General Overview of Genetic Research & Experimentation on Coconut Varieties Tolerant/Resistant to Lethal Yellowing Diseases

Survol Général de la Recherche et de l'Expérimentation sur les Variétés Résistantes/Tolérantes au Jaunissement Mortel

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Abstract:
The Lethal Yellowing disease is one of the main threats to coconut industry in many parts of Africa and the Caribbean. Planting resistant varieties has long been recognized as one of the most promising ways of controlling it. Considerable efforts have been devoted throughout the world to screening suitable varieties and have often involved international co-operation. It has proven to be a lengthy and difficult task. We present an overview of these efforts with special mention to Ghana, Jamaica and Mexico. Although no variety so far has been proven fully and permanently resistant, treating resistance level as a threshold trait makes it possible to demonstrate significant differences among varieties, which can be exploited effectively to make genetic improvement a component of an integrated control strategy. Based on past experience, we make a few suggestions to increase the diversity of resistance sources and increase the level and the sustainability of resistance to LY in coconut.
Key Words: Lethal Yellowing Disease, Coconut, Génétic, Breesing, Résistant/tolerant LYD

Résumé :
La maladie du Jaunissement Mortel est l'une des plus sérieuses menaces pour le secteur cocotier en Afrique et dans les Caraïbes. La plantation de variétés résistantes est considérée depuis longtemps comme l'un des principales méthodes de lutte. Des efforts considérables ont été consacrés à travers le monde à la sélection de variétés appropriées et on souvent fait l'objet de coopération internationale. Cette tâche s'est révélée longue et difficile. Nous présentons un survol de ces efforts, en particulier au Ghana, à la Jamaïque et au Mexique. Bien qu'aucune variété ne se soit révélée pleinement résistante sur le long terme, l'étude de la résistance en tant que caractère quantitatif a mis en évidence des différences significatives entre variétés qui peuvent être mise à profit, faisant de l'amélioration génétique une composante d'une stratégie de lutte intégrée efficace. En nous basant sur l'expérience passée, nous faisons quelques propositions, tendant à accroître la diversité des sources de résistance et à améliorer la durabilité de la résistance du cocotier au JM.
Mots Clés : Jaunissement Mortel du cocotier, Génétique, Sélection, Résistance/Tolérance au JM

Resumen : "Revisión general de investigación genética y experimentación con las variedades de Cocotero tolerantes/resistentes a la LY"
La Enfermedad Mortal del amarillamiento es una de las principales amenazas de la industria del cocotero en muchas partes de África y el Caribe. El cultivo de variedades resistentes se ha reconocido como una de las maneras más prometedoras de controlar la enfermedad. Se han dedicado esfuerzos considerables en todo el mundo para seleccionar variedades adecuadas y a menudo se ha implicado la cooperación internacional. Ha resultado ser una larga y difícil tarea. Presentamos una revisión de estos esfuerzos con especial mención a Ghana, Jamaica y México. A pesar de que ninguna variedad ha resultado ser por el momento totalmente resistente a largo término, el estudio de la resistencia en tanto que carácter cuantitativo ha puesto en evidencia diferencias significativas entre variedades que pueden ser aprovechadas, haciendo de la mejora genética un componente de una estrategia de lucha integrada eficaz. En base a experiencias pasadas, realizamos algunas sugerencias para aumentar la diversidad de fuentes de resistencia y aumentar la durabilidad de la resistencia del cocotero a la LY.

Introduction

Lethal Yellowing-like diseases are caused by insect borne intraphloemic phytoplasma and are a plague to the coconut plantations in several countries of the Caribbean, East and West Africa. They have been destroying millions of trees for decades and there is a serious concern in several countries that haven't yet been affected. The use of resistant varieties has long been recognised as a promising way of controlling it and succeeded in revivifying a severely affected coconut industry in Jamaica. However, the varieties that give good results in a country are not necessarily suitable in another and, even in Jamaica, a new outbreak is destroying the varieties that were formerly considered as resistant. This urges coconut researchers to reconsider past experience in the domain of resistance testing and to identify new pathways to providing farmers with suitable planting material, cumulating a good resistance level, a high productivity and good fruit quality. An update of resistance screening tests will be presented during this session. In this presentation, we will make a brief overview of the field testing experiment series, which were set up in the last decades. After considering the difficulties encountered, we will try to summarize the main lessons from already published results and make a few proposals for further research.

1. Genetic experiments for LY resistance worldwide

Genetic experiments were conducted in several countries affected by the disease:

1.1. Jamaica

In Jamaica, 6 resistance trials were planted from 1961 to 1970. All, except for one were planted in replicated blocks. The results of these trials, representing some 5,700 palms were published by Been (1981) and a re-evaluation was presented at the Elmina workshop by (Ashburner and Been 1995). The results of these experiments made it possible to restore the profitability of the Jamaican coconut industry for some 20 years, but a new outbreak occurred at the turn of the century and provoked massive destructions (Broschat *et al.* 2002).

1.2. Tanzania

In Tanzania, 5 trials were planted from 1981 onward by the National Coconut Development Programme (NCDP) with GTZ and World Bank funds. They involved 33 varieties (including 15 hybrids) imported from Ivory Coast and 4 local varieties. Schuilling *et al.* (1992) present results based on a total of nearly 10,000 palms.

1.3. Ghana

The Ghanaian form of LY is called Cape Saint Paul Wilt Disease (CSPWD). The history of genetic research in this country is summarized in Dery *et al.* (2008). The first genetic experiments were planted in 1956 and involved Malayan Dwarfs. After another trial planted in 1977, a series of seven trials involving 27 varieties was planted in 1981-1982. Partial results of these trials were published in Sangaré *et al.* (1992) and in Mariau *et al.* (1996). Two more trials were planted in 1995, raising the number of tested varieties to 38. In total, some 5,000 coconut palms were involved in this series of resistance trials. The results of these trials are analyzed in Dery *et al.* (2008) and will be presented in the present workshop by R. Quaicoe. Experimentation for LY resistance in coconut is a continuing story and, in addition to a set of adaptive trials with the MYD VTT hybrid (1995), a rehabilitation/replanting scheme was launched in 1999 on a total of 1,300 ha with the same variety (it was, at that time, the only promising hybrid that could be produced at a large scale). Finally, three trials involving seven Dwarf varieties (1,400 palms) were planted in 2007. This entire programme would not have been possible without the contribution of the French Embassy, the European Union and Cote d'Ivoire. Technical assistance was provided by the former IRHO and CIRAD during the whole duration of the trials.

1.4. Other countries

Screening trials for resistance to LY have also been planted in Mexico (Zizumbo Villarreal *et al.* 1999; Zizumbo Villarreal *et al.* 2006). Varietal resistance level is also being monitored in Mozambique, Cuba, Nigeria and the USA (Florida).

2. Difficulties encountered

2.1. As a tree crop, coconut is bulky and has a long life cycle.

The experiments cited in the present paper represent 150 to 200 ha, that were monitored for periods of time ranging from 10 to 25 years. This long duration requires long term commitment into coconut research and has adverse consequences on funding since donors often expect results to be produced within a few years. The delay between infection and the expression of the symptoms is 3 to 6 months in young trees and 7 to 15 months in mature palms, making an analysis of the favourable conditions for infection rather difficult.

2.2. The symptoms are well characterized but need to be observed carefully

The full sequence of the symptoms, from premature nut fall to yellowing and disappearance of the crown take a few months and, according to the stage of observation, may be confounded with symptoms of other diseases such as hartrot and red ring. As a result, trials need to be monitored regularly and assessing the presence of phytoplasma through PCR assay using universal phytoplasma primers (Deng and Hiruki 1991) and specific LY primers (Tymon *et al.* 1998) is recommended.

2.3. The disease tends to develop in an erratic way

The pattern of evolution of the disease depends highly on the behaviour of the vector and on the presence of the pathogen. Although environmental factors, such as climate and the surrounding flora are thought to influence these parameters, this influence is not well understood for the moment and the variability of environmental factors is reflected in a great diversity in the evolution of the disease. In addition, it has been argued that poor growing condition will affect adversely resistance (Harries 1995).

Although all trials were located in or near disease foci, two out of the nine Ghanaian trials are still unaffected after more than 20 years. The delay between planting and a) the first symptom and b) the full epidemic period varies greatly. For example in Tanzania, symptoms appearing as early as 10 months after planting were confirmed by electron micrograph (Schuilling *et al.* 1992). In Ghana, little or no losses to LY were observed before 10 years and some trials remained unaffected for 15 years and were eventually destroyed within a few years.
Although in a few trials, all trees were killed, the disease usually spares a variable percentage of the trees. Attempts have been made to reproduce these survivors to produce resistant progenies. Although this approach is likely to improve the mean resistance level in a population, it generally failed to produce resistant progeny. This is due to the fact that survival doesn't necessarily reflect resistance, but may result from a reduction of the inoculum pressure, due to the lowered coconut density. The spatial pattern of the epidemics is also difficult to predict: in most trials, the first cases appear to be distributed at random, but at later stages, patches of diseased palms suggest the effect of short distance contagion.

2.4. Adequacy of the planting designs

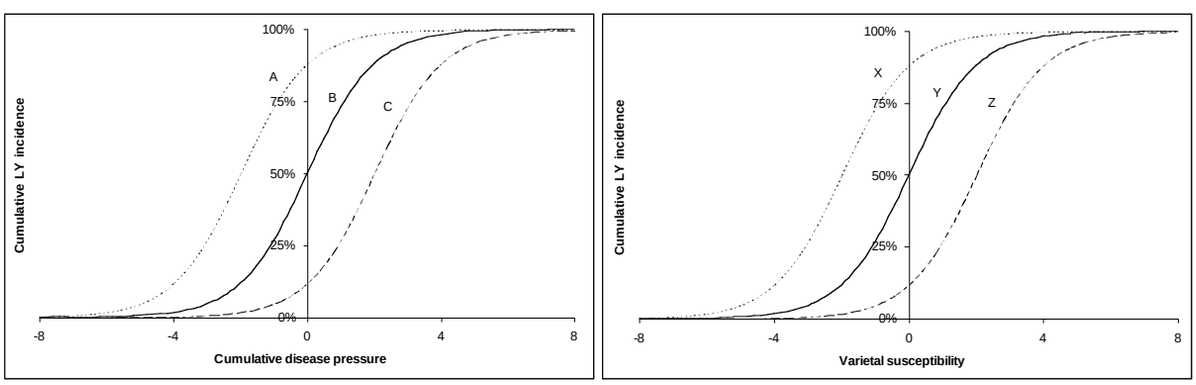
Most trials were planted with randomized blocks with a variable number of trees per elementary plots. Using elementary plots of, e.g. 3 4=12 trees, is usual in coconut breeding since it reduces competition effects among different varieties and provides a more accurate estimation of yield related traits. However, this may complicate the interpretation of the results due to the contagious nature of the disease: if a cluster of diseased and/or dead trees is centred on an elementary plot, it may reflect the intrinsic susceptibility of the variety or the partly random propagation pattern of the disease, described above. As a result, distinguishing genetic and environmental effects may become tricky.

In addition, due to limited available area and to seednuts shortage, the number of individuals initially planted was often low. This number was further reduced due to various causes, including drought, bushfire and diseases other than LY. As a result, the statistical exploitation of the intended field became often impossible. When possible, this problem was overcome by grouping several trials, considering each trial as a block.

An alternative solution is to plant only one palm per block and per variety. Smaller blocks ensure a better control of environmental factors, while randomization within block ensures a homogenous distribution of susceptible and resistant varieties. The main constraint is that a very rigorous control of the genetic identity of the trees has to be maintained for all the duration of the trial. In case of doubt, microsatellite marker analyzes can be useful to assess genetic identity (Baudouin *et al.* 2008; Lebrun *et al.* 2008).

3. Interpreting LY resistance trial results: the generalized linear model

The difficulties described above make the exploitation of the results of resistance trials particularly difficult. Since we don't control inoculation, we are forced to consider the response of a variety as the result of a combination of genetic and environmental factors. Moreover, this response evolves with time and the final percentage of losses is very high in most varieties. However, the rate at which the palms are destroyed varies greatly. As a result, we have to treat resistance level as a threshold trait rather than as a binary trait. (In a threshold trait, the response of an organism is one of two possibilities – e.g. dead or alive – but, unlike a binary trait, the probability of an outcome depends on a quantitative underlying variable). The variability of such traits can be analyzed using a generalized linear model (GLM – McCullagh and Nelder 1989).



a) Expected cumulative incidence as a function of the disease pressure in three varieties. The resistance level increases from variety A to variety C.

b) Expected cumulative incidence as a function of varietal susceptibility at three sites. The cumulative disease pressure increases from site X to site Z.

Figure 1: The logistic model: modelling the variations of the cumulating incidence as a function of disease pressure and of the resistance level.

Some of the features of this model are represented graphically in figure 1. In this model, losses to LY depend on the susceptibility of the tested variety and on the cumulative disease pressure at the trial site. The cumulative disease pressure always increases with time (the trees never recover) and its evolution depends on environmental factors, such as the density of the vector population, their rate of contamination by the phytoplasma, their feeding behaviour etc.

3.1. Modelling resistance levels in varieties

If we consider a given variety, the cumulative incidence (the percentage of dead trees at the time of observation) is assumed to be a logistic function of the disease pressure. The resulting sigmoid curve (figure 1a) expresses that the mortality rate is comprised between 0 and 100% and increases rapidly for values comprised between -4 and +4, (for variety B) while it remain almost constant when the disease pressure is either very low (left side of the graph) or very high (right side). Different varieties will be described by the same functions except that the curves are shifted to the left for a susceptible variety (curve A) and to the right for a resistant one (curve C).

3.2. Modelling disease pressure in sites

If, at present, we consider the cumulative incidence in an environment as a function of the susceptibility of the variety, we have exactly the same logistic function (figure 1b). In this case, the curve will be

shifted to the left for sites with a high disease pressure (curve X) and to the right for a low disease pressure (curve Z). In a given trial, the curve will move progressively to the left as time passes.

3.3. Statistical analysis

To summarize, each variety can be described by a single parameter S_i which characterizes its susceptibility and each site (at a given time) by a single parameter D_j which characterizes the cumulative disease pressure. We can thus include them in a model:

$$Y_{ij} = M + S_i + D_j$$

Where M is an arbitrary constant.

This is a classical quantitative genetic model except that Y_{ij} cannot be observed directly. Instead, we assume the observed cumulative incidence to have a binomial distribution whose parameters are the predicted incidence $P_{ij} = e^{Yij} / \left(1 + e^{Yij}\right)$ and the number of useful trees initially planted. This assumption makes it possible to take into account the uncertainties resulting from limited numbers of trees.

Appropriate software, such as *R* (R Development Core Team 2004) and its *glm* function can estimate the S_i and the D_i parameters as well as test the degree of significance of the genetic and environmental effects. If F_1 hybrid varieties are tested, additive values for the parental varieties can also be estimated. It is also possible to model the effect of a variation in disease pressure on the behaviour of a variety of known susceptibility. This approach was used in Ashburner and Been (1995) and, more recently in Dery *et al.* (2008)

Considering figure 1b, it is clear that the optimal time to analyze the results of a trial is when the mean losses are in the 25%-75% range: this maximises the influences of the genetic differences (in terms of S_i) on the observed responses (in terms of incidence). But it has to be kept in mind that the disease will continue to evolve and that the figures cited in (Been 1981) or in Dery *et al.* (2008) don't represent the final stage of the trials but a "snapshot" taken at the most convenient moment to reveal difference in susceptibility levels.

4. Genetic diversity of coconuts and the distribution of resistance factors

An update of the results of LY resistance trials in various countries will be presented during this session. We propose now to examine the distribution of resistance factors in the coconut species, essentially based on the already published results from Jamaica, Ghana, Tanzania and Mexico.

A common feature of these results is that no coconut variety was found absolutely and permanently resistant to LY. Even the most resistant varieties can be affected, provided that a sufficient number of individuals are exposed to the disease for a sufficiently long period of time. However, the mortality curve differs greatly according to the variety. GLM analyses presented in Ashburner and Been (1995) and in Dery *et al.* (2008) show that these genetic differences are significant and that about 80% of the deviance is accounted for by additive effects. Non-additive effects were significant, but quantitatively limited. Attempts made to account for them produced diverging results: in Jamaica, hybrids tended to behave better like the mid parent while it was the contrary in Ghana and Mozambique. In any case, the additive effects represent the main part of the diversity of coconut varieties for resistance level to LY.

Figure 2 is based on the results published in Been (1981) and illustrate the losses predicted by the additive model for 29 varieties (in an average environment). The cultivars are grouped according to the microsatellite based classification published in Lebrun *et al.* (2005). This classification concords partly with that proposed by Harries (1978), which was based on response to LY, germination and a combination of morphologic traits. With a few exceptions, Indo-Atlantic cultivars tend to have the *Niu Kafa* phenotype while the Pacific cultivars tend to have the *Niu Vai* phenotype. However, the microsatellite-based classification is more adapted to reveal the probable phylogeny relationships between cultivars.

At the time of observation, all Dwarf varieties but one (from the Pacific) presented very low disease incidences. Losses were quite significant (26 to 94%) in all Tall varieties but remained moderate in the

Tall from Southeast Asia and from the Pacific coast of America. Contrastingly, all the Indo-Atlantic had losses above 65% except for the Mozambique Tall and cultivars from the Pacific Ocean (Melanesia, Micronesia and Polynesia) had quite variable behaviours. To summarize, the Jamaican data suggest that the main source of genetic resistance factors is Southeast Asia. This is indicated by the behaviour of the Tall and especially the Dwarfs from this region. This is further illustrated by cultivars from other regions, but related to the Southeast Asian stock, which tend to be less susceptible than others: The Panama and Peru Tall, which reached the American continent in pre-Columbian times, had a Filipino origin (Baudouin and Lebrun 2008). Likewise, introgression of Southeast Asian genes into the Mozambique Tall (and into all East African cultivars) seems to explain why it was found much less susceptible than the other Indo-Atlantic cultivars. The presence of both susceptible and mildly resistant varieties in the rest of the Pacific could reflect the complexity of past germplasm exchange in the region.

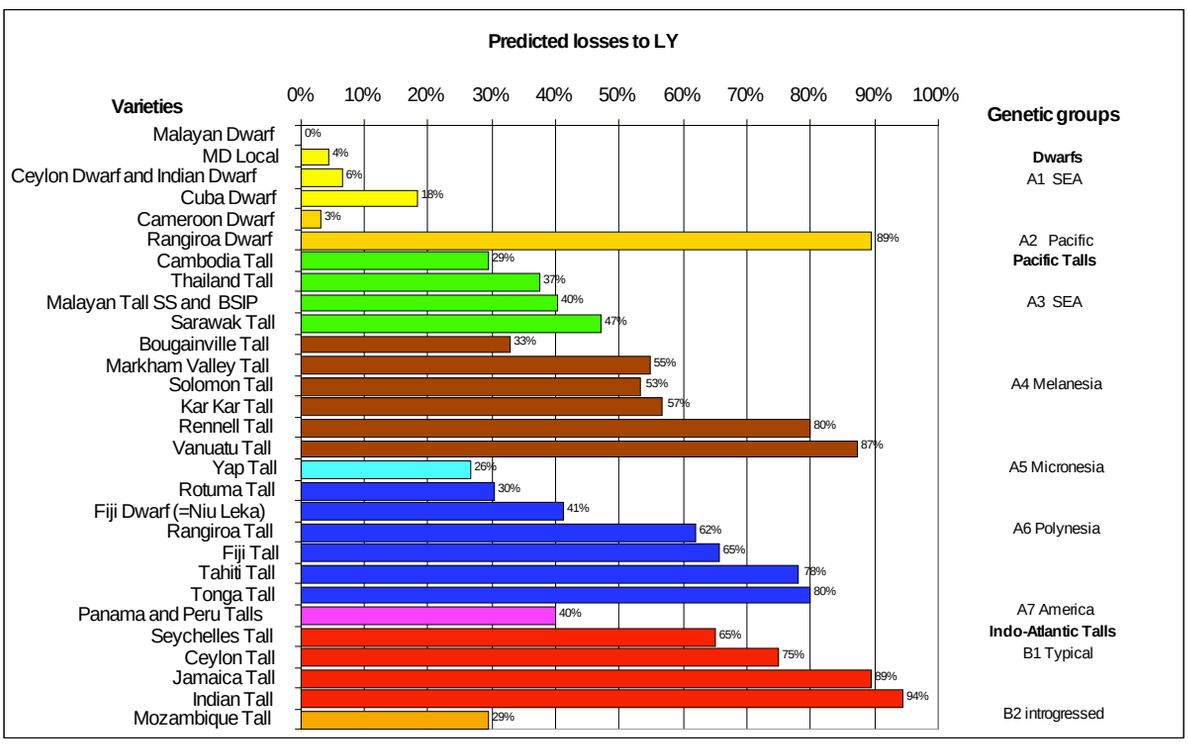


Figure 2: Results of GLM analysis in 6 trials of Jamaica (data from Been 1981). The predicted losses in an average environment were calculated based on the additive components of S_i.

Results in Mexico involve the susceptible Mexican Atlantic Tall, which is similar to the Jamaica Tall and the (partly) resistant MYD. They are compared with three groups of Mexican Pacific Tall, resulting mainly from importation from the Philippines at historic times. Under severe incidence of the disease, the rate of mortality is correlated with the genetic distance to the MYD (Zizumbo Villarreal *et al.* 2006).

The main common point between the results obtained in Ghana and in the Caribbean is the high susceptibility of the Indo-Atlantic West African Tall and a better level of resistance in the Dwarfs and in other Southeast Asian cultivars. In spite of this broad agreement, there are many differences in the detail: in Ghana, the Malayan Yellow Dwarf is much less resistant than the Sri Lanka Green Dwarf and the Vanuatu Tall is the most resistant Tall (Dery *et al.* 2008).

In Tanzania, the results differed from those obtained in other countries: the imported varieties didn't behave well but some of the local East African Tall populations had comparatively lower losses. The microsatellite profile of the East African Tall is quite similar to that of the Mozambique Tall: both belong to the "introgressed" subgroup of the Indo-Atlantic cultivars, meaning that a significant proportion of their genes are from Southeast Asia.

One possible explanation of the variations in the distribution of resistance level according to the country as well as in time is that they could result from interaction between a small number of resistance genes in coconut and of virulence genes that would differ in the various phytoplasma strains. Another explanation would involve differences in the behaviour of the vector(s) in response to the variations of their

environments (including range of accessible coconut varieties). In both cases, increasing the genetic diversity of resistant varieties would probably be an effective way of increasing the sustainability of LY resistance. Increasing the number of resistance factors will make it more difficult for the pathogen and/or the vector to invade the whole coconut population.

Conclusion

Experience from LY resistance screening tests shows that the response of cultivar to the disease is quantitative: an appropriate statistical analysis reveals clear differences for resistance level among coconut varieties but none of them can be qualified as truly and permanently resistant. Moreover, neither the mechanism of resistance nor the reasons why it happens to be overcome is known as yet. Identifying and mapping resistance factors remains a challenge for the future.

Selecting varieties for high resistance level is a good approach to reduce the risk associated to LY. It contributes to lowering the chances of contaminations, reducing the rate of losses and increasing the duration of the productive period of a coconut plantation.

Experiments conducted in Jamaica in the 70' lead to the identification of a hybrid, which had a high resistance level and a high productivity, the Malayan Yellow Dwarf x Panama Tall or MAYPAN. This was a partial success: the Jamaican coconut sector stayed profitable for some 20 years, but a new outbreak occurred at the turn of the century and provoked massive destructions. Directly or indirectly, this apparent breakdown of resistance seems to be related to low genetic diversity in the MAYPAN. To secure long term profitability in coconut industry, it will be necessary 1) to associate genetic control to environmental control measures including early eradication (Philippe et al, 2004) and 2) to diversify the sources increase the diversity of resistance factors.

Comparing the results obtained at different sites, we could identify Southeast Asia as the main source of genetic resistance factors. This suggests that ancestral populations of this region have been confronted to this or a similar disease in the past. In Jamaica, the resistance levels of Southeast Asian Tall cultivars were quite comparable to that of the Panama Tall but microsatellite markers show that their diversity is much higher. As a result, resistance should be more sustainable. Further genetic testing should be done, involving Southeast Asian Dwarfs and Talls in priority. This doesn't mean that other varieties are devoid of interest. Carefully chosen introgressed Indo-Atlantic varieties existing in East Africa and in the Indian Ocean may be of interest, since they present useful traits that are not in the Southeast Asian germplasm. Finally, although the varieties from the South Pacific as a whole seem to have a low resistance level, a few of them seem to be promising, including the NLAD.

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