étuve
- ☞ Laisser refroidir dans un dessiccateur pendant 30 minutes
- ☞ Broyer (moudre) les feuilles dans un mortier en porcelaine pour éviter l'enrichissement de la matière par des éléments notamment éléments minéraux. Le mortier en bois peut être également utilisé
- ☞ Conditionner immédiatement après broyage dans un sachet opaque (ou em ballage en cellophane) pour éviter la lumière
- ☞ Conserver au réfrigérateur à 4°C

NB : Une fois les feuilles cueillies veuillez à les exposer moins au soleil.

En complément du protocole sur l´échantillonnage des feuilles de baobab
Voilà quelques informations que vous avez sollicitées pour la réalisation des analyses :

* Pour le type de feuilles à prélever cela dépend de l´objectif visé par le projet.
  Si c´est pour voir l´héritabilité de mère – fille : il faut prélever le tout (jeunes et vielles).

  Si c´est pour voir l'apport alimentaire il faudra voir à quelle stade les feuilles sont généralement cueillies pour la consommation alimentaire (pas très jeunes, pas vieilles).

On peut envisager aussi de faire un suivi pour voir à quel stade de développement les feuilles sont concentrées en carotènes (mais c´est une autre étude).

* Pour les quantités, nous pensons que 100g de produit séché sont suffisant (c´est à dire 3 x 10g pour les carotènes et de 3 x 5g pour les humidités) si les feuilles sont moyennement riches en carotènes. Si elles en sont faibles il faudra augmenter la prise d'essai.

Il s´agira de déterminer les carotènes totaux qui seront exprimés en équivalent beta carotène (Provitamine A d´origine végétale). Mais si vous désirez avoir uniquement la beta carotène (sans les autres carotènes), il faudra une séparation et une purification sur colonne; ce qui est encore plus compliqué et demandera encore des dépenses supplémentaires (achat de colonne et d´autre produits chimiques).

* Pour la période d´acheminement du premier lot d'échantillons de feuilles sèches de baobab aux laboratoires DTA Ouagadougou, cela dépend de l´évolution au niveau des pépinières. Car pour éviter encore des attentes, perte de temps et d´autres problèmes qui peuvent être liés à l´attente pour un stagiaire académique (DEA, DESS) nous proposons l´option de prendre un technicien en prestation de service (immédiatement disponible).

* Pour le stockage :
  Maximum 1 mois à 4°+ ou - 2°C dans un emballage opaque à la lux
  (comme vous avez utilisé pour la pulpe de baobab)
  Maximum: 12 mois à -20°C, emballage opaque

* Y´aura-t-il des échantillons du Burkina pour les feuilles de baobab?

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