Ministerio Da Agricultura E Desenvolvimento Rural
Direcção Nacional De Agricultura

Report No. CP-2025 on the mission from 16 to 24 November 2006 by Dr Roland Bourdeix

Coconut genetic resources and breeding in Mozambique.
Preparation for the planting of two field experiments to screen coconut varieties for tolerance of Lethal Yellowing Disease.

Mission order No. 06-624

REPÚBLICA DE MOÇAMBIQUE

CIRAD

Contents

Acknowledgements

I would like to thank here the people who helped me and contributed to the success of this study in Quelimane and Maputo.
Special thanks to Serafina Mangana (Crop Protection: ZADP), who accompanied me throughout each visit, to Luis Tomo, from the Quelimane provincial Directorate for Agriculture and to Emmanuel Lourenço from the Madal Company who showed me all the aspects of coconut cultivation during the field visits.

Terms of reference

This CIRAD mission took place under a technical assistance agreement, launched in November 2002 between the National Directorate for Agriculture (DINA) of the Ministry of Agriculture and Agricultural Development (MADER) and the French Agricultural Research Centre for International Development (CIRAD). The objective of this mission was to ensure technical and scientific assistance to the Project called "Projecto De Apoio Ao Sector Coqueiro Em Moçambique" (PASCOM). The terms of reference for this mission were the following:

- To evaluate the activities linked to the improvement and genetics of the coconut palm, as well as the production of coconut seednuts currently conducted in Mozambique.
- To propose experimental designs for two experiments aiming at testing the tolerance of Lethal Yellowing Disease of imported and local coconut varieties.
- To propose new experiments enabling quick identification of material tolerant of Lethal Yellowing Disease.

Summary

The aim of this mission was to evaluate the activities linked to the improvement and the genetics of the coconut palm, as well as the production of coconut seednuts currently conducted in Mozambique; to choose geographical sites and to propose experimental designs for two experiments aimed at testing the tolerance of Lethal Yellowing Disease (LYD) of imported and local coconut varieties. The Madal company is currently producing a large amount of green-coloured seedlings (Mozambique Tall and a few hybrids), but there is no scientific evidence that this material will be tolerant of LYD. A total of 8,921 seednuts from 21 varieties were imported from Ivory Coast; from those imports, 2,429 seedlings are still available for planting. Some mixes between imported varieties have occurred in the nursery. Those varieties are to be tested against the disease together with local varieties collected in Mozambique. In any case, the two planned experiments can be planted in January 2007. Two sites have been chosen inside a large plantation located close to the Magromane seed garden and belonging to the Madal Company. Site 1 is the "Estacao Inhangulue", near the Madal husk processing Factory (S 17°56'32"03; E 36° 56'15"08); and site 2 is "Estacao Machimbui" (S 17°53'41"89 E 37°03'28"22). The main problem will be to protect the young seedlings from attacks by insects which proliferate in the stems of palms killed by the disease. The most efficient technique against insect attacks is to remove dead stems from the planting sites. Two experimental designs, each containing 8 replications of 196 palms have been proposed for planting in 2007. Some possible development of research activities is discussed for breeding and disease resistance.

Mission Schedule

Monday October 16. Departure from Montpellier for Mozambique.
Tuesday October 17. Arrival in Maputo. Met by and discussion with Mrs. Serafina Mangana.
Wednesday October 18. Departure for Quelimane with Mrs. Serafina. Met by Mr. Luis Tomo. Meeting with Mr. Rafik Mahomed Kala, Director of Agriculture for the province of Zambezia. Visit to the Nicoalada nursery where the seeds introduced from Ivory Coast and those collected in Mozambique were sown. Discussions with Mr. Liria Sambo Nhaquila, agronomist and Mr. Brendan Kelly, adviser, both working in the coconut sector at the Centre for the promotion of Agriculture (CEPAGRI).
Thursday October 19. Visit to the Madal plantation. Tour of the Inhangulue site where one of the experiments will be planted. Visit to the husk processing unit at the Madal Company. Tour of the site of the hybrid test funded by the Cogent Network. Presentation of documents about the Mozambican Coconut Research project prepared by Dr Bourdeix for the 2005 Steering Committee Meeting of the Cogent network. Brief presentation of the lecture on the coconut palm prepared by Dr R. Bourdeix.
Friday October 20. Visit to a putative second site for setting up the experiments (near the village of Inhassunge).
Saturday October 21. Discussions and decision-making about the location of the experiments and the experimental designs to be chosen. Return from Quelimane to Maputo.
Sunday October 22. Drafting the report. Visit to the island of Inhaca for possible establishment of a safeguard coconut collection linked with ecotourism.
Monday October 23. Visit to AFD and French Embassy in Maputo. Departure for Paris.
Tuesday October 24. Arrival in Paris.
Wednesday October 25. Arrival in Montpellier.

1 Evaluation of the genetic aspects of the project

1.1 The Nicoalada coconut nursery

This nursery, located in the district of Nicoalada, contains the seedlings of varieties imported from Ivory Coast and the tall varieties collected locally in Mozambique. The appearance of this nursery is satisfactory, with apparently very good maintenance at the time of this visit.
Some of the seedlings are derived from seednuts collected more than two years ago. The seedlings display weak development bearing in mind their age. They probably suffered in the past from drought, which limited their development. A boron deficiency was seen on some seedlings; it is therefore necessary to provide that nutrient by suitable fertilization (Boracine 2 g per seedling).
The seedbed contains a mixture of badly identified varieties. No selection has been carried out based on sprout colour by the Mozambican technicians, whereas they have that skill, which has existed for a long time in the Madal Company. Thus, on a batch of 17 seedlings referred to as "Malayan Yellow Dwarf", no more than 4 displayed the highly characteristic yellow colouring of that variety. According to the document provided by the Mozambican staff of the project, the "Malayan Yellow Dwarf" variety was not included in the list of varieties imported from Ivory Coast, and was not collected locally. We took leaf samples to try and specify from which varieties those seedlings really are.
There have therefore been varietal mixtures. This mixture occurred accidentally in Mozambique after receipt of the seednuts. The seednuts produced by artificial pollination from Ivory Coast each carried an artificial pollination number engraved on a metal or plastic label attached to the seed. That number made it possible to precisely find the identity of the male and female parents of each seednut. The labels were systematically removed by the Mozambican technicians. That is detrimental for the experimental work. Moreover, it prevents any identity checking of the seedlings by any other means but molecular analysis. Those labels would have in particular made it possible to know when the accidental mixture of the varieties had been made.
Molecular analysis will make it possible to confirm that there was a mixture and to specify the variety of each analysed seedling. It was asked for the seedlings to be numbered not only by line (as it is presently done) but individually, seedling by seedling with a label hung on each seedling.
A total of 8,921 seednuts from 21 varieties were imported from Ivory Coast; 2,429 seedlings are now available. So the percentage of good seedlings as compared to seednuts is 25% for the material imported from Ivory Coast[1]
A total of 1,880 seednuts from 9 populations of Mozambique tall palms were collected in Mozambique; 728 seedlings are now available. So the percentage of good seedlings as compared to seednuts is 39% for the material collected locally.

[1] According to the first inventory made by the Mozambican technicians during the mission by Dr R. Bourdeix, the percentage of good seedlings as compared to collected seednuts was 22% for the material imported from Ivory Coast. But some mistakes were detected (see annex 1), and a second inventory was requested. According to the second inventory sent by Mr. Luis Tomo in December 2006, the percentage of good seedlings as compared to collected seednuts is 25% for the material imported from Ivory Coast.

1.2 Seednuts and seedlings produced by the Madal Company

The Madal company continues to produce coconut seednuts in great quantity. In the Magromane seed garden, approximately 120,000 hybrid seednuts are produced annually. They are hybrids between Malayan Yellow and Red dwarfs, used as females, and green or brown Mozambique tall palms, used as males. Currently, only green hybrids are used in Zambezia. The number of green hybrids does not exceed 10,000 out of the 120,000 seednuts produced.

Some of the brown-sprouted hybrids are sent to other Mozambican provinces free of Lethal Yellowing Disease. As the disease is active close to the Magromane seed garden, we advise against sending again these seednuts to disease-free zones.

Madal also produces green Mozambique tall seednuts in large quantities. 780,000 seednuts were thus sown by Madal for its own plantations and the GTZ programme, with a view to producing approximately 200,000 seedlings.

The selection method is as follows: fruits are only taken from palms with green fruits. Seedbeds of 100 fruits are sown. That seedbed gives 45 green sprouts on average, 40 of another colour, and 15 fruits which do not germinate. Only the first 25 green seedlings that germinate are kept as seedlings. Then, one or two more seedlings are eliminated (short and thick seedlings, displaying slow development). The fruits not used as seednuts (75 out of 100) are recovered to make copra.

In Quelimane, green-fruited palms are said to be more tolerant of LYD. This is not proven, and it needs to be confirmed by scientific studies. Some observations seem to show that some of the green-fruited hybrids die more slowly from the disease and insect attacks than other hybrids. This may indicate that some of the green-fruited palms (Mozambique tall used as male parents for hybrids) from the Magromane seed garden transmit better tolerance of LYD than brown-fruited palms. This does not indicate that all green-fruited Mozambique tall palms have better tolerance of LYD.

The danger of this method (only taking green-sprouted fruits from green-fruited palms) is to select fruits arising from self-pollination. In the case of the coconut palm, selfing provokes an inbreeding depression that reduces yield by 20 to 30% (for the Tall-type varieties). So, the result of such a selection could be palms producing 20% fewer fruits than the normal Mozambique variety, and not tolerant of the disease.

So it would be extremely useful to evaluate the rate of selfing by molecular biology. Approximately 300 DNA analyses are necessary. A request would be made to choose 10 mother palms and to analyse batches of 30 green-coloured seedlings obtained from each of those palms, seedlings selected or not by Madal. Such an analysis will indicate the selfing rate of the parent palms.

Up to now, no coconut variety has been found that is tolerant of Lethal Yellowing Disease in Mozambique. So, all the material planted in Quelimane, including the Green Mozambique tall, will have a high probability of dying from the disease if it is still active.

2 Proposal of experimental designs for the disease resistance field tests.

2.1 Choice of planting sites for the experiments.

Several sites were visited, close to the village of Marumbune in the district of Nicoalada, and close to the village of Tumbouine in the district of Inhassunge. In those zones, most of the coconut palms have been destroyed by Lethal Yellowing Disease and insect attacks; the stems of the dead coconut palms have not yet been cut down. That option is acceptable because they are zones with strong disease pressure.
The principal problem will be to protect young seedlings from attacks by insects which proliferate in the stems of dead coconut palms. Various techniques will have to be used to ensure that protection: the most efficient is to remove dead stems. Other techniques are the removing of insect larvae from dead stems, protection of the seedling heart by fragments of fishing net, sand poured at the base of leaves (to damage insect joints), manual collecting of insects from the seedlings.
The track leading to Inhassunge is bad and becomes almost impracticable in the rainy season. Setting up an experiment in that zone is not recommended, because systematic monitoring would be impossible.
It is therefore recommended that the two experiments be set up in the district of Nicoalada, conducting those experiments in plots belonging to the Madal company, which will be able to ensure regular follow-up. The two experiments could be carried out one at Estacao Inhangulue 2 (Site 1 in the vicinity of the coconut husk processing unit of the Madal company), the other on the land Madal had me visit in the same district (village of Toumbouine). The two sites are 13.1 km apart.

[2] Site 1: Latitude 17°56'27" S Longitude 36°56'35" E

Figure 1.
Location of existing and future coconut experiments

Caption:

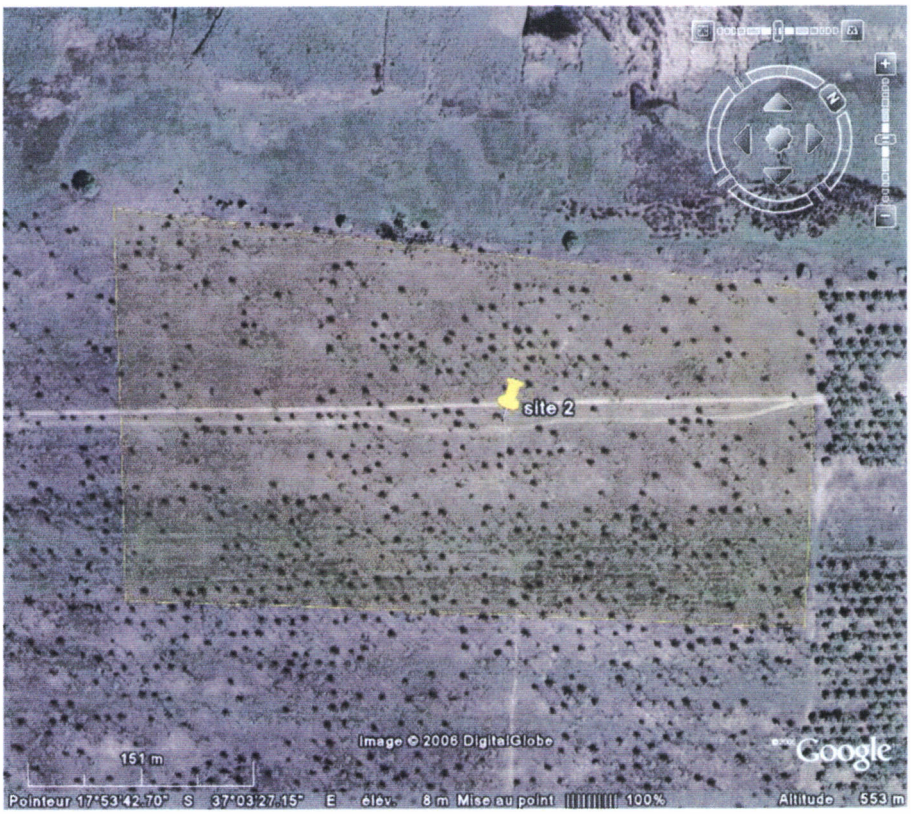
Cogent; site of the genetic experiment planted as part of the Cogent network.
(S 17° 50'26"85 ; E 36°58'05"47).

Site 1: Estacao Inhangulue (near Madal husk processing factory) (S 17°56'32"03; E 36° 56'15"08)
Site 2: Estacao Machimbui (S 17°53'41"89 E 37°03'28"22)

Seed Garden: location of the Magromane coconut seed garden belonging to the Madal Company

Figure 2.
View of site 1

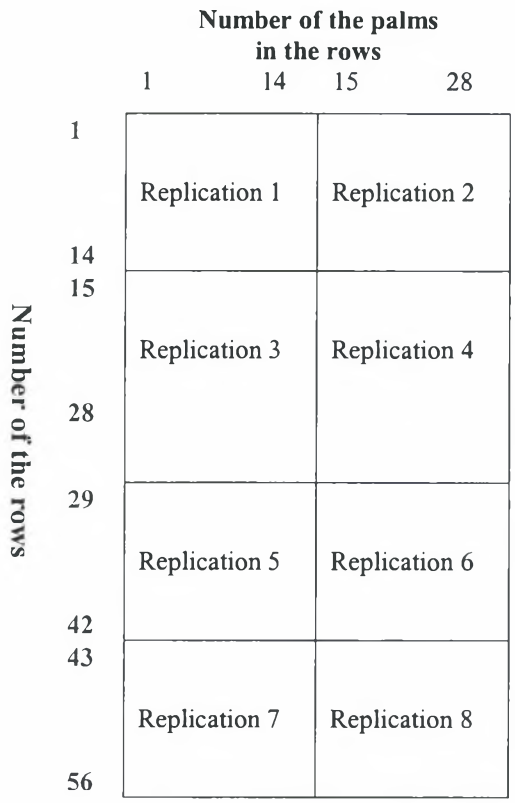
Figure 3.
View of site 2



2.2 Choice of experimental designs for the field experiments

Given the uncertainties about the genetics of the seedlings, very sophisticated experimental designs are not necessary. Molecular analysis of all the seedlings, which would make it possible to find their identity, would be much too expensive.
It is recommended that two separate designs be set up with a planting density of 143 palms per hectare. Each design will consist of 8 randomized replications.
3,775 plants are planned for the planting of these 2 experiments located at 2 geographical sites. The plants belong to 30 different coconut varieties. All the varieties except 3 will be planted in January 2007. The 3 remaining varieties, not yet available, will be planted in January 2008.
Only 3,136 plants will be planted at the beginning in the 2 experiments. The remaining 639 plants will be kept to replace any plants killed by insects in the first year after planting.
1,568 plants will be planted in each experiment with a planting density of 143 palms per hectare. Each experiment will contain 56 rows of 28 palms. Each experiment will contain 8 replications of 196 palms (a square of 14 x 14), as shown in Figure 4.
In each replication, each of the 30 varieties will be represented by 2 to 10 palms, according to the availability of varieties, as shown in table 1.

Figure 4. General map of an experiment



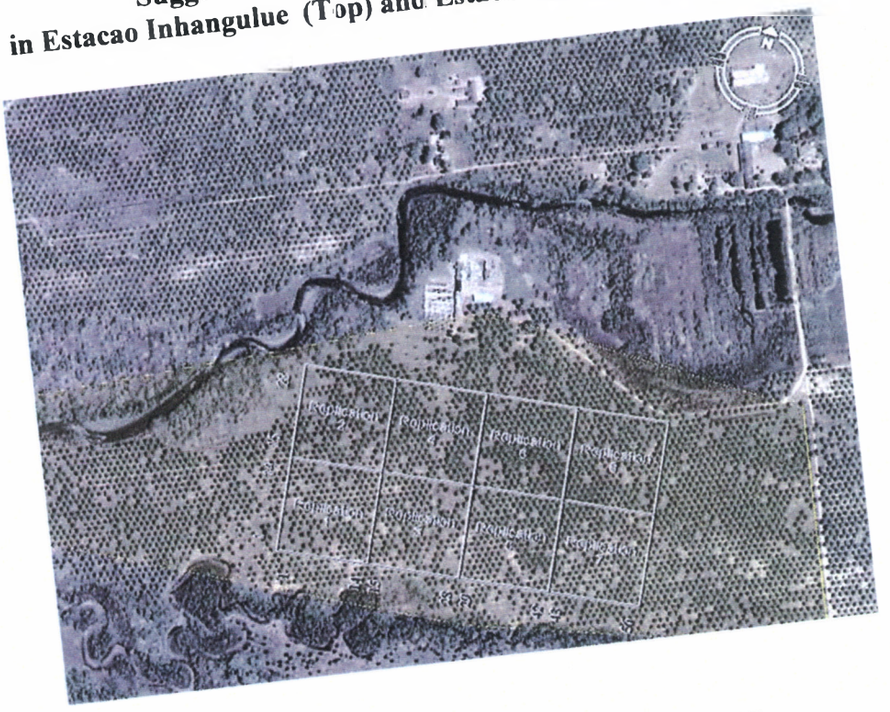
In each replication, the 30 varieties will be randomized. Figure 5 gives an example of a possible layout of the experiments at the chosen sites.

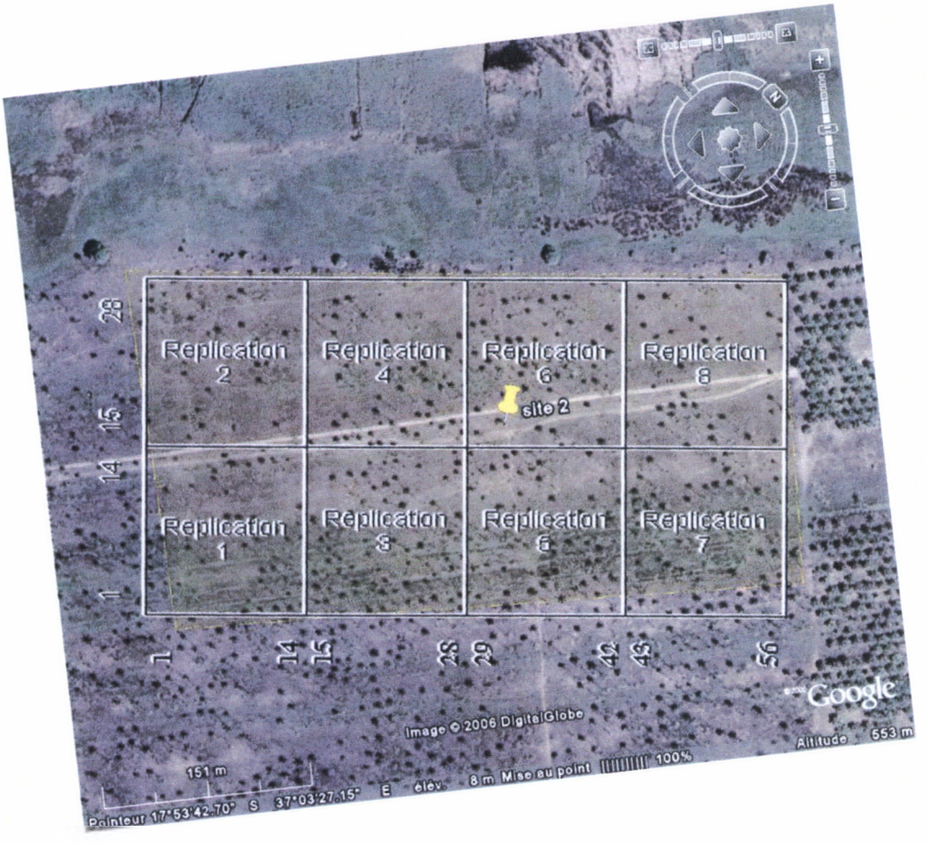
Table 1. List and code of varieties to be planted in 2007 and 2008 in 2 experiments, each containing 8 replications.

Code	French codes	International codes	International names	Number of seedlings available	Number of seedlings in each replication
01	GRLxGRL	RIT	Rennell Island Tall	257	10
02	NRCxNRC	CRD	Cameroon Red Dwarf	108	6
03	GPA2xGPA2	PNT02	Panama Tall Monagre	190	10
04	NRCxGRL	CRDxRIT	Hybrid PB113 (Cameroon Red Dwarf x Rennell Island Tall)	169	9
05	NNLxNNL	NLAD	Niu Leka Dwarf	162	9
06	NVP2xNVP2	CATD	Catigan Green Dwarf	75	4
07	NJMxGOA6	MYDx WAT06	Hybrid PB121 Benin (Malayan Yellow Dwarf x West African Tall Benin)	119	6
08 (1)	NJM	MYD	Malayan Yellow Dwarf	226	10
09	GTNxGTN	TAGT	Tagnanan Tall	154	9
10	GVTxGVT	VTT	Vanuatu Tall	40	2
11	NVSxNVS	PGD or SLGD	Sri Lanka Green Dwarf	58	3
12	NVSxGVT	PGDxVTT	Sri Lanka Green Dwarf x Vanuatu Tall	126	7
13	NBNxNBN	MBD	Madang Brown Dwarf	113	7
14	GTH4xGTH4	THT04	Thailand Tall Ko Samui	123	7
15	GNG4xGNG4	GPT	Gazelle Peninsula tall	144	7
16	GOA6xGOA6	WAT06	West African Tall Benin	109	6
17	NVSxGMZ	PGDxMZT	Sri Lanka Green Dwarf x Mozambique Tall	59	3
18	NVP5xNVP5	PILD	Pilipog Green Dwarf	78	4
19	NRYxNRY	TRD	Tahiti Red Dwarf	68	4
20	NVSxGPA2	PGDxPNT02	Sri Lanka Green Dwarf x Panama Tall monagre	34	2
21		MZTa	Mozambique tall Inhassunge	137	7
22		MZTb	Mozambique tall Micaune	130	7
23		MZTm	Mozambique tall Mocimboa da Praia	59	3
24		MZTu	Mozambique tall Ulo	63	3
25		MZTd	Mozambique tall Ilha de Mocambique	160	9
26		MZTi	Mozambique tall Morrumbene	214	10
27 (2)		MYD x MZT brown	Hybrid Malayan Yellow Dwarf x Mozambique tall (brown colour)	150	8
28 (3)		MYD x MZT Green	Hybrid Malayan Yellow Dwarf x Mozambique tall (green colour)	150	8
29 (4)		MZT Green Magromane	Mozambique tall Magromane (green colour)	150	8
30 (5)		MZT Green Madal	Mozambique tall Madal Green	150	8
Total				3775	196

Notes:
(1) the material referred to as "Malayan Yellow Dwarf", although of uncertain origin, will be planted in the 2 experiments
(2), (3) and (4): these varieties will be prepared by the Madal company but they will be planted in 2008 only.
(5) This variety is presently produced on a large scale by the Madal company. It will be planted in 2007.

Figure 5.
Suggested disposition of the 2 experiments in Estacao Inhangulue (Top) and Estacao Machimbui (Bottom)





The proposed planting method is as follows: in the nursery, each plant will receive a number, firmly attached to the stem or at the base of a leaf: that number will indicate the number of the variety (From 01 to 30) together with the number of the parent palm (for varieties collected locally).
Plants will be distributed according to the randomized maps shown in Annex 2 and then planted. After planting, two technicians or researchers will draw up separately a map of the experiments, recording on a drawn map all the numbers attached to the plants; the maps obtained by the two men will be checked, first one against the other, and then with the randomized maps in Annex 2. If necessary, recording and planting mistakes will be corrected.
One year after the experiments have been planted, it is recommended that leaf samples be collected from each of the coconut palms in the field. Those samples will be dried and kept in a dry room for future use. When most of the seedlings have been destroyed by Lethal Yellowing, it will be possible to carry out a molecular analysis on some of the samples to determine with certainty their variety of origin. Each dry leaf sample weighs approximately 10 grams.
As soon as the new introduced varieties start bearing, they should be reproduced and planted again in the same zone in plots not yeat attacked by LYD. Indeed, those varieties constitute an invaluable capital, even if some are susceptible to Lethal Yellowing. It is difficult and expensive to introduce new varieties into a country; it would be a pity for them to be completely destroyed by Lethal Yellowing.

As the question of transmission of LYD by seednut and embryos is not solved, it must be avoided to send seednuts from regrions with LYD to regions free of LYD, When this problem will be solved, by using quarantine precautions, a duplicate safeguard collection could be planted on the island of Inhaca in the bay of Maputo, in connection with the hotel existing on the island and with ecotourism activities. This island is free of LYD.

3 Proposals for new research projects

3.1 Observations on the impact of the disease and the tolerance of local material

In 2004 and 2006, a large number of plots attacked by the disease were visited: those plots where planted with the "Mozambique Tall" variety, aged from 70 to 100 years in estate plantations, and with the Mozambique Tall of all ages in mixtures on smallholdings, or with hybrids between Malayan Dwarf and Mozambique Tall palms aged from 10 to 30 years.
The senile plantation of the company in Zambezi (Zalala) looks like hundreds of "telegraph poles", dead coconut palms of which only the stems remain. However, according to L. Tomo and E. Lourenço, no case of Lethal Yellowing Disease was recorded there; all the palms died from senility or following attacks by insects proliferating in dead stems.
Throughout the attacked zones, it is not easy to see whether there are any disease-resistant palms because, when Lethal Yellowing occurs, it induces an outbreak of insects (*Oryctes*) in the stems of the dead palms. Those insects attack the surviving palms and all the population is then unrelentingly and rapidly destroyed.
In the Madal Company estates, all the palms under twenty-five years old are hybrids, which amounts to considerable areas. The hybrids using Malayan red and yellow dwarfs are generally planted in a mixture, and give populations with varying fruit colour, but where the brown colour dominates over the green colour. In some rare cases, there are plots planted with green hybrids only, or planted with brown hybrids only, probably resulting from a selection at seedbed level based on sprout colour.
In at least three geographically distinct locations, it was found that some hybrids with green fruits seemed to die later than hybrids with brown fruits; and some hybrids with green fruits seemed to die more from insect attacks than from Lethal Yellowing. This was observed following a comment by Mr. Lourenço from the Madal Company, on the road to Inhassunge, in the Micaune plantation, close to the Magromane seed garden.

Several assumptions might explain that observation. All the hybrids came from the Magromane seed garden. Crossing a Malayan Yellow dwarf with a green Mozambique tall always gives a green hybrid.
But the green hybrids may come from crossing a Malayan Yellow dwarf, as the female parent, and a Mozambique tall (MZT), green or brown in colour, as the male parent. The hybrids with brown fruits come from crossing a Malayan Red dwarf as the female parent and a green or brown MZT as the male parent, or from crossing a Malayan Yellow dwarf with a brown MZT.

The hypotheses are the following:

1) The Malayan Yellow Dwarf transmits better tolerance of the disease than the Malayan Red Dwarf.
2) It is not a phenomenon of tolerance but rather phenomenons of varietal preference. One can imagine that the insect vector of LYD (not identified yet) prefers the brown hybrids to the green hybrids; or that the insects killing directly the palms (Oryctes and Rynchophorus) prefers the brown hybrids to the green hybrids.

3) In the Magromane seed garden, some MZT palms, probably green in colour, transmit tolerance of the disease. This last assumption is worth checking by molecular biology techniques (see chapter 3.3.).

3.2 About coconut seeds currently produced in Mozambique

In 2006, the Madal company is producing a large quantity of green "Mozambique Tall" palms. The special selection which is carried out risks increasing the selfing of parent palms. Self-pollination has a strong depressive effect (20 to 30% loss) on the yield of the progeny. It is thus necessary to check the percentage of seeds resulting from selfing in the batches sold by the Madal company.
The fact that some green hybrids seem to resist the disease better does not mean that all green Mozambique tall palms are tolerant of the disease. On the other hand, this probably indicates that, among the green Mozambique tall palms planted in the Magromane seed garden, some of the palms have better disease tolerance.
It would be extremely useful to evaluate the rate of selfing by molecular biology. Approximately 300 DNA analyses are necessary. A request would be made for 10 mother palms to be chosen, analysing batches of 30 green coloured seedlings obtained from each of those palms, seedlings selected or not by Madal. That analysis will indicate the selfing rate of the parent palms.

3.3 A possible way of identifying local material tolerant of the disease

From an experimental point of view, the situation is very interesting in Mozambique because all the hybrids planted come from the same small seed garden

These two possible studies would be based on epidemiological approaches and genealogy using molecular biology analyses.

First study: genealogical approach. Search for the male parents of apparently tolerant hybrids.

This study will consist in:
- harvesting leaf samples for DNA analysis on 300 hybrids seeming to resist the disease in severely attacked zones.
- harvesting leaf samples for DNA analysis on all the 241 Mozambique Tall parents located in the Magromane seed garden.
- establishing the genealogical correspondences between putative tolerant hybrids and their male parents (All parent palms of the hybrids are located in the Magromane seed garden.).

<u>If this analysis shows that most of the putative tolerant hybrids come from the same male parental material, some tolerance of the disease would have been successfully identified.</u> This constitutes one of the only approaches making it possible to detect tolerance of the disease in a 2 to 4 year period.

Second study: epidemiological and genealogical approach.

This study will consist in:

- Harvesting leaf samples in 5 plots each containing 1,000 hybrids, and located in zones not yet devastated by the disease but located closed to attacked zones; or located in zones where 1 or 2 % of the palms have already died from LYD. Those leaf samples will be dried without carrying out a DNA analysis and kept for future use.
- Then carrying out an epidemiological survey. Wait for the disease to intensify in some of the sampled plots. The first 2 plots most attacked by the disease will be chosen as locations for the study. The remaining 3 plots will be discarded and the corresponding leaf samples eliminated.
- Harvesting leaf samples for DNA analysis on all the 241 Mozambique Tall parents located in the Magromane seed garden.
- Carrying out a DNA analysis on all the 2,000 hybrids located in the two zones selected for the study.
- Establishing the genealogical correspondences between all the 2,000 hybrids and their male parents using DNA analysis.

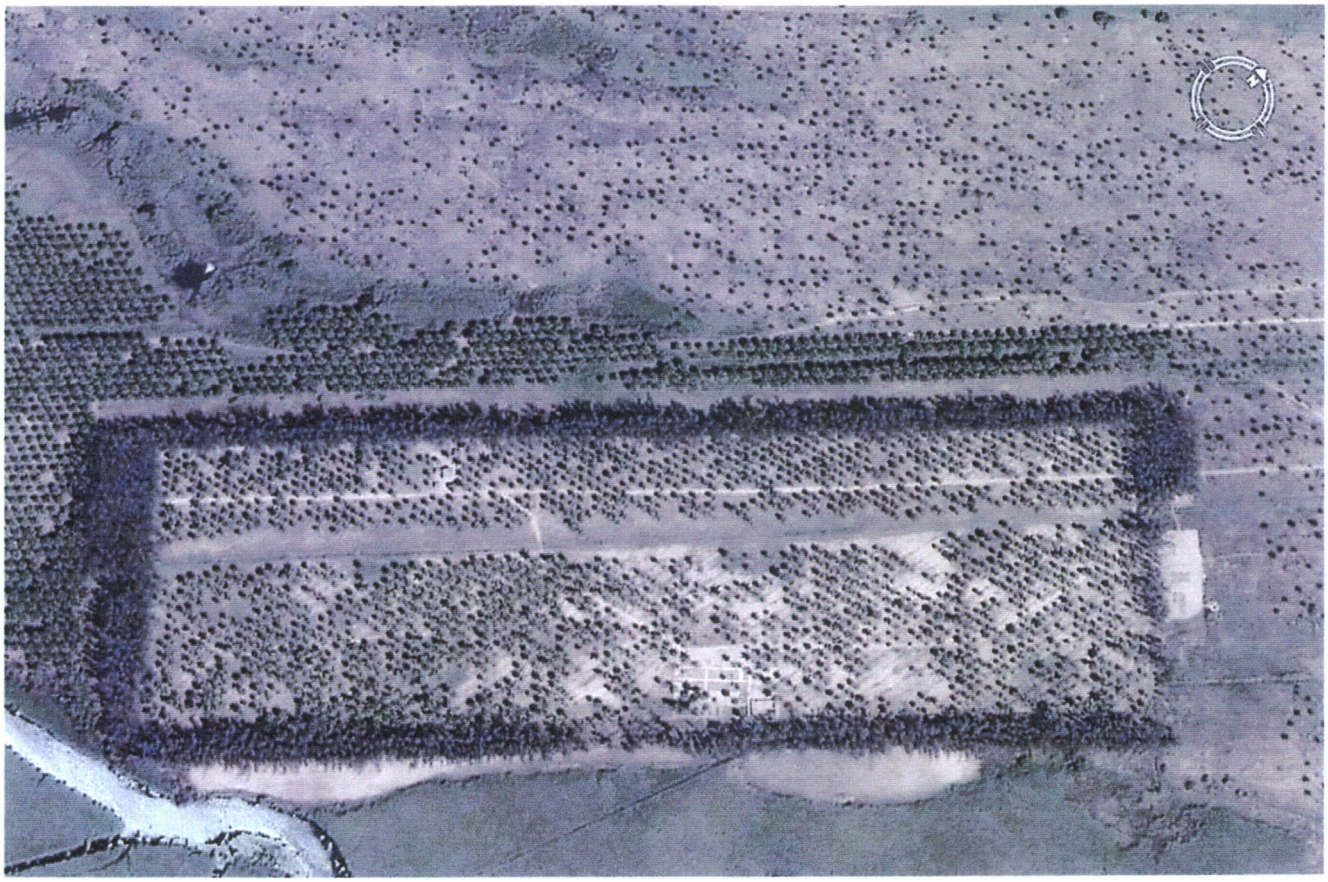
Carrying out an epidemiological study of the 2 selected plots. If the putative tolerant hybrids all come from the same male parental material, disease-tolerance sources would have been identified.

4 Annex 1. Map of the Magromane seed garden

Table 2. Inventory of the palms in Magromane seed garden as of 2005

Variety Name	Colours	Colour range	Number of living palms	Code
Mozambique Tall	Green		112	●
Mozambique Tall	Light brown, green brown		84	●
Mozambique Tall	Strong grown, red brown		45	●
Malayan Yellow Dwarf	Yellow-green		924	●
Malayan Red Dwarf	Orange		559	x
Brazil Green dwarf	Green		45	v
Total			1769	

Figure 6.
Picture of Magromane Seed Garden



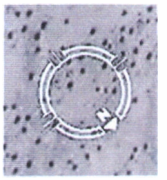


Figure 7. Map of the Magromane Seed garden
First part. (for caption see table 2 on the previous page)

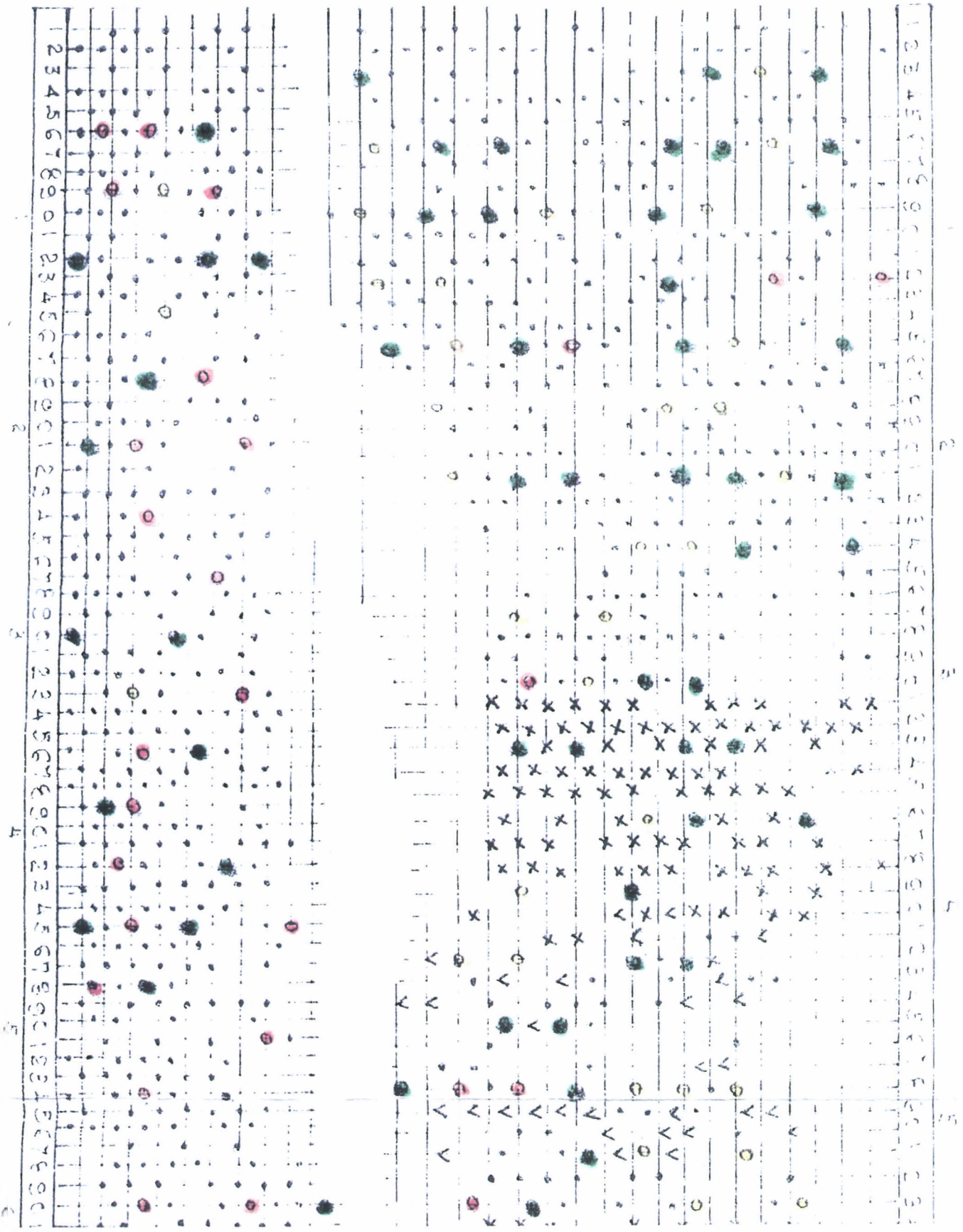
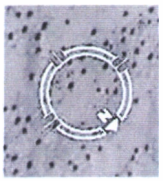
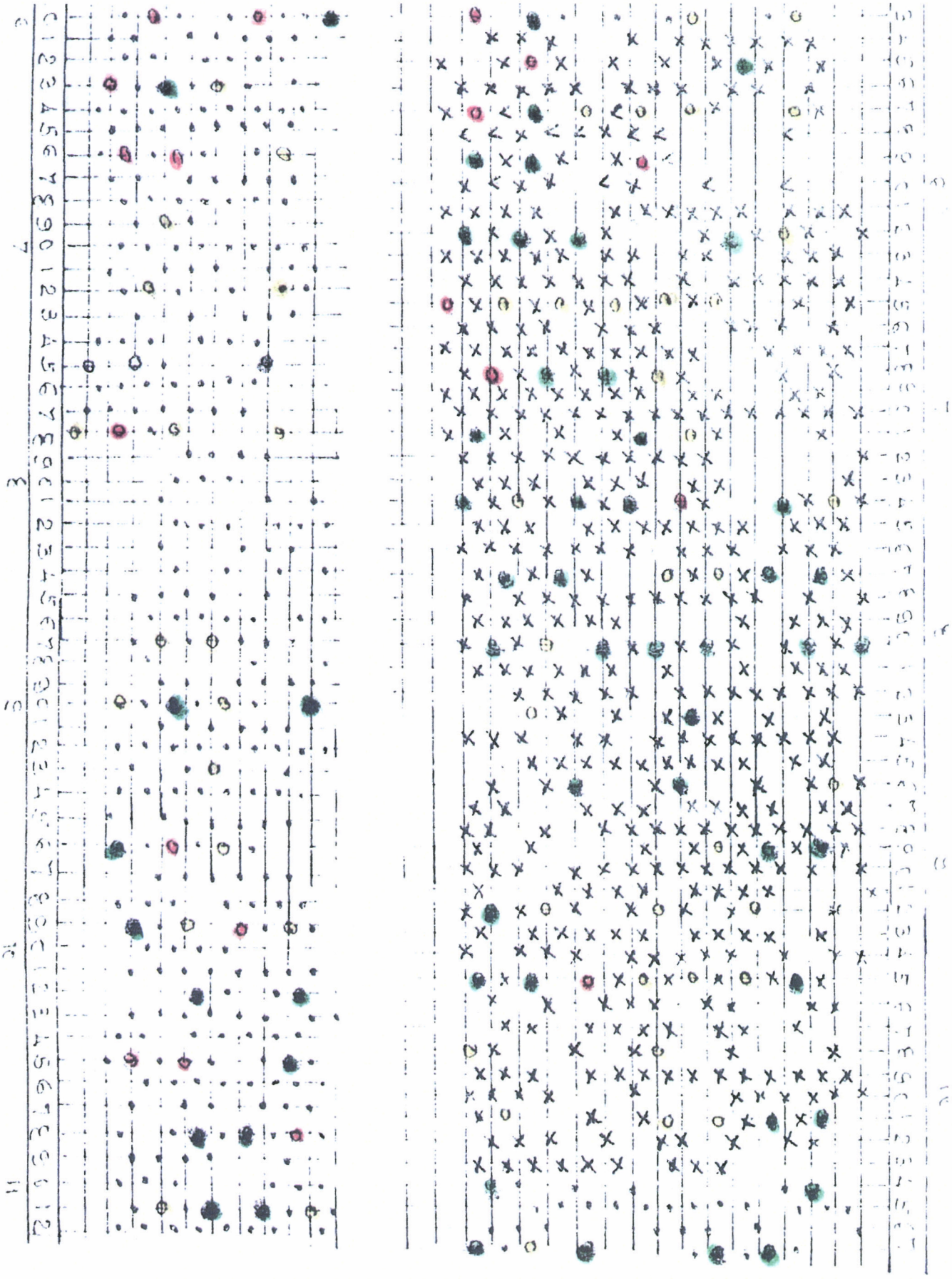


Figure 7. Map of the Magromane Seed garden
First part. (for caption see table 2 on the previous page)





5 Annex 2. Detailed maps of randomized replications for experiments 1 and 2

The numbers in the tables are coconut variety numbers as given in table 1.

Experiment 1 Replication 1

Number of the rows	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	13	01	04	21	15	09	06	30	16	22	08	19	12	14
2	15	08	03	23	02	17	25	26	04	22	01	13	25	18
3	12	06	11	27	18	22	03	10	01	15	26	28	27	21
4	21	09	16	01	20	12	28	13	14	08	02	30	29	11
5	19	25	14	17	21	26	04	26	05	09	16	23	03	25
6	24	29	03	26	08	29	21	05	01	05	17	27	22	09
7	25	16	26	04	27	12	07	08	06	03	14	05	28	13
8	26	01	27	24	02	19	15	30	07	13	08	03	29	15
9	22	09	25	28	08	07	27	02	21	30	05	29	04	28
10	14	29	04	22	18	30	07	01	05	09	05	25	30	26
11	25	12	03	29	30	28	16	20	05	29	28	02	27	12
12	13	01	26	28	09	07	04	25	27	03	26	24	08	18
13	09	03	10	02	23	08	15	01	22	09	07	06	04	14
14	08	30	04	11	14	05	13	21	03	19	01	16	12	15

Experiment 1 Replication 2

Number of the rows	15	16	17	19	19	20	21	22	23	24	25	26	27	28
1	04	28	24	29	03	26	08	29	13	01	26	28	09	07
2	19	25	14	17	21	26	04	26	05	09	16	23	03	25
3	13	01	04	21	15	09	06	30	16	22	08	19	12	14
4	14	29	04	22	18	30	07	01	05	09	05	25	30	26
5	04	14	22	09	25	28	08	21	09	16	01	20	12	28
6	13	14	08	02	30	29	11	25	12	03	29	30	28	16
7	20	05	29	28	02	27	12	08	30	04	11	14	05	13
8	26	01	27	24	02	19	15	30	07	13	08	03	29	15
9	07	27	02	26	28	27	21	21	03	19	01	16	12	15
10	21	05	01	05	17	27	22	09	25	16	26	04	27	12
11	03	10	01	15	10	02	23	08	15	01	22	09	07	06
12	15	08	03	23	02	17	25	26	04	22	01	13	25	12
13	06	11	27	18	22	18	07	08	06	03	14	05	28	13
14	04	09	03	21	30	05	29	25	27	03	26	24	08	18

Experiment 1 Replication 3

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

Experiment 1 Replication 4

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

Experiment 1 Replication 5

Number of the rows	1	2	3	4	5	6	7	8	9	10	11	12	13	14
29	16	30	8	4	24	28	8	3	29	3	9	26	25	21
30	15	4	7	3	21	13	6	10	29	21	6	16	4	23
31	8	29	13	21	5	25	18	13	8	15	6	28	12	27
32	9	27	2	12	25	5	30	29	4	3	26	28	11	4
33	7	10	17	25	9	27	1	22	22	22	28	1	27	29
34	3	26	19	22	26	26	16	2	5	9	25	29	4	29
35	26	30	2	12	16	7	26	20	14	21	22	30	4	9
36	27	25	8	14	1	9	8	29	16	28	1	4	5	14
37	15	26	2	27	9	21	17	13	24	7	18	19	1	30
38	28	12	9	5	9	30	23	1	8	11	12	15	13	14
39	26	12	2	22	27	5	8	20	28	7	30	15	12	14
40	11	14	23	25	26	8	5	17	18	25	3	3	30	25
41	1	1	15	19	3	13	2	16	1	15	4	22	27	6
42	7	14	13	19	21	3	5	5	18	1	24	3	28	8

Experiment 1 Replication 6

Number of the rows	15	16	17	19	19	20	21	22	23	24	25	26	27	28
29	27	30	5	10	14	8	8	8	26	8	8	12	6	4
30	2	5	3	11	9	25	15	13	28	18	4	22	14	16
31	5	8	25	12	11	1	18	28	13	21	27	15	4	1
32	7	23	3	19	27	21	15	7	25	29	9	27	7	20
33	3	25	14	4	9	1	25	1	5	24	19	16	4	2
34	2	14	6	19	28	23	13	30	26	28	25	18	3	25
35	5	26	15	4	22	3	25	21	13	27	2	23	18	5
36	21	2	7	16	14	10	14	27	6	20	3	16	8	16
37	9	21	22	26	2	28	15	29	7	7	15	30	17	26
38	8	17	9	5	26	12	29	30	26	17	4	3	25	28
39	27	29	1	29	12	30	16	8	29	8	22	1	22	12
40	29	9	5	3	30	29	4	27	3	1	5	21	30	14
41	9	12	26	1	26	28	13	4	13	6	30	21	1	11
42	24	22	15	28	3	13	26	1	9	22	12	24	9	19

Experiment 1 Replication 7

Number of the rows	1	2	3	4	5	6	7	8	9	10	11	12	13	14
43	28	19	14	4	5	29	12	6	26	30	30	27	23	12
44	30	7	13	6	22	9	27	23	3	2	30	26	14	21
45	16	1	27	7	27	29	25	8	22	2	3	25	8	1
46	14	16	4	25	30	29	11	29	5	1	9	25	13	3
47	5	3	9	14	29	15	8	15	2	21	3	3	13	26
48	2	7	10	21	27	20	15	4	11	5	18	10	27	19
49	28	18	14	29	26	22	12	12	23	16	12	9	5	15
50	21	9	13	1	30	28	19	4	17	30	25	14	17	14
51	7	8	25	13	4	5	28	26	28	25	8	18	3	3
52	1	8	5	6	1	2	21	22	29	15	17	16	22	3
53	1	27	26	26	13	26	13	4	30	5	12	24	8	7
54	25	20	9	8	3	1	4	29	9	24	4	11	18	1
55	21	8	1	8	16	2	9	26	4	16	7	25	28	24
56	26	28	9	5	28	22	22	19	6	27	21	15	15	12

Experiment 1 Replication 8

Number of the rows	15	16	17	19	19	20	21	22	23	24	25	26	27	28
43	12	9	2	19	29	28	2	6	16	30	15	8	26	9
44	27	1	11	14	4	4	15	29	30	27	20	22	14	24
45	23	5	4	21	22	4	29	8	18	14	26	9	8	25
46	7	11	15	17	7	1	1	22	19	27	1	13	9	16
47	25	16	22	5	30	4	2	3	6	5	21	29	5	4
48	26	19	25	28	28	5	30	14	27	10	27	26	28	21
49	6	3	28	1	1	2	13	22	1	7	3	9	25	13
50	21	9	23	15	30	8	30	26	27	12	22	21	1	16
51	13	25	4	12	12	3	27	26	14	24	26	8	2	3
52	4	28	12	7	7	3	5	28	3	29	4	9	5	11
53	25	3	16	13	18	13	28	8	13	21	29	8	3	27
54	19	12	26	8	26	18	8	17	5	2	29	15	25	10
55	17	14	15	1	25	21	23	24	5	26	7	16	30	15
56	9	1	30	12	3	18	29	14	22	8	6	20	9	25

Experiment 2 Replication 1

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

Experiment 2 Replication 2

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

Experiment 2 Replication 3

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

Experiment 2 Replication 4

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

Experiment 2 Replication 5

Number of the rows	1	2	3	4	5	6	7	8	9	10	11	12	13	14
29	27	25	8	14	1	9	8	29	16	28	1	4	5	14
30	11	14	23	25	26	8	5	17	18	25	3	3	30	25
31	7	10	17	25	9	27	1	22	22	22	28	1	27	29
32	15	4	7	3	21	13	6	10	29	21	6	16	4	23
33	26	30	2	12	16	7	26	20	14	21	22	30	4	9
34	8	29	13	21	5	25	18	13	8	15	6	28	12	27
35	1	1	15	19	3	13	2	16	1	15	4	22	27	6
36	28	12	9	5	9	30	23	1	8	11	12	15	13	14
37	3	26	19	22	26	26	16	2	5	9	25	29	4	29
38	15	26	2	27	9	21	17	13	24	7	18	19	1	30
39	26	12	2	22	27	5	8	20	28	7	30	15	12	14
40	16	30	8	4	24	28	8	3	29	3	9	26	25	21
41	9	27	2	12	25	5	30	29	4	3	26	28	11	4
42	7	14	13	19	21	3	5	5	18	1	24	3	28	8

Experiment 2 Replication 6

Number of the rows	15	16	17	19	19	20	21	22	23	24	25	26	27	28
29	7	8	25	13	4	5	28	26	28	25	8	18	3	3
30	28	18	14	29	26	22	12	12	23	16	12	9	5	15
31	16	1	27	7	27	29	25	8	22	2	3	25	8	1
32	1	27	26	26	13	26	13	4	30	5	12	24	8	7
33	1	8	5	6	1	2	21	22	29	15	17	16	22	3
34	28	19	14	4	5	29	12	6	26	30	30	27	23	12
35	14	16	4	25	30	29	11	29	5	1	9	25	13	3
36	5	3	9	14	29	15	8	15	2	21	3	3	13	26
37	30	7	13	6	22	9	27	23	3	2	30	26	14	21
38	26	28	9	5	28	22	22	19	6	27	21	15	15	12
39	21	9	13	1	30	28	19	4	17	30	25	14	17	14
40	25	20	9	8	3	1	4	29	9	24	4	11	18	1
41	2	7	10	21	27	20	15	4	11	5	18	10	27	19
42	21	8	1	8	16	2	9	26	4	16	7	25	28	24

Experiment 2 Replication 7

Number of the rows	1	2	3	4	5	6	7	8	9	10	11	12	13	14
43	26	19	25	28	28	5	30	14	27	10	27	26	28	21
44	23	5	4	21	22	4	29	8	18	14	26	9	8	25
45	12	9	2	19	29	28	2	6	16	30	15	8	26	9
46	7	11	15	17	7	1	1	22	19	27	1	13	9	16
47	21	9	23	15	30	8	30	26	27	12	22	21	1	16
48	27	1	11	14	4	4	15	29	30	27	20	22	14	24
49	6	3	28	1	1	2	13	22	1	7	3	9	25	13
50	25	3	16	13	18	13	28	8	13	21	29	8	3	27
51	13	25	4	12	12	3	27	26	14	24	26	8	2	3
52	9	1	30	12	3	18	29	14	22	8	6	20	9	25
53	19	12	26	8	26	18	8	17	5	2	29	15	25	10
54	4	28	12	7	7	3	5	28	3	29	4	9	5	11
55	25	16	22	5	30	4	2	3	6	5	21	29	5	4
56	17	14	15	1	25	21	23	24	5	26	7	16	30	15

Experiment 2 Replication 8

Number of the rows	15	16	17	19	19	20	21	22	23	24	25	26	27	28
43	22	13	4	19	21	3	29	9	2	26	19	17	3	2
44	28	26	25	7	1	14	5	16	28	25	4	16	22	9
45	3	29	12	26	9	21	8	28	13	12	10	8	10	9
46	7	5	12	11	25	8	19	3	3	27	29	13	11	27
47	5	9	18	2	4	15	4	9	22	27	8	30	27	23
48	15	1	22	26	30	23	12	8	5	8	22	13	18	3
49	21	1	3	20	8	27	12	25	12	21	7	5	21	1
50	20	26	16	8	8	29	26	8	26	16	5	4	13	14
51	29	27	28	7	30	28	4	21	30	11	28	14	7	25
52	5	14	15	24	28	29	25	6	22	25	1	17	30	30
53	19	6	4	9	14	15	7	24	21	29	3	4	2	9
54	25	14	14	1	16	26	15	17	30	12	16	9	29	6
55	27	1	18	15	3	15	23	2	4	1	5	1	5	27
56	25	30	3	26	13	6	18	26	22	2	24	1	13	28

6 Annexe 3. Presentation made at the 2004 Cogent meeting.

CIRAD

Compte rendu de mission

Collecting local Coconut varieties in Mozambique in order to screen them for Lethal Yellowing Disease Tolerance

R. Bourdeix, M. Racaud, L. Tomo, A. Uaciquete and F. Rossi

October 2004

A component of the Pascom Project (AFD funding)

1) To collect 9 Coconut populations in 4 provinces: Zambezia (3 pop.), Nampula, Cabo Delgado and Ihambane

2) To analyse morphometrics and molecular data from these 9 populations

3) To plant the 4 more interesting populations in two disease resistance trials (at Chinde and Gonhane, Zambezia)

Material and methods

- 50 to 70 palms harvested from each population in order to get 350 seednuts each.

- 30 palms observed per population for morphometrics data and sampled for DNA analysis (palms producing 5 seednuts or more).

- Number of parent palm was written on seednuts in order to keep information on female genealogies.

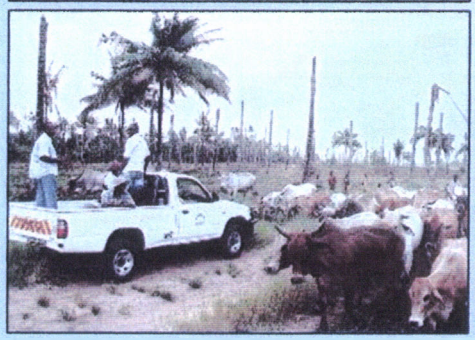
- DNA analysis will be conducted both on parent palms and progenies planted in the LYD Trials (reproductibility and estimation of selfing rate).

CIRAD

LYD Early Symptoms

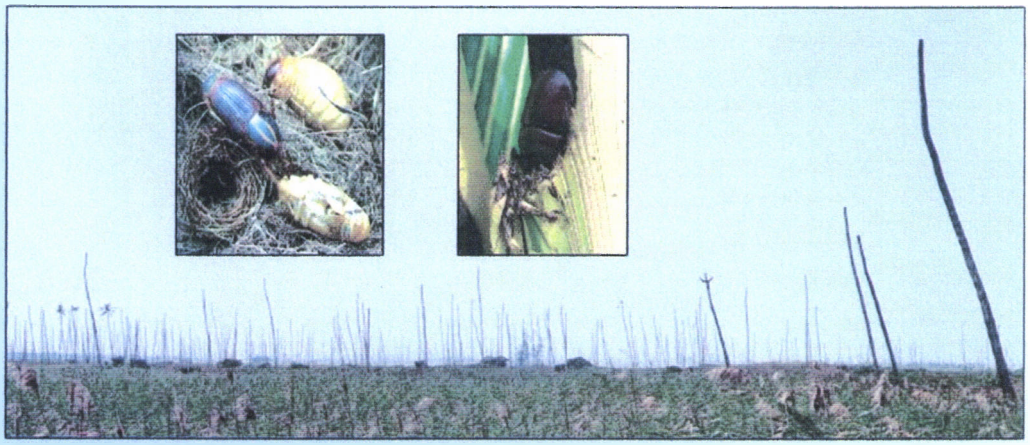
CIRAD

LYD Symptoms



CIRAD

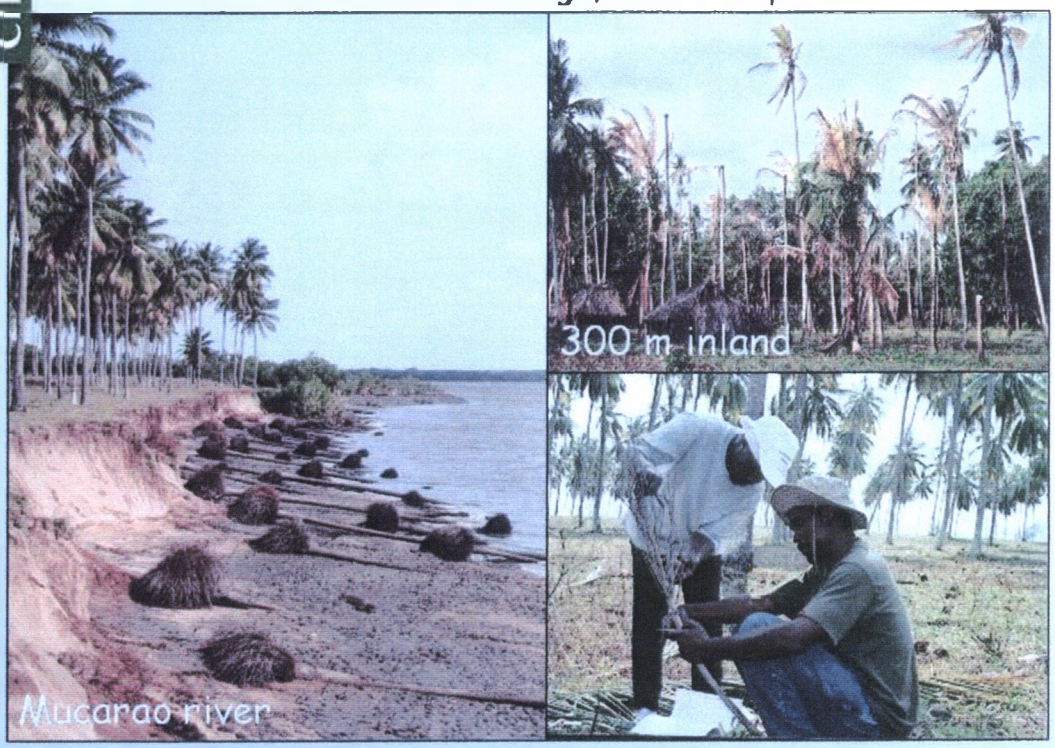
Not what it seems to be...



Old plantation from Companhia do Zambezia, Zalala, 2004

CIRAD

Collection site 1 : Inhassunge, Zambezia province



CIRAD

Collection site 2 : Micaune, Zambezia province

CIRAD

Collection site 3 : Mocimboa da praia, Cabo DelGado province

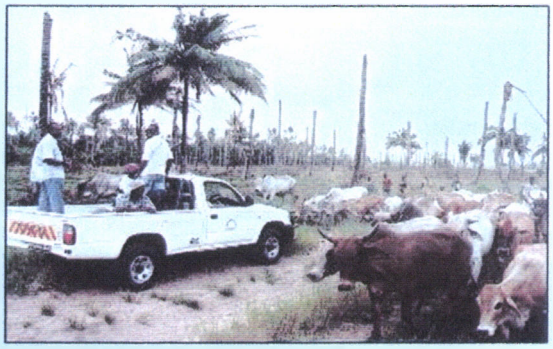
CIRAD

Collection site 4 : ilha de Moçambique, Nampula province

CIRAD

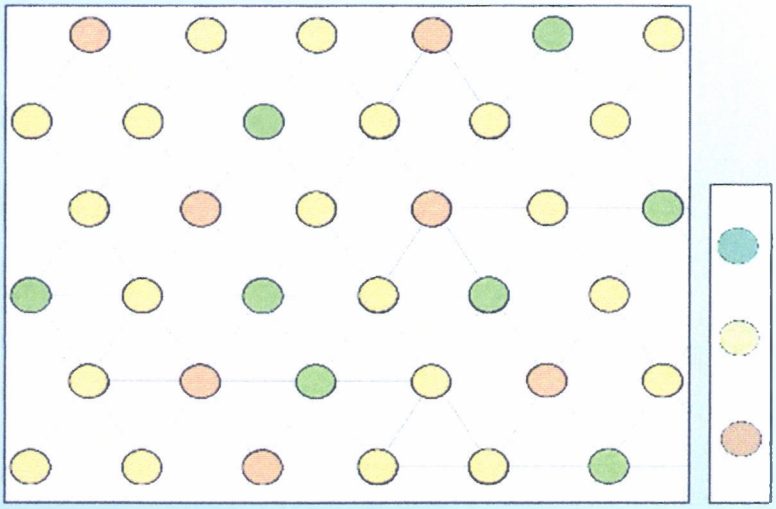
Partipatory approach

- Urgency feeling: need for immediate replanting
- No time to wait for a 15 years studies
- Not all the palms dye from LYD
- Few varieties identified by farmers (to be confirmed)
- In hybrids plots, palms bearing green fruits dye slower



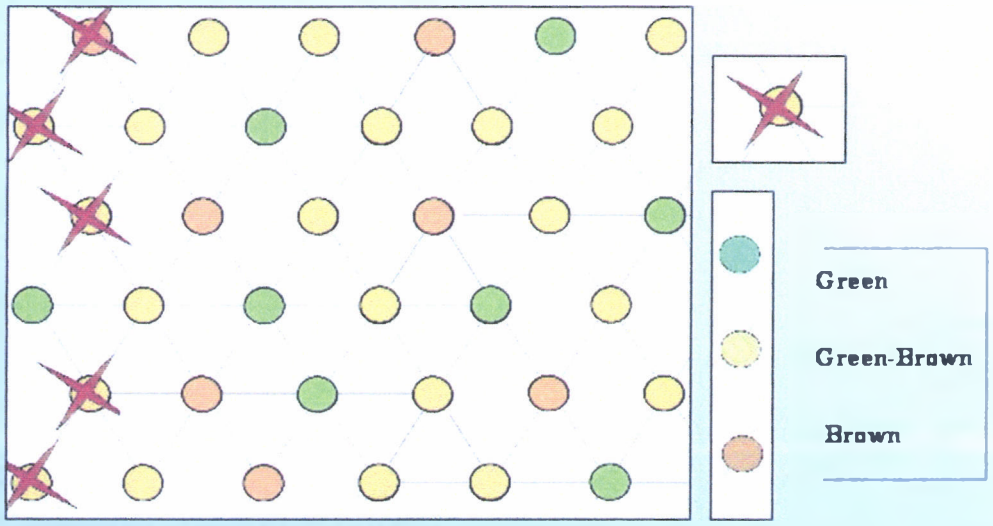
CIRAD

Map of a plot of healthy hybrids Malayan Dwarfs (Yellow and Red) crossed by Mozambique Tall



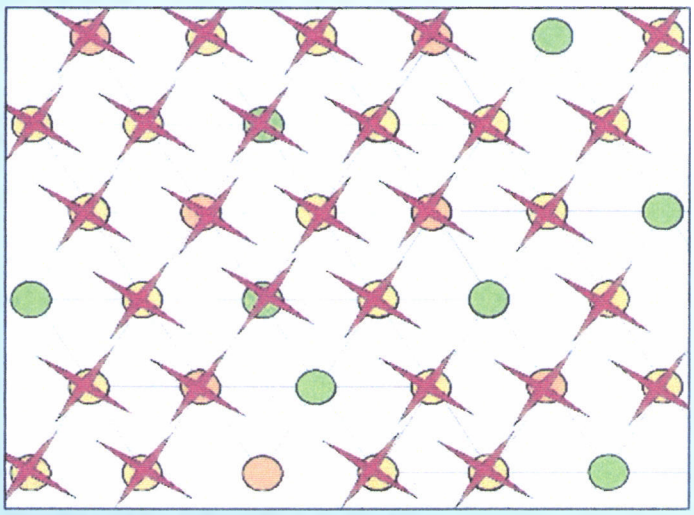
CIRAD

Beginning of LYD attack in a plot of hybrids
Malayan Dwarfs (Yellow and Red)
crossed by Mozambique Tall



CIRAD

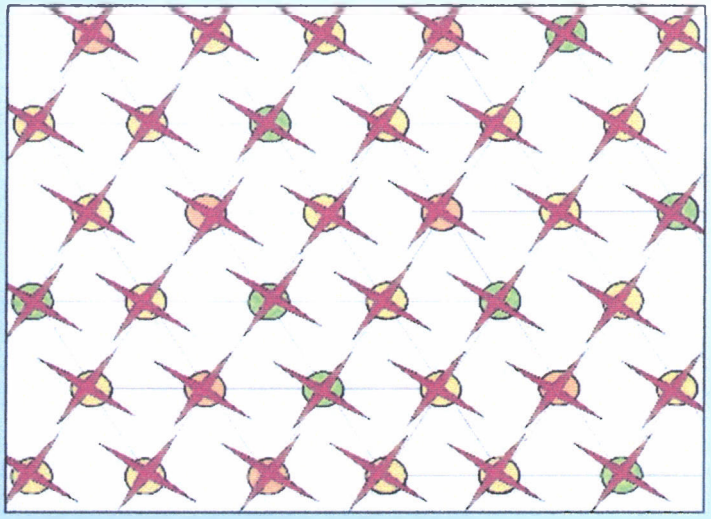
Green hybrids seems to dye slower
during LYD and Oryctes attacks



?

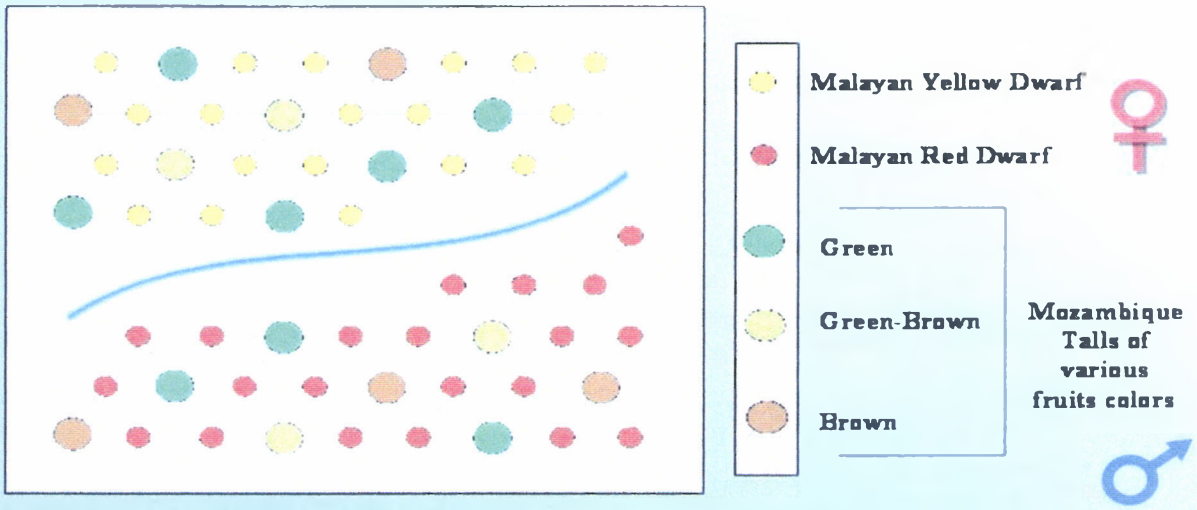
CIRAD

All hybrids finally die,
but not necessarly from LYD



CIRAD

Map of the Magromane coconut seed garden,
Quelimane, Mozambique



CIRAD

Fruits colours of coconut hybrids
and its parents from the Magromane
coconut seed garden, Mozambique

Hybrids colours	♀ Female parent	♂ Male parent MZT
	Malayan Yellow Dwarf	Green, Green-Brown or Brown
	Malayan Red Dwarf or Malayan Yellow Dwarf	Green, Green-Brown or Brown
	Malayan Red Dwarf or Malayan Yellow Dwarf	Green, Green-Brown or Brown

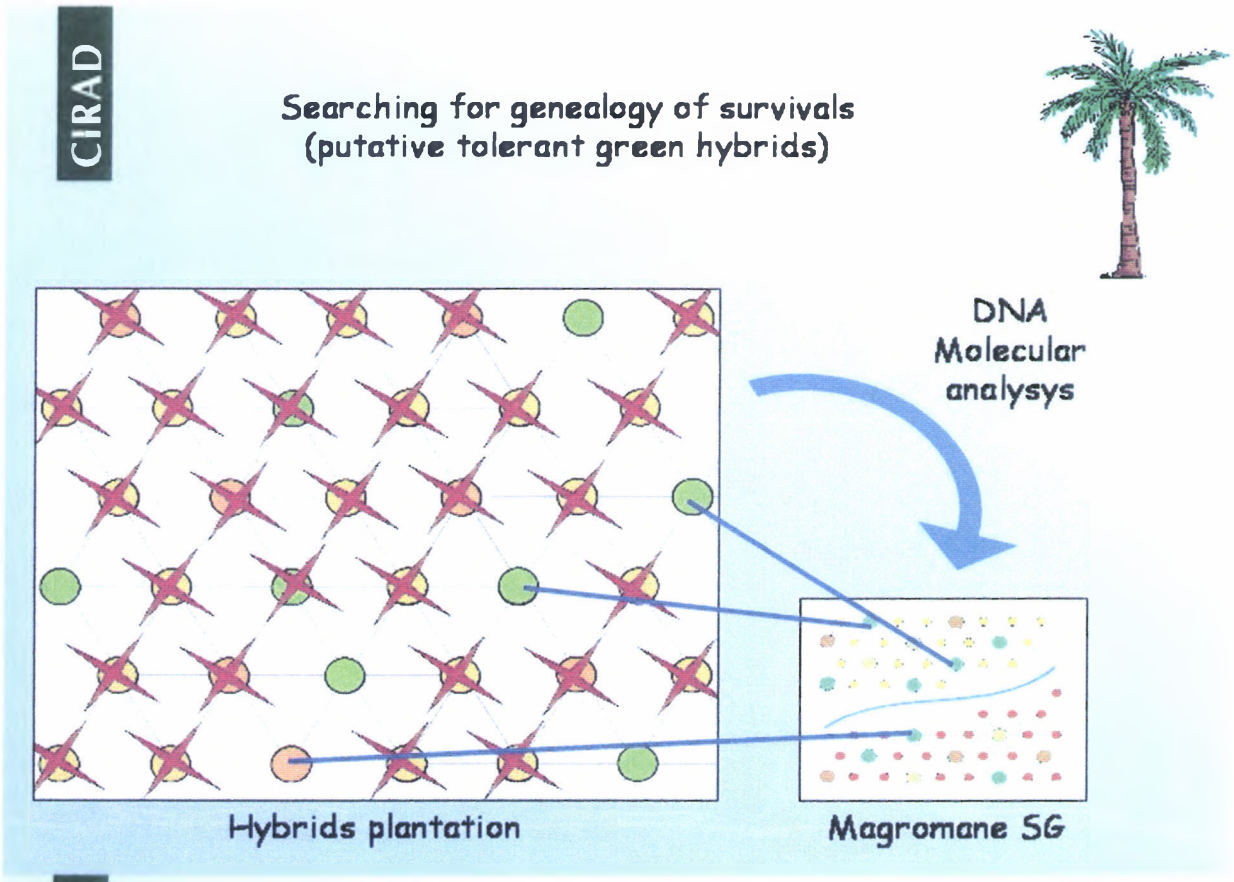
CIRAD

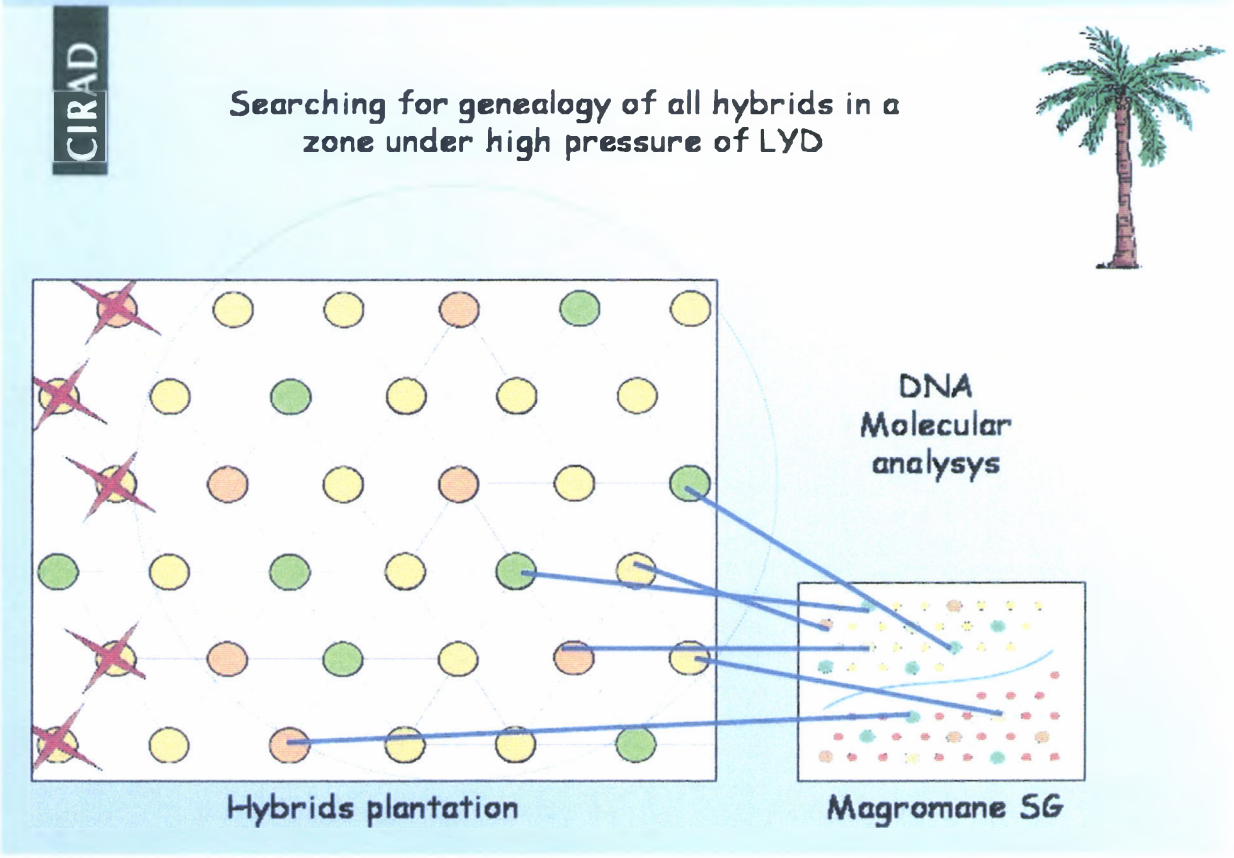
Search for hypothesis to explain the better
survival of green hybrids

- The Yellow Dwarf parent transmits better « tolerance » to LYD than the Red Dwarf parent

- Phenomenons of varietal preferences may explain the apparent better survival of green hybrids

- Some MZT male parents, from the seed-garden and preferably Green-fruited, transmits a better « tolerance » to LYD than other MZT male parents.





CIRAD

Ethnological approach – Cabo del Gado

CIRAD

Ethnological approach – Cabo del Gado