Doras-Rubber Project
Towards the improvement of the productivity of rubber tree
Kasetsart University, Rrit-Doa, Cirad

Mission in Thailand
1st – 14 June 2003

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- at Rrit-Doa : Ms Kannikar Teerawatanasuk, Ms Kanlaya Prapan, Ms Napawan Lekawipat, Ms Somjintana Ruderman, Mr Krissada Sangsing, and staff
- at Kasetsart University : Dr Somvong Tragoonrung, Ms Unakorn Silpi,
- at Cirad : Dr Marc Seguin, Ms Maguy Rodier-Goud, Dr Philippe Thaler, Dr Eric Gohet,
- at Inra-Piaf : Dr Hervé Sinoquet and his team,
- at Cirad-Ecotrop unit : Dr Michael Dingkühn, Dr Georges Nizinski.

Résumé

Le projet Doras-Rubber intitulé « Vers l'amélioration de la productivité de l'hévéa » (Kasetsart University, Rrit-Doa, Cirad-cp/hévéa, Inra-Piaf) est soutenu financièrement par le Comité Mixte Franco-Thai. Il comporte quatre composantes : a/écophysiologie(Ecophysio), b/systèmes de saignée (Tapping), c/génétique et physiologie (Genmap), and d/qualité du caoutchouc (Rubberqual). Il vise à développer la recherche, ainsi que la formation par la recherche de jeunes chercheurs thais, sur la compréhension du fonctionnement physiologique de l'hévéa et l'identification de facteurs de qualité, afin d'améliorer les systèmes de culture à base d'hévéa, avec une attention particulière portée aux clones, aux systèmes d'exploitation, et au caoutchouc naturel. Une réflexion sur la production du bois d'hévéa est initiée actuellement.

La composante Genmap porte sur l'étude d'une famille de pleins frères issue de deux parents physiologiquement contrastés, afin de caractériser la base génétique et la variabilité de paramètres morphologiques et architecturaux, de paramètres physiologiques associés au métabolisme du latex, et de paramètres liés à la qualité du caoutchouc. Cette mission avait pour principal objet de faire le point des mesures réalisées sur l'essai en champ Genmap (étude de 196 descendants) un an après sa mise en place.

Ce rapport présente :

- Un résumé de l'état d'avancement du projet
- La présentation d'une nouvelle proposition d'action portant sur la relation parcelle-climat, sous la supervision du Dr Georges Nizinski
- Une note sur la possibilité d'introduction, d'échange et d'étude de nouveaux clones dans le cadre du projet
- Le compte-rendu de la visite au Dr Somvong Tragoonrung à Kamphaengsen
- Concernant Genmap, la mise à jour par Marc Seguin du rapport de Ms Kanlaya Prapan portant sur la cartographie génétique du croisement RRIM600 x PB217, et l'état d'avancement des mesures dans l'essai en champ (incluant les Appendix 14 à 20 décrivant le détail des analyses de données)

Pour achever la cartographie Genmap, un financement reste à trouver pour la prise en charge de l'accueil au laboratoire Cirad- Biotrop à Montpellier de Ms Napawan Lekawipat.

Abstract

Doras-Rubber project, entitled « Towards the improvement of the productivity of rubber tree » (Kasetsart University, Rrit-Doa, Cirad-cp/Hevea, and Inra-Piaf) is financially assisted by the French-Thai Committee Programme. This project is made of four components : a/ecophysiology (Ecophysio), b/tapping systems (Tapping), c/genetics and physiology (Genmap), and d/quality of rubber (Rubberqual). It aims at developing research, as well as training to research for young Thai researchers, focussed on the better understanding of rubber physiological functioning and identification of the factors responsible for the rubber quality, with emphasis on clones, tapping systems and rubber. Improving rubberwood production is being addressed just now.

The Genmap component deals with the study of a full-sib family issued from the cross between two physiologically contrasted parents, in order to characterize the genetic base and the variability of morphological and architectural factors, of physiological factors associated with latex metabolism, and of factors related with rubber quality. This visit to Thailand was to take stock of field measurements carried out in the Genmap field trial since its setting-up one year ago.

This report presents :

- A synthesis of the advancement of the project
- A new proposal of Dr Georges Nizinski for studying the plot-climate relationship
- A note suggesting the introduction, exchange, and study of new clones within the framework of the project
- The report of the visit to Dr Somvong Tragoonrung at Khamphaengsen campus
- Concerning Genmap, the up-dating of the report of Ms Kanlaya Prapan by Marc Seguin, relating to the genetic mapping of the family RRIM600 x PB217, and the report of field measurements and analysis (including Genmap Appendixes 14 to 20 describing the details of analyses and of first results).

In order to achieve the saturation of Genmap genetic mapping, some funding is still to be obtained for covering the Cirad-Biotrop laboratory costs of a planned 5 months period of Ms Napawan Lekawipat in Montpellier.

Schedule of the mission and people met

Schedule

Sun, June 1, 2003	- Departure from Montpellier-France at 03 pm
Mon, 2	- Arrival in Bangkok-Thailand at 01 pm
	- Meeting with Cirad colleagues at Cirad office, Kasetsart Univ.
	- Meeting with Jacques Morcos (SCAC, French Embassy)
Tue, 3	- Field research on Genmap component at Chachoengsao Rubber Research Center (Crrc/Rrit-Doa)
Wed, 4	- Field research, Genmap, Crrc
Thu, 5	- Field research, Genmap, Crrc
Fri, 6	- Field research, Genmap, Crrc
Mon, 9	- Reception of Dr Georges Nizinski (Ird)
	- Meeting with Dr Christian Bellec at Ird office in Bangkok
	- Meeting with Dr Poonpipope
Tue, 10	- Meeting with Dr Somvong at Kamphaegsen
	- Meeting with Dr Kumut and Mr Tiwa at Kamphaengsaen
Wed, 11	- Visit to Crrc with Dr Georges and Mr Tiwa
Thu, 12	- Field research, Genmap, Crrc
Fri, 13	- End-of-mission meeting with Dr Sornprach and Dr Poonpipope in Ku
	- End-of-mission meeting with Dr Prasat and Dr Suchin in Rrit-Doa
	- Visit to the lab of Laurent Vaysse in Ku-Kapi
	- Departure from Bangkok at 11 pm
Sat, 14	- Arrival at Montpellier at 02 pm.

People met

Ku
Dr Sornprach Thanisawanyangkura,
Dr Poonpipope Kasemsap (Ku), Dr Somvong Tragoonrung (Ku),
Mr Tiwa, Ms Unakorn, Mr Krissada

Rrit-Doa
Dr Prasat Kesawapitak, Chamnong Kongsin
Dr Suchin Maenmeun, Arak Chantuma, Pisamai Chantuma, Kannikar Therawatanasuk, Kanlaya Prapan, Somjintana Ruderman, Napawan Lekawipat

Cirad
Drs Eric Gohet, Philippe Thaler, Laurent Vaysse (Cirad)

Ird
Dr Christian Bellec (Ird)
Dr Georges Nizinski (Ird, assigned to Cirad-Ecotrop unit)

French Embassy, Scac
Mr Jacques Morcos

Contents

A. Overview of the Doras-Rubber project in Thailand

1. Advancement of the project, in short

Doras-Rubber project was launched in July 1998 for a first period of six years.

- From July 1998 to June 2001, Dr Pascal Montoro (Cirad), based in Thailand, coordinated research devoted to rubber genetic engineering.
- Since March 1999, Dr Eric Gohet (Cirad) coordinated the setting up of tapping systems trials, the development of the use of latex diagnosis, and the ecophysiology research, in close cooperation with Dr Poonpipope (Kasetsart) and Rrit-Doa team.
- From May 1999 to April 2000, Dr Marc Seguin (Cirad) developed the identification of microsatellites for rubber genetic mapping in Thailand.
- In July 1999, agreement was passed with Inra-Piaf (Hervé Sinoquet, André Lacointe, Hervé Cochard, Thierry Améglio) for cooperation in rubber ecophysiology research within the framework of the Doras-Rubber project.
- In year 2000, three Ph.D. theses were initiated, one in genetics (Ms Napawan Lekawipat), and two in ecophysiology (Ms Unakorn Silpi and Mr Krissada Sangsing). These theses will be defended before the end of year 2003, or just at the beginning of year 2004. These three young researchers spent some short periods in France at Inra-Piaf and Cirad.
- In June 2001, a mid-term project seminar was organised at Kasetsart University.
- In August 2001, Dr Philippe Thaler (Cirad) settled in Thailand to strengthen ecophysiology research.
- In year 2002, Ms Kanlaya Prapan (Rrit-Doa) spent a 12-months period at Cirad-Montpellier for developing the genetic map of the Genmap family.
- In year 2002, a new thesis was initiated with Ms Pisamai Chantuma, focussed on carbohydrate reserves in the rubber tree.
- In October 2002, Dr Laurent Vaysse (Cirad) settled in Thailand for developing research about the identification of factors responsible for the variability of rubber quality.

Funding of the project is provided by :

- the four partners (Kasetsart, Rrit-Doa, Inra-Piaf, and Cirad), since 1998 : wages, equipment, and operating costs,
- a specific Inra-Cirad funding for operating costs (two years),
- the French Embassy in Bangkok (French Ministry of Foreign Affairs) : missions, training periods, workshop,
- the Thai-French Committee Programme (since 2002) : missions and training periods,
- Agropolis (12-months period in France).

2. Ecophysiological research

Ecophysiology applied to rubber, at the level of the whole tree or of the field plot, is a logical follow-up of research devoted to the functioning of the laticifer. As a matter of fact, the two components of latex production, flow and regeneration, depend on the water status in the trunk (and consequently on the general water balance), and on the photosynthetic assimilation of carbohydrates and of their distribution among the different sinks. Moreover, the growing interest on total biomass and on rubberwood production leads us to address the soil-plant atmosphere continuum. This research makes possible the application to the rubber tree of methodologies already applied to other plants and trees, and to continue former works carried out by Ninane (1960'), Barbier and Monteny (1980'). Beyond the production of knowledge, this research will help in enriching the concept of clonal metabolic typology with an enlarged agro-physiological characterization of the behaviour of the main cultivated clones, in order to choose the clones more adapted to specific ecological contexts (water stress) or to specific production objectives (short or long cycles, joint latex and rubberwood production), and to optimize tapping systems according to the clones and to the production objectives.

A methodological gap is more often lying between breeders and physiologists : breeders use parameters which are easily measurable for application to a wide range of genotypes, whereas physiologists use parameters which need more time and equipment for measurement on a narrow range of varieties. Physiologists often argue that their work will provide the breeder with new tools, whereas breeders use more often statistical methods globally applied to a « black box ». But for a tree crop such as rubber, breeding often identifies interesting clones which lay poorly characterised at the level of their response to the variability of environment (interaction genotype x environment) ; consequently, it seems very interesting to apply to rubber some joint breeding and physiology efforts in order to provide a better characterization and use of the main existing clones in the short and middle term, and to suggest new possible ways for breeding in the long term.

Rubber particularities

As other annual crops and tree crops, rubber has to provide its production under some ecological limitations (climate, soil) which affect photosynthetic assimilation. One main limiting factor is water which depends on the climatic demand (energy = radiation) on one hand, and on transpiration regulation (soil water availability, stomatal functioning, hydraulic conductance from roots to leaves). One specificity of rubber tree is linked with the artificial introduction of a new carbon sink which is generated by tapping, and which is added to the other sinks such as growth, reserves storing and release, and respiration (plus fruits, refoliation, etc.). Another specificity is linked with the qualitative role of water, the amount of which is not significant in latex exportation, but the influence of which is determinant on latex flow (turgor pressure in the laticifer, and dilution effect).

Ecophysiological research (« Ecophysio ») can be considered as the heart of the Doras-Rubber project, with downstream applications in agro-physiology such as choice of clones, optimisation of tapping systems, adaptation to drier areas, optimisation of the joint latex and rubberwood production. Upstream of « Ecophysio », the « Genmap » component of the project is addressing the variability analysis of many agro-physiological parameters, within the restricted genetic framework of a full-sib family issued from physiologicaly contrasted parents. Downstream of « Ecophysio », the « Rubberqual » component is addressing the issue

of the quality of the natural rubber material, including its links with cropping and agro-physiology of the rubber tree.

The three Ph.D. theses (Unakorn, Krissada, Pisamai) are going on, with particularly intensive activity at the level of carbon reserves analysis at Crrc lab. The Ph.D. theses defences of Unakorn and Krissada, initially planned for December 2003, could be postponed to March 2003. Unakorn spent a one-month period at Inra-Piaf in France in July 2003 for data analysis, and Krissada could spend a one-month period at Inra-Piaf in October 2003 for addressing the application of the RATP-RER coupled models to rubber. These researches are focussed at organ and plant level.

Researches being carried out

- Krissada thesis is addressing different aspects of water and carbon related functioning of young rubber trees which are not tapped :

- Vulnerability to embolism (cavitation) at the scale of peripheric (distal) organs such as midribs, petioles, and small branches. The loss of hydraulic conductivity, compared between clones, provide information about stomatal functioning (confirmation of the hypothesis that stomates close in order to avoid cavitation which can cause long-lasting disturbances to the trees). RRIT251 seems more susceptible than RRIM600 in case of water stress (publication 2003). Use of a Inra-Piaf patented equipment, methodology developped by Dr Tyree, and by Inra-Piaf (Dr Cochard).

- Hydraulic conductance of different parts of the tree. Use of equipment HPFM developed by Tyree and Inra-Piaf. The higher resistance would be due to small branches ; leaves would have an intermediate resistance, whereas petioles poorly contribute to the total hydraulic resistance (publication in preparation).

- From the photosynthesis model of Farquhar (biochemically based upon nitrogen content in the leaves), taking into account stomatal conductance and climatic data, and from the stomatal functioning model of Jarvis (based on light, temperature, water vapor pressure deficit, and CO^2 content) at the scale of the leaf, parameterization of these models for rubber in order to assess leaf photosynthesis and stomatal conductance (and so, carbon assimilation) depending on climate, in a situation free from any water stress. Comparison of RRIT251 and of RRIM600 (publication in preparation).

- Dynamics of leaf gas exchanges, depending on water stress (transpiration, photosynthesis) for two clones (PB260, PB217, glasshouse experience in Inra-Piaf Clermont-Ferrand/France in year 2000).

- Study of maintainance and growth repiration costs for leaves, depending on the location of leaves and of leaflets, and on clones (PB235, PB260, RRIM600, GT1), for implementing a carbon balance study, a growth model, and a study about clones response to the environment. The greeness of leaves (indicating chlorophyll content, and measured by SPAD metodology) was studied together with respiration. Growth respiration did not seem to vary from one clone to the other, whereas maintainance respiration seemed lower for PB235. A strong positive correlation was found between respiration rate and growth rate relative to leaf area. Maintainance respiration was poorly correlated with leaf temperature ; growth respiration was not correlated with leaf temperature.

- Study of the respiration of trunks and of branches of different sizes on untapped young trees of three clones (RRIM600, RRIT251, PB260) in order to assess respiration costs for growth and maintainance, with view to estimate carbon balance for rubber.

- Application of Inra-Piaf models, RATP and RER, to rubber, in order to assess exchanges of carbon and water at the scale of whole young trees. RATP (Radiation, Absorption, Transpiration, Photosynthesis) simulates the spatial distribution of transpiration and of photosynthesis within a canopy, taking into account the geometry of the tree (according to Amap methodology) and microclimatic data. Implementation at the level of two trees (age 2.5 years) for clone RRIT251. RER (Regulation of Embolism Resistance) describes the relationship between hydraulic parameters of the tree and regulation of water loss. The coupling of the two models is planned.

• Unakorn thesis (under supervision of André Lacointe from Inra-Piaf) is studying adult trees, with three main treatments : not tapped, tapped and not stimulated, tapped and intensively stimulated, mainly on clone RRIM600, but also on PB235 and on GT1. The objective is to describe the daily and seasonal dynamics of carbon assimilates among the four main sinks : growth (biomass and wood), latex production, reserves storing and release, and respiration, according to the following approaches :

- Estimation of actual energetic costs of latex production and of growth, by way of continuous respiratory CO^2 exchanges and radial growth measurement (LVDT and RS equipments). From the analysis of radial growth data, it can be seen that the impact of tapping on the growth reduction is very fast, as it can be observed just after a few days. The different parts of the trunk are not affected in the same way. Data analysis at the pace of hours remains to be done in order to know if this method can be adapted to the study of water fluxes within the trunk along the day and in relationship with tapping.

- Assessment of the cumulated effect of latex production on the dynamics of carbon reserves storage and release, with localisation in the different parts of the trunk (histological slices) and quantitative biochemical analysis (starch and soluble sugars). First indications are the followings : accumulation of starch in wood and bark from May to January, release of reserves during refoliation in January-February ; the vertical gradient is strongly disturbed by tapping. Starch stored in the wood at the level of the regenerating bark is not released ; tapped trees store more starch than non-tapped trees.

- Assessment of trunk areas involved in metabolic activation due to latex regeneration, and localization of sinks (accumulation or use) by way of the « latex diagnosis mapping » ; the area affected by tapping is more extended that the sole drained area. Some areas are « accumulating » (carbon received is higher than carbon used) and some areas are « using » (consumption higher than reception).

- Estimation of total biomass of trees, from empiric relationship with dimension and shape. This work is based on measurements and weighing of 24 trees « extracted » (destroyed) from three production potential trials (8 trees per clone for PB235, GT1, and RRIM600).

• Pisamai thesis (initiated in year 2002) aims at describing, at the scale of the whole tree, the dynamics of carbon reserves storage and release, in response to tapping systems and to the phenology of the trees, at the tissue level (wood, bark). This research might be

focussed on some tapping system trials devoted to the evaluation of the new DCA system (Double Cut Alternate system). Another approach is to be included, taking into account the study of sucrose trans-membrane transfer from the apoplasm to the laticifer, by way of immuno-cytolocalization of sucrose transporters. This methodology is currently being developed at Université Blaise Pascal of Clermont-Ferrand (Dr Souleiman Sakr), which belongs to the « Piaf » Joint Research Unit (associated with Inra-Piaf) ; the first approach was carried out with an heterologue probe of sucrose transporter, but this probe did not match with the rubber transporter ; consequently, implementation of this technique on rubber would require the isolation of homologous probes.

3. A new proposal : Impact of climate on a rubber plot (Dr Georges Nizinski)

As the first researches were developed at the level of the tree, in cooperation with Inra-Piaf, there was a wish to implement another approach at the level of one rubber stand, for assessing the impact of edaphic conditions on the production of the rubber crop (biomass, wood, latex), in cooperation with Cirad-Ecotrop unit.

Dr Georges Nizinski, belonging to the Ird-Clifa bioclimatology unit, and currently assigned to the Cirad-Ecotrop unit, made a proposal about the modelisation of energy balance and water balance at the scale of a monoclonal rubber plot in East-Thailand. He participated to the mission, from June 9 to 13, and had discussions with Dr Poonpipope and Dr Kumut.

A short note describing the proposed project is provided in Annex.

4. Tapping systems

From the different trials devoted to tapping systems and set by the project, the new system « DCA » (Double Cut Alternate), which has now reached three years of tapping, appears able to provide smallholders with a better productivity of the plot as well as of the tapping work, of about 25-30 %. A communication is currently being prepared for presentation to Irrdb in Chiang Mai in September 2003.

5. Introduction of new clones to Thailand, exchange of clones

Exchange of clones between rubber research partners, in the form of budwood or of budded stumps, is of mutual benefit for development, but also for research as it makes possible the testing of available planting material in different ecological areas and so, the improvement of the physiological and agronomical characterization of these clones. But such transfers are not so easy as they may involve varying costs and strongly depend on plant material available at the adequate stage.

In May 2001, just before a mission to Thailand, Cirad had some budwood from different clones ready for grafting and so available for a possible transfer. Consequently, after receiving the agreement of Rrit-Doa, Cirad took the initiative of transfering 16 clones to Thailand, in the form of budwood, in respect with international regulations (phytosanitary certificate, presentation of the plant material at Thai customs office at arrival, on May 29, 2001. The intention of Cirad is to include this transfer as one of its contributions to the outputs of the

Doras-Rubber project for the benefit of the Thai rubber industry ; consequently, oral information was provided to Kasetsart University about this transfer, during the same mission of André Clément-Demange (May 28 – June 9, 2001) . At research level, the interest of Cirad is :

- to make possible the characterization of these clones in Thailand
- to gain a possible access to some clones issued from Rrit breeding programme.

The 16 clones are :

- IRCA 18 , 41 , 101 , 109 , 122 , 144 , 145 , 317 , 321 , 323 , 523 , 631, 825, 871
- PB314 and RRIM901.

The grafting of the 16 clones at Rrit-Crrc was successful. In Cirad classification of clones, three of them are currently classified in class 2 (recommended for planting) and eight of them are classified in class 3 (promising clones). More detailed informations can be provided for these clones. Rrit-Doa informed us about the possible planting of one first Large Scale Clone Trial (LSCT) for the study of these 16 clones in Thailand in June-July.

A request was made by Cirad to Rrit-Doa for receiving 5 RRIT clones. Rrit-Doa agreed to this request and suggested the possible provision of clones RRIT218, RRIT226, RRIT251, RRIT404, and RRIT408. A request is being submitted by Rrit-Doa to DOA for export authorization.

This transfer of clones from France to Thailand could be improvised because there was a good opportunity at that time to do it with no real cost for Cirad and no commitment required from the Thai partners. This point was discussed at the « end-of-mission » meeting at Kasetsart University (June 13, 2003). For the future, the question of renewing such action, under the framework of the Doras-Rubber project, would have to be formally discussed and agreed by the partners of the project.

We underline the interest of the exchange of clones : it is of mutual benefit for the partners and for rubber development ; for the project in Thailand, it makes possible a better association between breeding and physiology by introducing new clones with some already known characteristics into the physiological characterization process of the project.

6. Genetics. Meeting with Dr Somvong Tragoonrung (DNA technology) at Kamphaengsen

The evolution of the Genmap component had been presented to Dr Tragoonrung in June 2002, at the time when the Genmap field trial was being set. One year after, the meeting of June 10, 2003 was an opportunity for A. Clément-Demange to precise the current status of the mapping of the family RRIM600 x PB217, at the end of Ms Kanlaya 's period in Montpellier, based on Ms Kanlaya's report. Since then, a detailed updated report was established by Dr Marc Seguin (cf Annex).

As a complementary period in Montpellier of one Thai researcher would be necessary to complete the genetic map, it was suggested that Ms Napawan Lekawipat could be assigned to this work for a five month period, taking into account her abilities in this field, provided that

funding is made totally available for that action : for now, a budget of 6300 euros for lab functioning costs is still to be found.

Ms Napawan Lekawipat, who attended this meeting, presented the results of her Ph.D thesis : « Comparison of gene and non-gene specific molecular markers for evaluating genetic diversity in rubber ». The part of this work which is devoted to non-gene markers (microsatellites) was carried out in cooperation with Dr Marc Seguin, especially during two one-month periods in Montpellier funded by the French Embassy in Bangkok (MAE-Scac), and is now published :

> « *N. Lekawipat, K. Teerawatanasuk,M. Rodier-Goud, M. Seguin, A. Vanachit, T. Toojinda, S. Tragoonrung (2003). Genetic diversity analysis of wild germplasm and cultivated clones of Hevea brasiliensis Müll. Arg. by using microsatellite markers. J. Rubb. Res. Vol. 6 (1), 36-47* » .

The other part of this research (SSCP gene specific molecular markers) was submitted to Sabrao for publication. This thesis was jointly funded by Rrit-Doa and by Kasetsart University.

Leaf diseases were examined as a possible cooperation topic within the Doras-Rubber project. As a matter of fact, Dr Somvong is supervising the Ph.D. thesis of Ms Phayo Romruensukharom (Rrit-Doa), devoted to the « *Hevea x Corynespora* host-pathogen system », which is totally funded by Rrit-Doa. André Clément-Demange explained that the Cirad team concerned by genetics and leaf diseases was now focussed on Microcyclus within the framework of a Cirad-Michelin project (CMB = Cirad-Michelin-Brésil). Two actions can be considered :

- organisation of a visit of Dr Somvong to the CMB project (which makes necessary a quarantine stay of 7 days in Europe or North America before coming back to Thailand),

- provision of the toxin of *Corynespora*, cassiicoline, for application to Phayo's work (Phaya is currently practising artificial inoculation of the inoculum of different strains over different rubber clones for studying the interaction, and would be happy to test the direct effect of the toxin).

7. Organization of a Rubberwood Workshop

During the mission, we had oral information that the funding of the workshop project about rubberwood would be secured. This funding has now been officially confirmed. Due to the short time left between this confirmation and the end of the year 2003, the partners of the project expressed the wish that the workshop would be postponed to March 2004 ; however, Cirad thought that postponing the workshop could make the funding uncertain again ; consequently, we propose to maintain the initially planned period of November 12-14, 2003 for the organisation of the workshop in Bangkok by Cirad and Kasetsart University

The main idea was that the worksop would be oriented towards research ways for developing the volume of rubberwood and rubber biomass production to be offered to the market. However, Dr Prasat, Director of Rrit-Doa, expressed the wish that wood quality and preservation aspects could be taken into account too.

The workshop would be mediated as : « RWCR Bangkok 2003 » : RubberWood : Cropping and Research. Adaptation of rubber cropping and rubber research in South-East Asia. November 12-14.

B. The Genmap component

1. Presentation of Genmap

The Genmap component of the Doras-Rubber project is analysing a promising full-sib family, RRIM600 x PB217, issued from two parents with a high genitor value and which have contrasted and possibly complementary physiological characteristics. One part of the work is devoted to the building of a genetic linkage map of this family, by the main use of 276 microsatellites (molecular genetic markers belonging to the « targeted PCR » methodology). Field analysis of 196 progenies of this family will be carried out over a period of 8-10 years : it will make possible the exploration of the genetic determinism of the studied characteristics, by Qtl approach (number and localization of genome areas concerned by each characteristic, relative impact of additivity and dominance). But some physiological characteristics are too long and difficult for being measured with good accuracy over the whole family ; consequently, they will be studied only on a sample of 30 progenies + 2 parents, in order to assess their variability within this genetic framework of this full-sib family, to assess the possibility to find some specific levels for these characteristics (such as high level of sucrose ratio in the laticifer). Selection of elite clones from this family for development is planned.

Genetic mapping was carried out at Cirad-Biotrop lab in Montpellier by Ms Kanlaya Prapan, with a funding from « AGROPOLIS Plateforme de Recherches Avancées en Génomique », during a one-year period (December 2001-November 2002). The current status of this work is presented in the reports of Ms Kanlaya and of Dr Marc Seguin (cf Annex). The completion of the map could be carried out at Montpellier by Ms Napawan, provided that the lab costs for five months can be found (6300 euros) : this work includes the mapping of some more 137 microsatellite markers, and then of the saturation of the genetic map with AFLP markers.

First analyses of field measurement heva been made (cf Report of missions 2002, Clément-Demange A., Cirad cp n° 1568-03).

2. Main results after one year of measurements

From June 2002 (planting of the trial) to June 2003 (end of first year), the RRIT research station had a rainfall of 1357 mm with four months below 50 mm (from November to February). By comparison with evaporation, the level of which is rather stable at around 150 mm per month, we can deduce the occurrence of an important water deficit during 5 months (October to February). Insolation is of 2400 hours for the year (Appendix 20).

After one year, a trial planted in August 2002 (around 2.5 months after Genmap) appears to be late in height growth (Appendix 19).

The length of the different growth units was measured in November 2002 (5 months old) over all the trees. The second GU, and to a lesser extent the third GU, appear to be much smaller than the other ones, due to transplanting stress (planting of one-whorl plants).
In June 2003, there are 2992 living trees (within 3200 tree-sites). During the first year, replacements of died trees have been carried out for an estimated number of 438 trees. Measures of base diameter, at 10 cm high, and of height have been carried out in July 2002, September 2002, December 2002, and June 2003 (Appendix 15). Along time, base diameter and height exhibit fast increase as well as an increase in variability between genotypes. Over the first year, heritabilities for the two measurements repeated along time show a reducing trend, from about 0.40 to 0.11, which seems to reveal an increasing influence of the variability of microlocal environment on the growth of individual trees ; this heritability level appears to be low. At one year old, mean base diameter and height for the 198 genotypes are of 2.04 and 207 cm. The maximum base diameter is of 2.68 cm (genotype rritcode = 779), and the maximum height is of 298 cm (genotype rritcode = 1307). The vigour of the two parents, PB217 and RRIM600, appears to be lower than the mean of the family.

The correlation between base diameter and height (calculated at the level of the adjusted means of the genotypes) appears to increase along time : r = 0.67 at one month old, 0.78 at three months old, 0.84 at six months old, and 0.89 at one year old (ddl = 198).

The relative height (height / base diameter) is of course positively correlated with height (r = 0.67), but it is also positively corelated with base diameter (r = 0.37).

In an outer part of the Genmap field, a sample of 25 trees with the same genetic origin, same age and same dimensions than Genmap trees was extracted, at one year old, for measuring dimensions, leaf areas, and dry weights. A set of indications was so provided about biomass of such trees. Based on the measurement of base diameter and height at one year old (V9, V10), and after some transformations, a regression coefficient was established for predicting Dry Weight of the Trunk (Dwt) with determination coefficient $R^2 = 0.98$. For predicting the Number of Leaves (Nbl) , the best determination coefficient was $R^2 = 0.74$; for predicting the Total Leaf Area (Tla), the best determination coefficient was $R^2 = 0.52$ (Appendix 16).

By using the regression equation for predicting Dwt (dry weight of the trunk) on each Genmap tree, we found that Dwt varied from 69 to 501 grammes among the 198 genotypes (Appendix 17).

The topology description of the trees for genotypes 1 to 30 + 198 and 199 (452 trees) at seven months old (January 2003) shows that ramification is still at a very low frequency. Over a total of 2090 GUs observed, it was found only 61 relays, and 807 axillary ramifications A2 distributed over 241 GUs.

3. Measurements to be planned for the second year

- Measurement of base diameter (at 10 cm high) and of height in September 2003, December 2003, March 2004, and June 2004 (use of a graduated telescopic rod as soon as possible).
- Continuation of topology description (Amap-mod) for genotypes 1-30, + 198-199 (GUs, diameter and length of GUs, number of ramifications per GU and description of ramifications). Identification of living branches with numbered tags.

- Measurement of girth at 1 meter high in June 2004.
- Extraction of trees in the outer part of the field (during a mission of Clément-Demange).
- Vulnerability to embolism, for some clones (Krissada).
- Other possible physiological measurements.

Conclusion

This mission of June 2003 and the present related report were an opportunity to summary the evolution and the present state of Doras-Rubber project, just one year before renewal of the agreement between the different partners. Three components, i.e. Ecophysiology, Tapping, and Genmap, go on at a good pace, whereas Rubberqual is still in the launching phase. The introduction of a new action within Ecophysiology, in order to study soil-plant-climate interactions at the level of the crop stand, is being prepared in cooperation with Dr Georges Nizinski (Ird-Cirad). Exchange of clones, as well as joint study of these clones, might be explicitly recognised as a possible output of the project. The Tapping component led to specific contribution of the project to Irrdb meeting in Chiang Mai (September 15-17, 2003). First academic publications are now issued from the project (Napawan et al., 2003 ; Krissada et al., 2003). Three Ph.D. theses will be defended within the next months (Napawan, Krissada, Unakorn), whereas another one is currently being developed (Pisamai). With the Heveawood workshop being held at Kasetsart University next November 12-14, thanks to the « Regional fund for assistance to education and research » of the French Embassy at Bangkok, a regional action is so initiated by the project.

Genmap is combining genetic mapping and field measurements for analysing the genetic variability and genetic determinism of different physiological traits. Just one year after the setting of the field trial, growth measurements have been made regularly, so providing first elements about the variability of genetic and environment factors within the trial, and architectural description was initiated on the young trees. The genetic mapping of the Genmap family, developed by Ms Kanlaya Prapan with Cirad team at Montpellier, could be completed by Ms Napawan Lekawipat within a five month period in France, with funding of the French-Thai Committee Programme for airflight and accomodation, but the budget for lab costs is still lacking for launching this action.

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ANNEX 1

Towards a new component in the Doras-Ruber Project

Climatic variations, functioning and production of a rubber plot

Energy balance, carbon and water balance modelized at the scale of a monoclonal rubber plot in East-Thailand.

(Georges Nizinski, Ird/Cirad-Ecotrop)

The Doras-Rubber project, «Towards the improvement of the rubber tree productivity », is currently carried out in Thailand by Kasetsart University, the Rrit-Doa, Inra-Piaf and Cirad. This project is made of a predominant part devoted to the ecophysiology of the rubber tree. The project theoretically ends in June 2004, but it will be discussed and up-dated by partners for continuation over a new six-year period.

This short note is presenting a new component which could be logically added to the current research activities, by moving from the tree-scale to the plot-scale.

Scientific context and objectives

One of the rubber development policy trends in Thailand is to relocate part of the rubber cropping area from the South to the North-East. North-East is affected by a longer dry season with seasonal water stress. Water stress and water use by the rubber tree are currently being studied at the scale of the young tree (hydraulic architecture of the plant, hydraulic resistance of organs, stomatal conductance, vulnerability to embolism), with impact on growth. Those studies are carried out in conjunction with carbon balance analysis and with the specific effect of the tapping of the rubber tree.

The present proposal is to provide a complementary approach at plot-scale of energy and water availability, water demand by the climate and by the tree, in order to develop a rationale about the necessary adaptations of rubber cropping to limiting areas.

It is assumed that the main limiting factors of the production (biomass, wood, and latex) are energy and water availability. Modelizing the variations of production depending on climatic variations will allow to estimate the limiting aspect of energy and water availability. It will be a first step towards prediction of potential productions under varied limiting conditions. It will also allow us to evaluate and adapt technics for optimizing the global production.

Details about the projected research activities

By reference to what was made by Monteny (1987), who carried out a joint bioclimatologic and ecophysiological research, this proposal is focussed on the ecophysiological component which mainly addresses the estimation of vegetal production at plot-level.

The first phase of the research would be applied to one sole clone inserted within a homogeneous rubber stand, rather flat and of around 15-20 hectares. This clone may be the most commonly used clone in the world and in Thailand (RRIM600), or a latex-timber clone potentially more adapted to the future practise of rubber cropping (PB260), or a clone reputed to have a high-medium and long term latex production potential (PB217).

One main experimental site will be developed at the site of Rrit-Crrc (Chachoengsao), with possible secondary sites in Rrit-Buriram or Rrit-Nongkhai if necessary.

Measurements will be carried out during specific periods of the year (rainy season, defoliation, dry season, refoliation, ...) over two successive campaigns. They will include the methods of water balance and energy balance, Bowen ratio, and Penman-Monteith equation.

Human means

The Cirad-Ecotrop unit would be involved in this project component, with main commitment of Dr. Georges Nizinski (belonging to Ird, and currently working at Cirad-Ecotrop unit).

This research would be carried out in Thailand by a student or a young researcher in the framework of a Ph.D study, under supervision of Kasetsart University (Dr Poonpipope) and of Dr. Georges Nizinski acting as scientific adviser.

Required equipment and functioning costs

A preliminary mission of Dr Nizinski will allow him to present the proposal and to take stock of available equipment and additional required equipment.

A search for funding will be initiated in order to allow implementation.

Calendar

This component could be part of the up-dating of the project for the period 2004-2010, with a first three-year phase over 2004-2007 (one Ph.D time period).

Measurements would be conducted over two full years. A third year would be devoted to processing, analysis and interpretation of data, before defending the Ph.D degree at Kasetsart.

The launching of this proposal, if agreed, is dependant of the availability of equipment and of complementary funding for additional equipment and functionning.

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ANNEX 2

Genmap genetic mapping

Genmap component :
« Variability analysis and genetic determinism
of some physiological characteristics of the productivity in Thailand ».

Development of a genetic map of RRIM600 x PB217 based on microsatellite markers.

Update of the mapping work of Ms Kanlaya Prapan work in Montpellier (Agropolis funding)

- M. Seguin July 2003 -

This report gives updated results on microsatellite genome mapping carried out by Ms Kanlaya Prapan at Cirad-Montpellier (December 2001-December 2002). After the departure of Kanlaya, Cirad team finalized some steps of the mapping work performed in 2002 : 1) finalization of primer pairs screening and 2) complete data analysis for mapping. During Kanlaya's course, the map construction performed was only a rapid and preliminary analysis. In addition, the number of usable microsatellite sequence being insufficient for a satisfying genome coverage, 100 more DNA sequences were obtained, under an additional grant from Cirad.

Material and methods

Plant material

The segregating population RRIM600 x PB217, used for genetic mapping experiments, was created by RRIT (CRRC, Thailand) and encompasses 335 progenies. The legitimacy of all these individuals have been previously confirmed by paternity testing using 10 microsatellite markers. Nevertheless, a mislabeling problem led to the wrong identification of one of the 335 progenies. This appeared during progress of additional microsatellite genotyping. The rogue individual (id = 216 = AA115-5) was discarded for data scoring. The genetic map was thus established on 334 progenies (table 1), with few missing data.

Sources of microsatellite markers

The microsatellite markers and sequences were obtained by Cirad from 3 different sources (Seguin et al. 2001):

1- Markers from the previously Cirad published map (Lespinasse et al. 2000) ; these markers were issued from a PstI genomic library (Besse et al. 1994; Seguin et al. 1997)
2- Sequences obtained by Cirad under a grant from Genoscope/CNS (Evry) in 1999
3- Sequences obtained by Cirad under a second grant from Genoscope/CNS (Evry) in 2000

The genomic DNA clones sequenced at Genoscope/CNS were selected from 3 microsatellite enriched libraries created by Cirad in Montpellier (Gay 1998; Seguin et al. 2001; Xiong 2000).

Genotyping using radio-labeled PCR products

This protocol was applied for genotyping the progenies with the microsatellite primers designed from sequences issued from the Génoscope/CNS 1999 project. It was also applied for the microsatellite markers previously mapped on the Cirad reference map.

Radio-labeled PCR products were obtained by labeling forward primers with [γ^{33}P]ATP using a T4 polynucleotide kinase.

PCR reactions were carried out in a 25 µl volume containing 100 ng of genomic DNA, 0.2 µM of each primer, 200 µM of each dNTP, and 1.5 U of Taq DNA polymerase in 1X reaction buffer (20µM Tris HCl, 1.5 mM $MgCl_2$).

PCR amplifications were performed in PTC 100™ (MJ Research, Inc.) using the following cycles: 5 min at 95 °C, then followed by 35 cycles of 1 min at 94 °C, 45 sec at 48, 50 or 55 °C, 1 min at 68, 70 or 72 °C, with the final step of 5 min at 68, 70 or 72 °C. Samples were prepared for polyacrylamide gel electrophoresis by adding 20 µl of formamide dye (containing 10 mM NaOH; 0.05% Bromophenol, 0.05 Xylenecyanol, 12.5% (P/V) Saccharose and 95% formamide). The amplification products were detected on 5% denaturing polyacrylamide gels (33 cm width of nucleic acid sequencer Model DDH-402-33) in 0.5XTBE buffer at 55 W constant power. The gels were dried and then exposed to X-ray film for 3-4 days.

Genotyping using fluorescent labelling and electrophoresis in DNA sequencer

IR-fluorescent PCR reactions were performed using the following strategy. One of the PCR primers had a 19 base extension at its 5' end with the sequence 5'-CACGACGTTGTAAAACGAC-3'. This sequence is identical to an IR-labelled universal M13 Forward (29-mer) primer, which is included in the reaction. During the PCR, the tailed primer generates a complementary sequence to the M13 primer which is subsequently utilised for priming in the amplification reaction thereby generating IR-labelled PCR products. All PCR were produced in 10 µl containing 20 ng of DNA, 1 µl 10X PCR buffer (200 mM Tris-HCL (pH 8.4), 500 mM KCl), 200 mM of each dATP, dCTP, dGTP, dTTP, 2 mM of $MgCl_2$, 0.05 mM of each the M13-tailed primer, 0.1 µM of the other primer, 0.1 µM of the IR-labelled (either with IR700 or IR800) M13 primer and 1U of Taq DNA Polymerase (Eurobio). Primers were synthesised by Eurogentec (France) and the IR-labelled M13 primer by Biolegio (The Netherlands). Cycling conditions consisted of an initial denaturing step of 4 min at 94°C, followed by 35 cycles consisting of 45s at 94°C, 45s at 50°C, and 45s at 72°C, and a final elongation step at 72°C for 10 min. All PCR reactions were performed on a PTC 200 MJResearch thermocycler. Gel electrophoresis and visualisation of the microsatellite alleles were accomplished using a LI-COR IR2 Model 4200 automated DNA sequencer (LI-COR, Inc., Lincoln, NE). Gels were 18 cm in length, 0.25 mm in thickness and composed of 6.5% KB+ acrylamide, 7M urea (LI-COR). Runs were performed in 1X TBE buffer, at 48°C and 40 W constant. A standard size ladder obtained from amplification of known band sizes was loaded regularly. The raw data depicting the microsatellite alleles is displayed as an auto-radiogram like image on the computer and analyzed as it.

Table 1: List of the 335 RRIM600 x PB217 legitimate progenies used for microsatellite genotyping. Individual 216+AA115/5 appeared to be illegitimate during mapping experiments and was discarded for linkage analyses.

1	AA68/2	51	AA78/6	101	AA90/4	151	AA103/2	201	AA112/5	251	AA122/4	301	AA133/6
2	AA68/4	52	AA78/8	102	AA90/5	152	AA103/3	202	AA112/6	252	AA122/5	302	AA133/7
3	AA68/5	53	AA78/9	103	AA90/6	153	AA103/4	203	AA112/8	253	AA122/7	303	AA133/9
4	AA68/7	54	AA78/10	104	AA90/7	154	AA103/5	204	AA112/9	254	AA122/8	304	AA134/4
5	AA68/9	55	AA79/3	105	AA91/2	155	AA103/7	205	AA113/2	255	AA122/9	305	AA134/5
6	AA68/10	56	AA79/5	106	AA91/4	156	AA104/4	206	AA113/3	256	AA123/3	306	AA134/6
7	AA69/3	57	AA79/6	107	AA91/7	157	AA104/6	207	AA113/5	257	AA123/4	307	AA134/7
8	AA69/4	58	AA79/7	108	AA91/9	158	AA104/7	208	AA113/8	258	AA123/6	308	AA134/8
9	AA69/6	59	AA79/8	109	AA92/4	159	AA104/9	209	AA113/10	259	AA123/8	309	AA134/9
10	AA69/8	60	AA79/9	110	AA92/5	160	AA104/10	210	AA114/4	260	AA123/9	310	AA134/10
11	AA69/9	61	AA80/4	111	AA92/7	161	AA105/2	211	AA114/7	261	AA123/10	311	AA135/2
12	AA70/3	62	AA80/5	112	AA92/10	162	AA105/3	212	AA114/8	262	AA124/3	312	AA135/3
13	AA70/4	63	AA80/6	113	AA93/2	163	AA105/4	213	AA114/10	263	AA124/5	313	AA135/4
14	AA70/5	64	AA80/7	114	AA93/4	164	AA105/6	214	AA115/3	264	AA124/8	314	AA135/7
15	AA70/7	65	AA80/8	115	AA93/8	165	AA105/8	215	AA115/4	265	AA124/9	315	AA135/8
16	AA70/9	66	AA80/10	116	AA93/10	166	AA105/9	216	AA115/5	266	AA124/10	316	AA136/3
17	AA71/2	67	AA81/3	117	AA94/2	167	AA105/10	217	AA115/10	267	AA125/2	317	AA136/5
18	AA71/3	68	AA81/5	118	AA94/5	168	AA106/2	218	AA116/3	268	AA125/5	318	AA136/6
19	AA71/4	69	AA81/6	119	AA94/6	169	AA106/3	219	AA116/5	269	AA125/6	319	AA136/7
20	AA71/5	70	AA81/7	120	AA94/7	170	AA106/4	220	AA116/6	270	AA125/8	320	AA136/9
21	AA71/7	71	AA81/8	121	AA94/9	171	AA106/5	221	AA116/7	271	AA125/9	321	AA136/10
22	AA71/8	72	AA81/9	122	AA95/8	172	AA106/8	222	AA116/8	272	AA126/9	322	AA137/2
23	AA71/9	73	AA82/6	123	AA95/9	173	AA106/10	223	AA116/9	273	AA126/10	323	AA137/3
24	AA72/3	74	AA82/7	124	AA95/10	174	AA107/6	224	AA117/2	274	AA127/4	324	AA137/4
25	AA72/4	75	AA82/8	125	AA96/3	175	AA107/7	225	AA117/3	275	AA127/5	325	AA137/6
26	AA72/6	76	AA82/10	126	AA97/4	176	AA107/8	226	AA117/4	276	AA127/6	326	AA137/7
27	AA72/9	77	AA83/2	127	AA97/5	177	AA107/9	227	AA117/5	277	AA127/10	327	AA137/9
28	AA72/10	78	AA83/4	128	AA97/6	178	AA108/4	228	AA117/6	278	AA128/3	328	AA137/10
29	AA73/2	79	AA83/7	129	AA98/4	179	AA108/7	229	AA117/7	279	AA128/6	329	AA138/5
30	AA73/4	80	AA83/9	130	AA98/7	180	AA108/10	230	AA117/8	280	AA128/7	330	AA138/7
31	AA73/7	81	AA83/10	131	AA98/8	181	AA109/3	231	AA117/9	281	AA128/9	331	AA139/8
32	AA73/8	82	AA84/3	132	AA98/9	182	AA109/5	232	AA117/10	282	AA129/5	332	AA140/5
33	AA74/5	83	AA84/4	133	AA98/10	183	AA109/6	233	AA118/2	283	AA129/8	333	AA140/10
34	AA74/7	84	AA84/6	134	AA99/2	184	AA109/9	234	AA118/4	284	AA129/9	334	AA141/9
35	AA75/3	85	AA84/9	135	AA99/5	185	AA109/10	235	AA118/5	285	AA130/2	335	AA141/10
36	AA75/4	86	AA85/2	136	AA99/7	186	AA110/3	236	AA118/8	286	AA130/4		
37	AA75/7	87	AA85/3	137	AA99/10	187	AA110/4	237	AA118/10	287	AA130/5		
38	AA75/8	88	AA85/4	138	AA100/5	188	AA110/5	238	AA119/3	288	AA130/6		
39	AA75/9	89	AA85/5	139	AA100/6	189	AA110/6	239	AA119/4	289	AA130/10		
40	AA76/2	90	AA85/6	140	AA100/7	190	AA110/7	240	AA119/5	290	AA131/2		
41	AA76/4	91	AA86/4	141	AA100/10	191	AA110/8	241	AA119/7	291	AA131/5		
42	AA76/5	92	AA86/8	142	AA101/2	192	AA110/9	242	AA119/8	292	AA131/6		
43	AA76/6	93	AA86/9	143	AA101/5	193	AA110/10	243	AA120/2	293	AA131/7		
44	AA76/7	94	AA86/10	144	AA101/6	194	AA111/3	244	AA120/3	294	AA131/8		
45	AA76/8	95	AA87/4	145	AA102/2	195	AA111/4	245	AA120/5	295	AA131/10		
46	AA76/10	96	AA87/10	146	AA102/4	196	AA111/7	246	AA120/9	296	AA132/4		
47	AA77/7	97	AA88/3	147	AA102/5	197	AA111/8	247	AA120/10	297	AA132/6		
48	AA77/9	98	AA88/4	148	AA102/7	198	AA111/9	248	AA121/5	298	AA132/8		
49	AA77/10	99	AA88/5	149	AA102/9	199	AA112/2	249	AA121/9	299	AA132/10		
50	AA78/4	100	AA88/10	150	AA102/10	200	AA112/3	250	AA122/3	300	AA133/3		

Screening of polymorphic markers

Before genotyping the complete progeny, all primer pairs were screened for PCR amplification efficiency and for genetic polymorphism, and were tested on a small sample of accessions : the 2 parents (RRIM600 and PB217) and the 3 available grand-parents (PB49, PB86 and TJ1).

Data analysis

For some of the markers genotyped on the LiCOR sequencer, segregation data were scored using automated detection from fluorescent pattern using SAGA software. But, for most of the markers, including all the ones genotyped by radio-labeling methods, data scoring was performed "manually".

Data were scored as 0/1 digit for presence/absence of the segregating bands in each progeny, and transformed in data file format for JoinMap V3 mapping software using the CP option. CP option allows linkage analysis for loci heterozygous either in only one parent (2 alleles abxaa or aaxab configurations) or in the 2 parents (4 alleles abxcd, 3 alleles abxac or 2 alleles - codominant or dominant - abxab configurations). In addition to the consensus map built from all segregating markers, the 2 parental maps were also built using segregation data for markers heterozygous in each parent respectively (pseudo-test cross analyses).

The comparison of marker orders in the two parental maps allows to control that each microsatellite markers reveals identical locus, with no great distortion, in the 2 parental genotypes. Markers revealing same locus in the 2 parents are then used as bridge locus between the 2 maps for consensus CP map establishment. In case of disagreement in map location between the 2 parents, the segregating data from one of the 2 parents were discarded, based on quality parameters (number of missing data, segregation distortion from the expected ratio : 1:1, 3:1 or 1:2:1, or in case of dominance in only one parent).

Linkage tests were performed at a LOD threshold of 3.0. Loci orders were established at LOD = 3.0 with thêta = 48% of recombination and min-LOD threshold = 0.1. Two additional criteria were used to identify linked markers with uncertain map location : Chi2 contribution and goodness of fit which estimate the quality of map position of individual markers.

Results

The mapping results are summarized in table 2 and 3, for the 3 maps established from segregating data in the RRIM600 x PB217 progeny. The detailed lists of mapped markers are given in table 4, 5 and 6. Map drawings are given in figure 1, 2 and 3.

The current consensus (CP) map encompasses 139 microsatellite markers clustered in 15 linkage groups. Eleven of these groups were assigned to reference linkage group (Lespinasse *et al.* 2000) thanks to loci in common with the published map. The consensus map length (table 3) represents about 50 % of the total *Hevea* genome length, and of expected number of linkage group is 18 in this species. Nevertheless, only half of the polymorphic markers were genotyped on the complete progeny and mapped (table 2). This suggests that a nearly saturated map could be reached, by achieving genetic mapping of all the 276 polymorphic markers in this RRIM600 x PB217 progeny.

Table 2: Summarized results of microsatellite mapping on the RRIM600 x PB217 progeny. Number of markers are given according to the genomic library source.

Microsatellite source	Reference map	CNS 1999	CNS 2000	Total
Screened for polymorphism	36	138	261	435
Polymorphic between parents	27	76	173	**276**
Mapped	17	74	48	**139**
Remaining to be mapped	10	2	125	**137**

Table 3: Synthetic results of microsatellite genetic mapping in the RRIM600 x PB217 progeny, for the two parental maps and for the CP consensus map. The CP map was built by merging the parental maps using CP option of JoinMap3 software. Map length is equal to the sum of linkage groups.

	RRIM600	PB217	RRIM600xPB217 (CP)
Number of segregating markers	107	103	139
Number of unlinked markers	3	7	5
Number of linkage groups	14	13	15
Number of assigned linkage groups	10	9	11
Map length	1220	1190	1380

Perspective

This work must be completed and achieved by :

1- mapping the remaining 137 microsatellite markers
2- complete map saturation using AFLP technique
3- mapping more bridge markers on the Cirad PB260 x RO 38 reference map

Due to limited funding and restricted access to leaf samples from RRIT-Thailand, items 1 and 2 would have to be carried out by a supplementary course of a RRIT researcher in Cirad-Biotrop-Montpellier. Item n°3 will be partly realized by Cirad team (M. Seguin and M. Rodier-Goud) in the frame of other genome mapping and linkage disequilibrium projects.

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Table 4: List of the bridge markers, i.e. mapped on the RRIM600 x PB217 progeny and present in the published map, allowing assignment to the reference linkage group (from Lespinasse et al. 2000)

no.	Primer	Product size (bp)	Forward	Reverse	Annealing temp/ extension temp
1	M574	238	69-2	70-2	50/70
2	MT65	196	175	176	50/70
3	MnSOD	200	5	6	55/72
4	MA66	275	195	196	55/72
5	M124	166	25-2	26-2	50/70
6	MA31	154	193	194	50/70
7	MT67	120	185	186	50/70
8	M256	258	123	124	55/72
9	M338	272	9	10	55/72
10	M412	258	51-2	52-2	50/70
11	Hmg1	272	1	2	55/72
12	M72	192	19	20	55/72
13	M127	292	29	30	55/72
14	M214	242	119	120	55/72
15	M264	228	125	126	50/70
16	M291	196	41-2	42-2	48/68
17	M340	304	21-2	22-2	50/70
18	M415-460	185	217	218	50/70
19	M508	262	73	74	48/68

Table 5: List and characteristics of the 74 microsatellite markers mapped in the RRIM600 x PB217 progeny, using [γ33 P]-ATP labeling protocol. The corresponding DNA sequences were obtained from the CNS 1999 / Cirad project.

no.	Marker name	Product size (bp)	Forward primer AGHE#	Reverse primer AGHE#	Annealing temp/ extension temp
1	t1067	260	377	378	52/72
2	t150	141	239	240	50/70
3	t152	163	257	258	50/70
4	t319	188	269	270	50/70
5	t595	251	291	292	50/70
6	t601	206	293	294	52/72
7	t615	230	295	296	50/70
8	t686	227	297	298	52/72
9	t728	228	301	302	50/70
10	t730	203	303	304	50/70
11	a036	264	243	244	50/70
12	a053	238	245	246	50/70
13	a058	174	247	248	52/72
14	a073	155	273	274	52/72
15	a078	183	251	252	52/72
16	a087	170	255	256	55/72
17	a090	228	275	276	52/72
18	a105	176	281	282	50/70
19	a107	147	283	284	55/72
20	a120	131	307	308	52/72
21	a123	309	310	195	55/72
22	a129	277	313	314	52/72
23	a131	185	315	316	52/72
24	a134	173	317	318	50/70
25	a135	237	319	320	52/72
26	a137	190	321	322	50/70
27	a144	152	327	328	55/72
28	a148	274	329	330	50/70
29	a156	244	333	334	52/72
30	a169	269	339	340	52/72
31	a170	259	341	342	52/72
32	a173	163	343	344	52/72
33	a174	126	345	346	52/72
34	a179	178	349	350	50/70
35	a188	222	353	354	52/72
36	a214	156	363	364	50/70
37	a215	289	365	366	50/70
38	a220	177	369	370	52/72
39	a235	280	387	388	52/72
40	a241	162	395	396	52/72
41	a245	111	397	398	50/70
42	a248	265	399	400	52/72
43	a251	205	401	402	52/72
44	a262	253	405	406	52/72
45	a268	242	411	412	52/72
46	a274	236	415	416	50/70
47	a275	178	417	418	52/72
48	a278	200	419	420	50/70
49	a282	101	421	422	52/72
50	a283	174	423	424	52/72
51	a286	178	425	426	52/72
52	a288	231	427	428	50/70
53	a289	173	429	430	52/72
54	a295	280	433	434	50/70

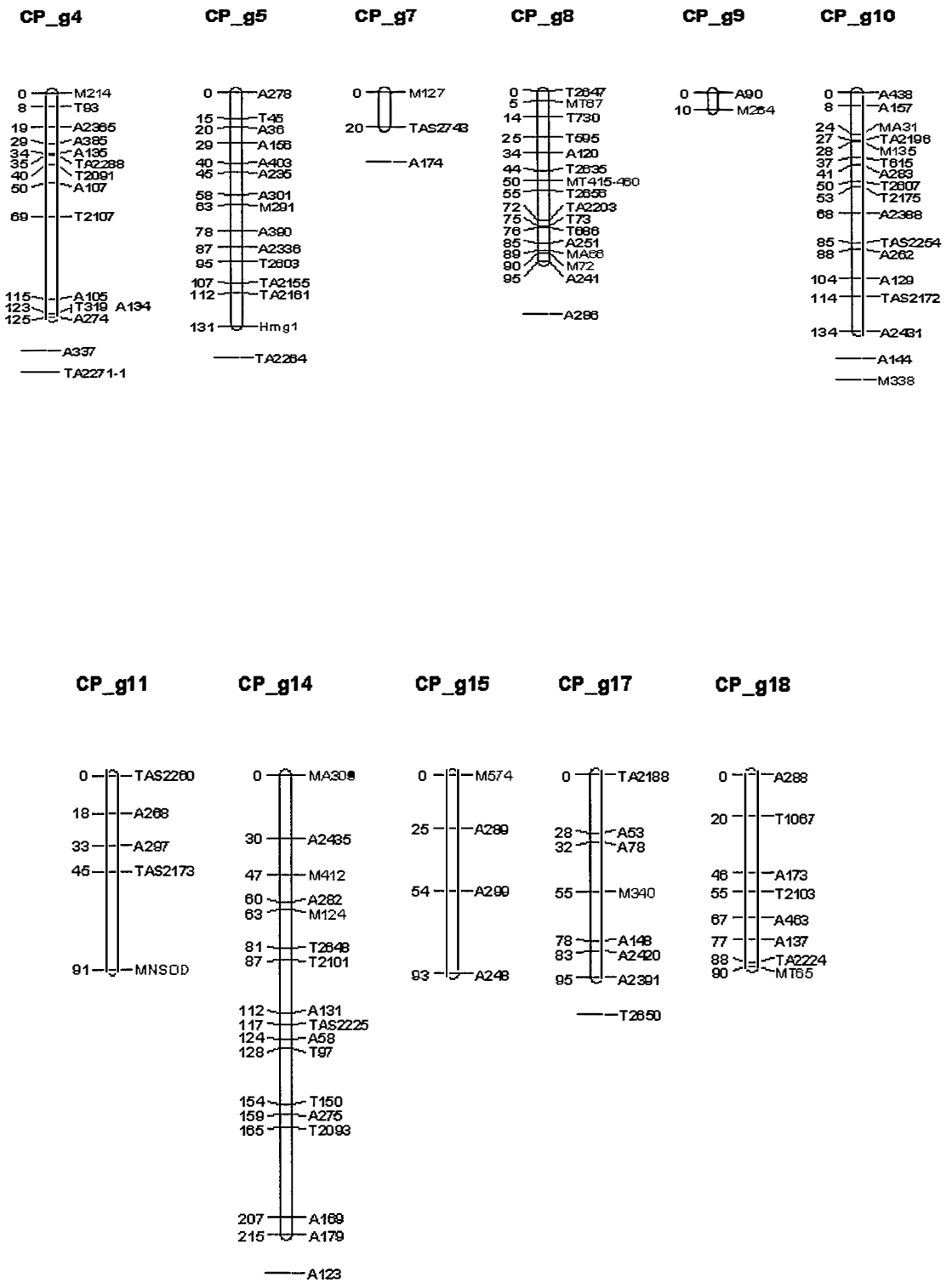
Table 5 (continued)

no.	Primer	Product size (bp)	Forward	Reverse	Annealing temp/ extension temp
55	a296	147	435	436	50/70
56	a297	209	437	438	52/72
57	a299	132	439	440	50/70
58	a301	282	441	442	50/70
59	a304	226	445	446	52/72
60	a337	123	449	450	50/70
61	a344	215	451	452	52/72
62	a365	219	461	462	52/72
63	a385	115	467	468	50/70
64	a390	230	469	470	55/74
65	a394	211	471	472	52/72
66	a403	161	475	476	52/72
67	a438	250	481	482	52/72
68	a451	275	483	484	52/72
69	a460	116	487	488	52/72
70	a463	196	489	490	52/72
71	t045	238	225	226	52/72
72	t073	143	231	232	50/70
73	t093	136	233	234	50/70
74	t097	158	235	236	50/70

Table 6: List and characteristic of the 48 microsatellite markers mapped in the RRIM600 x PB217 progeny, using fluorescent labeling in DNA sequencer protocol. The corresponding DNA sequences were obtained from the CNS 2000 / Cirad project.

No.	microsatellite	primer 1	primer 2	product size	No.	microsatellite	primer 1	primer 2	product size
1	A2298	Hb685	Hb686	264+19	28	T2635	Hb793	Hb794	315+19
2	A2336	Hb691	Hb692	90+19	29	T2647	Hb809	Hb810	221+19
3	A2348	Hb693	Hb694	322+19	30	T2648	Hb811	Hb812	295+19
4	A2388	Hb703	Hb704	127+19	31	T2650	Hb815	Hb816	246+19
5	A2391	Hb707	Hb708	267+19	32	T2656	Hb817	Hb818	148+19
6	TAs2743	Hb925	Hb926	113+19	33	TA2155	Hb857	Hb858	283+19
7	TAs2748	Hb929	Hb930	196+19	34	TA2158	Hb579	Hb580	298+19
8	A2405	Hb949	Hb950	173+19	35	TA2161	Hb581	Hb582	122+19
9	A2415	Hb963	Hb964	264+19	36	TA2172	Hb587	Hb588	112+19
10	A2420	Hb969	Hb970	206+19	37	TA2175	Hb589	Hb590	201+19
11	A2431	Hb987	Hb988	257+19	38	TA2188	Hb595	Hb596	145+19
12	A2433	Hb721	Hb722	102+19	39	TA2196	Hb607	Hb608	116+19
13	A2435	Hb991	Hb992	243+19	40	TA2203	Hb613	Hb614	216+19
14	TAs2216	Hb873	Hb874	162+19	41	TA2224	Hb629	Hb630	200+19
15	TAs2260	Hb887	Hb888	182+19	42	TA2225	Hb631	Hb632	181+19
16	TAs2464	Hb841	Hb842	213+19	43	TA2252	Hb653	Hb654	233+19
17	TAs2698	Hb915	Hb916	315+19	44	TAs2254	Hb885	Hb886	183+19
18	T2085	Hb849	Hb850	184+19	45	TA2264	Hb661	Hb662	121+19
19	T2091	Hb853	Hb854	277+19	46	TA2271	Hb665	Hb666	216+19
20	T2093	Hb547	Hb548	259+19	47	TA2288	Hb681	Hb682	225+19
21	T2094	Hb549	Hb550	313+19	48	TAs2173	Hb859	Hb860	186+19
22	T2101	Hb555	Hb556	138+19					
23	T2103	Hb557	Hb558	108+19					
24	T2107	Hb855	Hb856	95+19					
25	T2449	Hb729	Hb730	106+19					
26	T2603	Hb753	Hb754	293+19					
27	T2607	Hb757	Hb758	70+19					

Figure 1: Consensus map built on RRIM600 x PB217 progeny using CP option in JoinMap3. Bridge markers with the Cirad reference map, i.e. those mapped in the PB260 x RO38 published map are in red colour. Markers drawn outside the corresponding linkage group are linked markers with imprecise map location.



(Figure 1 continued)

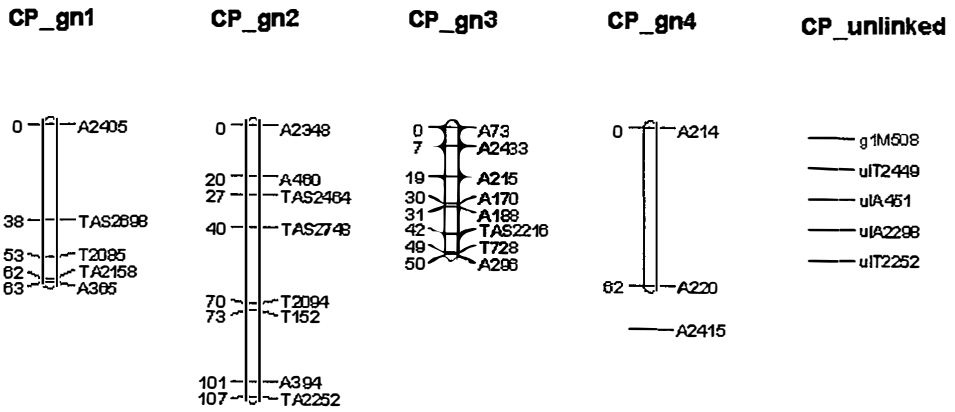


Figure 2: Parental map built on RRIM600 (female parent) using only markers heterozygous in RRIM600. Bridge markers with the PB260 x RO38 published map are in red colour. Markers drawn outside the corresponding linkage group are linked markers with imprecise map location.

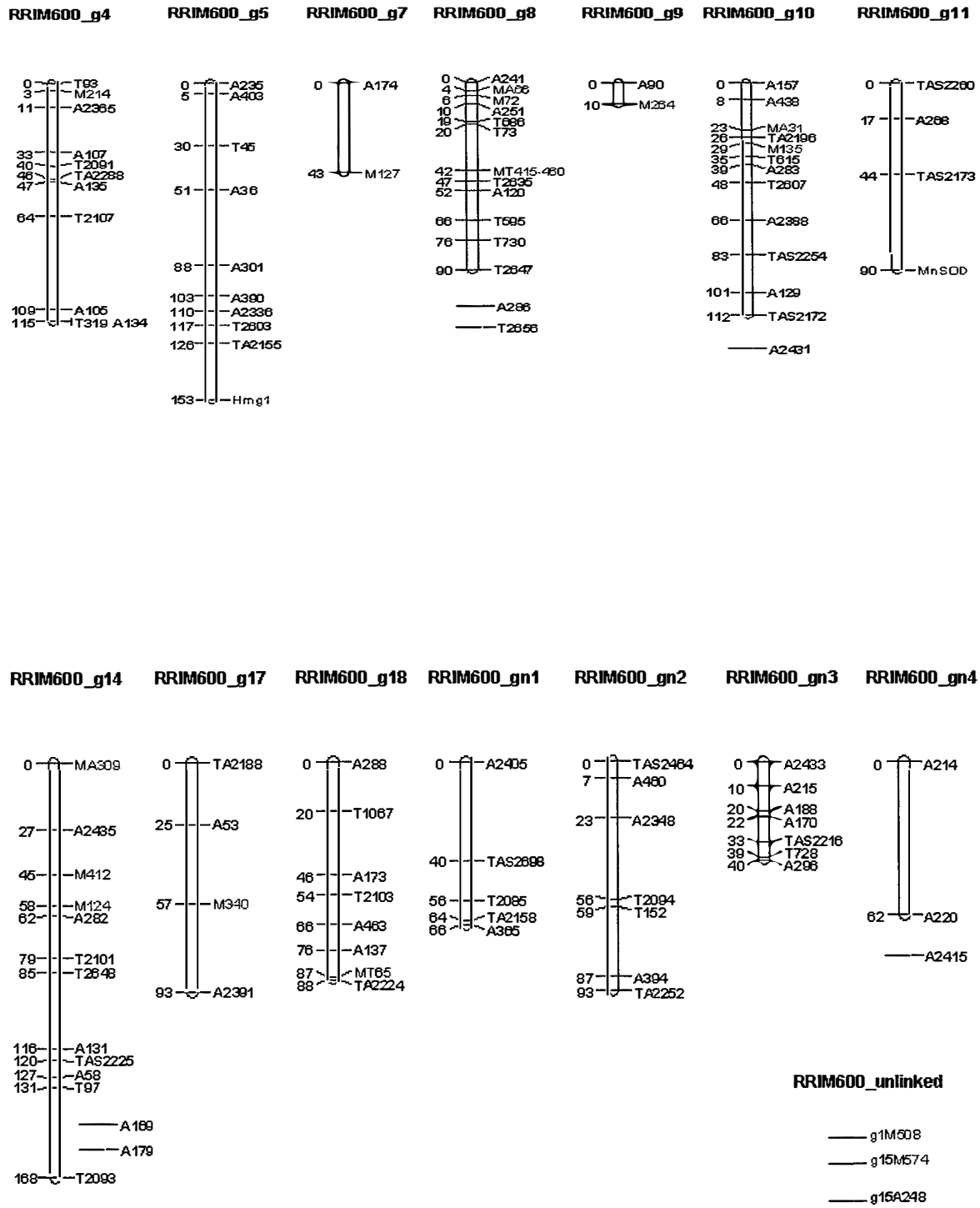
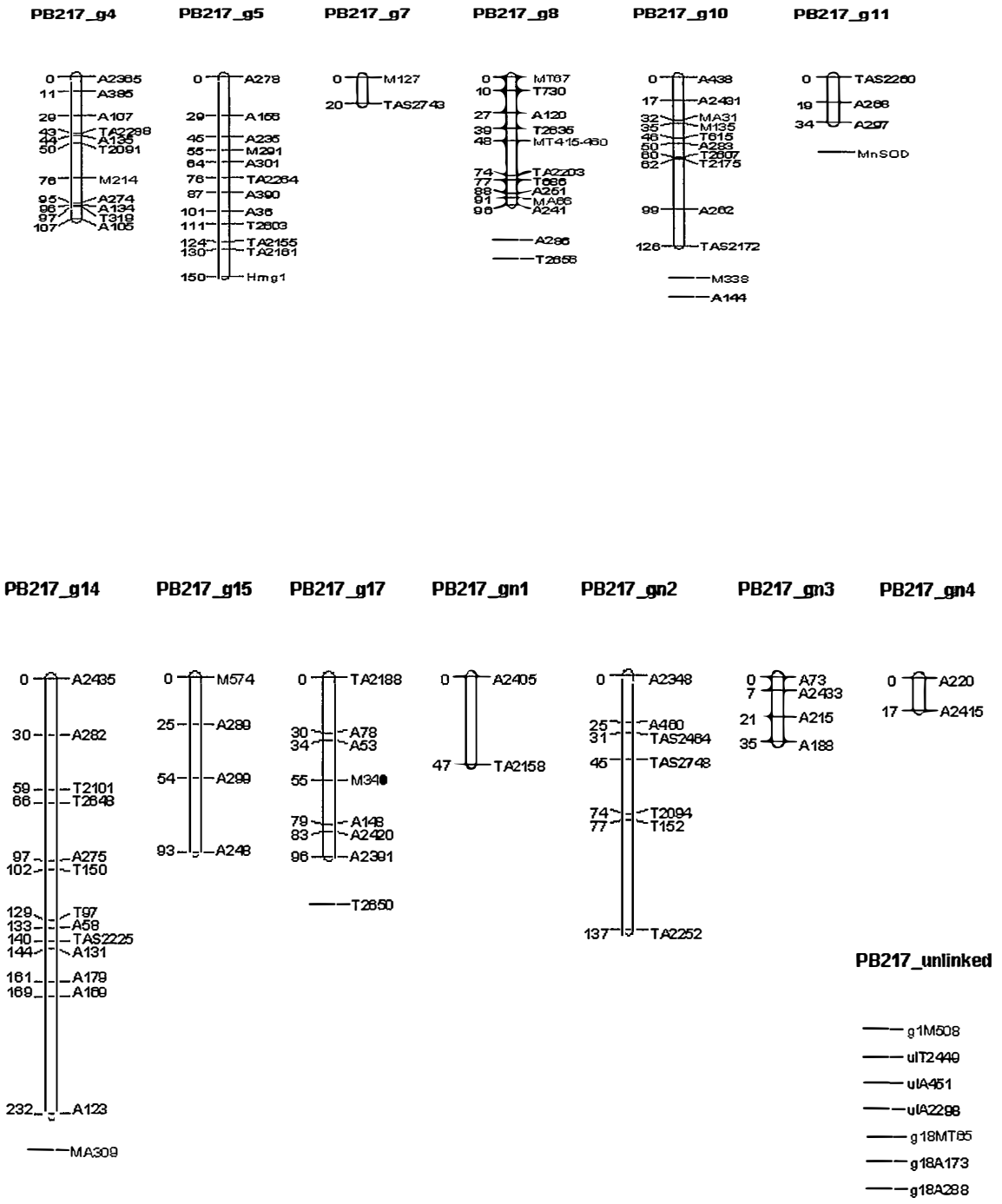


Figure 3: Parental map built on PB217 (male parent) using only markers heterozygous in PB217. Bridge markers with the Bridge markers with the PB260 x RO38 published map are in red colour. Markers drawn outside the corresponding linkage group are linked markers with imprecise map location.



Annex 3

Genmap Appendix

Appendixes 1 to 13 were included in Cirad report CP 1568-03. Successive Genmap Appendixes are aimed at cumulating observations and results from data analyses along time on Genmap field trial, just as a laboratory workbook. They can be written by any involved researcher, and they can be used as a common base for discussion about this trial. A common set of data files is associated to those appendixes.

The contents of this annex is described below :

14. (Ref Appendix 11), July 2003. Erratum and correction of analyses of V2, V3, V4, and V5 (height and diameter in July and September 2002).
15. State of the field trial at one year old, census of growth measurements in June 2003, and analysis.
16. Extraction of 25 trees taken in an outer part of the trial for indirect estimation of variables in Genmap.
17. Classification of the genotypes for vigour at one year old.
18. Description of architecture (topology) of 32 genotypes in January 2003 (7 months old).
19. Difference in growth between Genmap and a neighbouring plot.
20. Climate of the first year.

Genmap Appendix 14 (ref : Appendix 11)

Erratum and correction of analyses of V2, V3, V4, and V5 (height and diameters in July and September 2002).

Data analyses carried out with Sas Software.
Variance analysis on 800 plot-data issued from a set of 3150 tree-data :

	Genotype	Rep	Block(rep)	R^2	CV%	**Mean**	Min	Max	Std
V2	***	***	***	0.67	8.4	**0.51**	0.40	0.61	0.023
V3	***	***	**	0.67	11.6	**32.7**	21.44	41.96	2.06
V4	***	***	**	0.55	14.5	**0.74**	0.53	0.94	0.059
V5	***	***	*	0.56	18.7	**48.8**	32.81	64.31	5.14

Min and Max are the minimum and maximum adjusted means of the genotypes. Std is the average std of the adjusted mean of the genotypes.

Components of the variance and heritabilities
Large sense heritability = Var Progenies / (Var Progenies + Var Error)

	V2	V3	V4	V5
Progenies	0.00083	10.88	0.0033	26.51
Replications	0.00050	1.88	0.0004	6.92
Blocks/rep	0.00041	0.92	0.0011	4.88
Error	0.00180	14.47	0.0115	89.37
Total variance	0.00354	28.15	0.0163	127.68
Heritability	**0.32**	**0.43**	**0.22**	**0.23**

Impact of the design on R^2 of variance analyses :

	V2	V3	V4	V5
With reps and blocks	0.67	0.67	0.55	0.56
With reps	0.52	0.58	0.43	0.44
With no control	0.41	0.53	0.41	0.40

Impact of the design on heritabilities :

	V2	V3	V4	V5
With reps and blocks	0.32	0.43	0.22	0.23
With reps	0.28	0.41	0.22	0.22
With no control	0.21	0.38	0.21	0.20

In fact, the assessment of the impact of the design is minimized because randomization was carried out by distributing the plots among the replications and the blocks within each replication. A really randomized design would very probably have increased the error variance.

Genmap Appendix 15

State of the field trial at one year old, census of growth measurements in June 2003 and analysis.

Evolution of the trees along the first year (death and replacements) :

In June 2003 (one year old), the average base diameter V9 and height V10 are 2.04 and 207 cm respectively, for 2991 living trees including replacements at different dates.

In July 2002, after replacement of trees died just after planting, there were 3190 living trees on site. Among these trees, 3147 were living in September 2002, and 2988 trees increased their height between July and September 2002 ; from these 2988 trees, 2718 trees were living in December 2002, and 2680 trees increased their height between September and December 2002 ; from these 2680 trees, 2642 were living in June 2003, and 2553 trees increased their height between December 2002 and June 2003. This sample (Sjun03) of 2553 trees can be considered as homogeneous for the date of planting, although there is a wide variability for base diameter (from 0.43 to 4.30 cm, with average = 2.23 cm)) and for height (from 30 to 420 cm, with average = 229 cm).

Average base diameter and height for this sample (2.23 and 229 cm respectively) are a little higher than for the whole population of trees living in June 2003. The trees of this sample Sjun03 are identified as **V12** = 1 (other trees : V12 = 0) in Genmap3.xls. Sjun03 contains 80 % of the 3200 tree-sites of Genmap, and 85 % of the 2991 trees living in June 2003. Considering the 438 other trees living in June 2003 (2991 – 2553), not included in Sjun03, which are issued from later replacements or which met strong growth difficulties, average base diameter and height are 0.92 and 78 cm respectively. It was checked that heritabilities of V9, V10, V11 is not improved when analysis is applied to sample Sjun03 only (around h = 0.10). It must be concluded that low heritabilities at one year old are due to a strong variability of microlocal environment on the different trees.

Data (tree by tree) :

V1 Scoring of each tree on June 26, 2002 (before replacements).
V2 Diameter (cm) at 10 cm height on July 22, 2002
V3 Total height (cm) on July 22, 2002
V4 Diameter (cm) at 10 cm height on September 19 2002
V5 Total height (cm) on September 19 2002
V6a Length of GU1 (growth unit 1) in November 2002
V6b Length of GU2 (growth unit 2) in November 2002
V6c Length of GU3 (growth unit 3) in November 2002
V6d Length of GU4 (growth unit 4) in November 2002
V6e Length of GU5 (growth unit 5) in November 2002
V6f Length of GU6 (growth unit 6) in November 2002
V6T V6a+V6b+V6c+V6d+V6e+V6f = Total height in November 2002
V6Nb Number of growth units in November 2002

V6Lm = V6T/V6Nb, Average length of the growth unit of one tree, in November 2002
V6T1 = V6c+V6d+V6e (trees with at least 3 GUs)
V6Nb1= V6Nb –2
V6Lm1= V6T1/V6Nb1
V7 Diameter (cm) at 10 cm height on December 22, 2002
V8 Total height (cm) on December 22, 2002

June 2003 : End of replacements

V9 Diameter (cm) at 10 cm height on June 3, 2003 (2992 living trees)
V10 Total height (cm) on June 3, 2003 (2992 living trees)
V11 Estimated Dry Weight of the Trunk
V12 Identification of trees belonging to Sjun03 (V12=1)
V13 = V10/V9 (height/base diameter, relative height)

Adjusted means :

V1m	Means from V1
A2	From V2
A3	From V3
A4	From V4
A5	From V5
A6Nb	From V6Nb and from 3018 trees
A6Lm	From V6Lm and from 3018 trees
A6T	From V6T and from 3018 trees
A6Nba	From V6Nb and from 2656 trees
A6Lma	From V6Nb and from 2656 trees
A6Ta	From V6Nb and from 2656 trees
A6Nb1	From V6Nb1 (from 2656 trees)
A6Lm1	From V6Lm1 (from 2656 trees)
A6T1	From V6T1 (from 2656 trees)

A7, A8, A9, A10, A11, A13.

January 2003	Architectural description of 32 genotypes within the trial
June 2003	Extraction of 25 trees of the same family out of the trial and measurements

Physical state of the trial

In June 2003, there are 2991 one-year old living trees, with a base diameter ranging from 0.30 to 4.40 cm (average : 2.04 cm), and with a height ranging from 20 to 420 cm (average : 207 cm). Branching is being artificially pruned up to a height of 2.3 meters high ; consequently, the aspect of the trees is predominantly made of « One-axis A1 » trees. Due to glyphosate weeding, some trees have lost their rythmic growth and display « fox tail » aspect, some other trees display distorted leaves. In June 2003, pig manure has been brought to each tree. Inter-row is maintained mechanically with hoes and harrows driven by a tractor. Extraction of 25 trees (cf Appendix 16) and observation of taproots showed a negative effect of soil induration and the development of roots, which may be responsible for inter-tree variability and for low heritabilities.

Table 1 shows the state of the trial in November 2002 (at 5 months old), regarding the number of growth units per tree, the number of trees for each level of growth unit number, the corresponding diameters and heights. The average number of GUs is 3.67 (with GU1 grown in nursery, and GU2 issued from transplanting stress).

	November 2002				December 2002	
Nb GU	Nb trees	%	Cum %	Height (V6T)	Diam (V7)	H (V8)
0.5	10	0.3	0.3	28.6	0.216	11.8
1	70	2.3	2.6	27.3	0.415	24.9
1.5	4	0.1	2.8	26.7	0.425	25.5
2	279	9.2	12.0	37.3	0.522	34.6
2.5	8	0.3	12.3	39.7	0.758	49.2
3	689	22.8	35.1	54.5	0.860	65.4
3.5	40	1.3	36.4	68.7	1.006	88.4
4	**1417**	**46.9**	83.4	83.6	1.235	101.4
4.5	23	0.8	84.1	92.6	1.395	126.3
5	433	14.3	98.5	114.8	1.554	137.3
5.5	4	0.1	98.6	97.7	1.480	130.7
6	42	1.4	100.0	125.6	1.657	152.1
	3019	100.0			1.17	95.8

Table 2 shows the distribution in size of each rank of growth unit (from GU1 to GU6) in November 2002. GU1 was grown in nursery before planting. The reduced size of GU2 is clearly related with the transplanting growth stress of the young plants in the field. The relatively low average length of GU3 indicates that this unit may be also influenced by the transplanting stress, or by a subsequent water stress. A positive correlation of r = 0.38 was found between the lengths of GU3 and GU4 (ddl = 1955) ; this correlation is a little higher (r = 0.40) if we consider only the trees having 4 or 4.5 GU (GU3 and GU4 respectively grown during the same period). No relationship was found between the lengths of GU4 and GU5 (ddl = 499), and even also if we consider only trees with the same number of 5 GU. No relationship was found between the lengths of GU5 and GU6.

GU	Nb trees	Min length	Max length	**Average length**
V6a (GU1)	3018	7	62	**29.8**
V6b (GU2)	2938	0.5	48.5	**7.4**
V6c (GU3)	2655	1	105	**15.2**
V6d (GU4)	1957	1.5	94	**31.1**
V6e (GU5)	501	2	131	**30.3**
V6f (GU6)	46	1	65	**22.8**

Analysis of V6T, V6Nb, and V6Lm (number of growth units in November 2002)

After elimination of lacking trees, the data-file had 3019 trees. At the level of the trees, correlations V6T-V6Nb, V6T-V6Lm, and V6Nb-V6Lm are 0.74, 0.67, and 0.06 respectively.

There is no relationship between the number of growth units and their average length ; the relationship with the total height is higher for the number of growth unit than for the average length of the growth units.

This study can be carried out by taking into account only the trees with at least three GUs, and by using V6Nb1 (=V6Nb-2) and V6Lm1 calculated with GU3 to GU6 only (2656 trees). In this case, the correlation matrix is the following :

	V6T	V6NB	V6LM	V6NB1	V6T1	V6LM1
V6T	1.00	0.66	0.88	**0.66**	0.96	**0.82**
V6NB	0.66	1.00	**0.25**	1.00	0.73	0.28
V6LM	0.88	0.25	1.00	0.25	0.78	0.92
V6NB1	0.66	1.00	0.25	1.00	0.73	**0.28**
V6T1	0.96	0.73	0.78	**0.73**	1.00	**0.81**
V6LM1	0.82	0.28	0.92	0.28	0.81	1.00

Here (small trees with less than 3 Gus excluded), we find that there is a small positive relationship between V6Nb and V6Lm, or between V6Nb1 and V6Lm1. We also find that the total height (V6T, or V6T1) is a little more dependent on the average length of growth units than on the number of growth units.

Analysis is then carried out at the level of the means per plot (800 lines of data).

Variables V6Nb, V6Lm, and V6T from 3018 trees

Variance analysis :

	Genotype	Rep	Block(rep)	R^2	CV%	**Mean**	Min	Max	Std
V6Nb	***	*	*	0.48	14.2	**3.665**	2.65	4.71	0.285
V6Lm	***	NS	NS	0.52	17.0	**20.6**	13.4	28.7	1.91
V6T	***	***	NS	0.49	23.6	**75.7**	47.3	106.9	9.71

Min and Max are the minimum and maximum adjusted means of the genotypes. Std is the average std of the adjusted mean of the genotypes.

The adjusted data per genotype are stored in Genmap2.xls.

Results for the parents are the followings :

	PB2171	PB2172	**PB217m**	RRIM6001	RRIM6002	**RRIM600m**
A6Nb	3.15	3.81	**3.48**	2.77	2.99	**2.88**
A6Lm	20.35	15.89	**18.12**	16.06	20.78	**18.42**
A6T	64.82	61.52	**63.17**	48.66	63.90	**56.28**

Variables V6Nba, V6Lma, V6Ta, V6Nb1, V6Lm1, and V6T1 from 2656 trees
(means available for 799 plots)
a : with all GUs
1 : after discarding GU 1 and GU2

Results from variance analyses are the followings :

	Genotype	Rep	Block(rep)	R^2	CV%	**Mean**	Min	Max	Std
V6Nba	***	NS	***	0.53	10.3	**3.92**	3.09	4.93	0.22
V6Lma	***	**	NS	0.54	17.6	**20.4**	13.1	29.5	1.96
V6Ta	***	*	*	0.52	22.4	**81.0**	50.6	115.5	9.88
V6Nb1	***	NS	***	0.53	21.0	**1.92**	1.09	2.93	0.22
V6Lm1	***	NS	NS	0.52	30.6	**21.7**	11.0	39.7	3.61
V6T1	***	NS	*	0.49	38.4	**43.8**	18.1	77.8	9.16

Min and Max are the minimum and maximum adjusted means of the genotypes. Std is the average std of the adjusted mean of the genotypes.

Results for the parents are the followings :

	PB2171	PB2172	**PB217m**	RRIM6001	RRIM6002	**RRIM600m**
A6LMA	20.66	15.48	**18.07**	17.02	19.18	**18.10**
A6LM1	20.12	17.50	**18.81**	14.65	23.22	**18.94**
A6NBA	3.30	3.99	**3.64**	3.43	3.34	**3.38**
A6NB1	1.30	1.99	**1.64**	1.43	1.34	**1.38**
A6TA	68.91	62.55	**65.73**	58.63	66.46	**62.55**
A6T1	27.85	36.02	**31.93**	23.06	35.10	**29.08**

This table illustrates the variation that can be found between two estimations of the same genotype. The mean characteristics of the two parents appear to be a little lower than the means of the whole family.

The correlation matrix of adjusted means for the 200 genotypes is the following :

	A6LMA	A6LM1	A6NBA	A6NB1	A6TA	A6T1
A6LMA	1.00					
A6LM1	0.92	1.00				
A6NBA	**0.09**	0.04	1.00			
A6NB1	0.09	**0.04**	1.00	1.00		
A6TA	**0.88**	0.78	**0.55**	0.55	1.00	
A6T1	0.77	**0.78**	0.62	**0.62**	0.95	1.00

At the level of these adjusted means per genotype, total elongation A6Ta, or elongation after transplanting stress A6T1 seems a little more dependent on the average length of the GUs than of the number of GUs. However, the two factors contribute independantly to elongation.

The adjusted data per genotype are stored in Genmap2.xls. For each variable, the 200 adjusted data have a normal distribution.

Estimation of the variance components and large sense heritabilities :

	V6Ta	V6Nba	V6Lma	V6T1	V6Nb1	V6Lm1
Progenies	76.7	0.039	4.051	47.25	0.039	11.25
Replications	2.4	0.000	0.175	0.00	0.000	0.05
Blocks/rep	20.1	0.016	0.444	21.49	0.016	1.89
Error	327.9	0.162	13.042	280.01	0.162	44.08
Total variance	427.1	0.217	17712	348.75	0.217	57.27
Heritability	**0.18**	**0.18**	**0.23**	**0.14**	**0.18**	**0.20**

Analysis of V7 and V8 (diameter and height in December 2002 - 2852 trees, 799 plots)

Variance analysis :

	Genotype	Rep	Block	R^2	CV%	**Mean**	Min	Max	Std
V7	***	*	*	0.48	19.4	**1.17**	0.782	1.561	0.13
V8	***	***	*	0.51	24.2	**95.8**	60.0	143.8	12.7

Min and Max are the minimum and maximum adjusted means of the genotypes. Std is the average std of the adjusted mean of the genotypes.

Values for the two parents (they appear lower than the means) :

	PB2171	PB2172	**PB217m**	RRIM6001	RRIM6002	**RRIM600m**
A7	0.987	0.961	**0.974**	1.001	0.925	**0.963**
A8	76.95	73.67	**75.31**	74.88	69.85	**72.37**

The correlation between V7 and V8 is r = 0.84.

The adjusted data per genotype are stored in Genmap2.xls.

Estimation of the variance components and large sense heritabilities :

	V7	V8
Progenies	0.00592	98.8
Replications	0.00052	12.0
Blocks/rep	0.00420	40.4
Error	0.05116	535.8
Total variance	0.06180	687.0
Heritability	**0.10**	**0.14**

Analysis of V9 and V10 (diameter and height in June 2003 – 2991 trees, 798 plots)

Variance analysis :

	Genotype	Rep	Block	R^2	CV%	**Mean**	Min	Max	Std
V9	***	***	**	0.49	20.7	**2.040**	1.14	2.68	0.23
V10	***	***	**	0.49	24.6	**207.7**	92.1	297.9	27.8

Min and Max are the minimum and maximum adjusted means of the genotypes. Std is the average std of the adjusted mean of the genotypes.

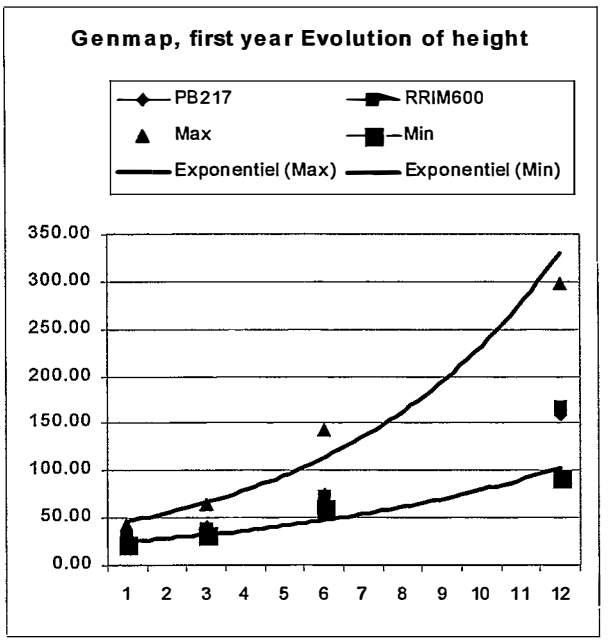
Values for the two parents (they appear lower than the means) :

	PB2171	PB2172	**PB217m**	RRIM6001	RRIM6002	**RRIM600m**
A9	1.86	1.75	**1.81**	1.59	1.73	**1.66**
A10	171.5	147.9	**159.7**	164.9	170.9	**167.9**

Replications 2 and 3 are medium, replication 4 is the most vigorous, replication 1 is the less vigorous.

The adjusted data per genotype are stored in Genmap2.xls.

Evolution of growth over the first year of the trial :



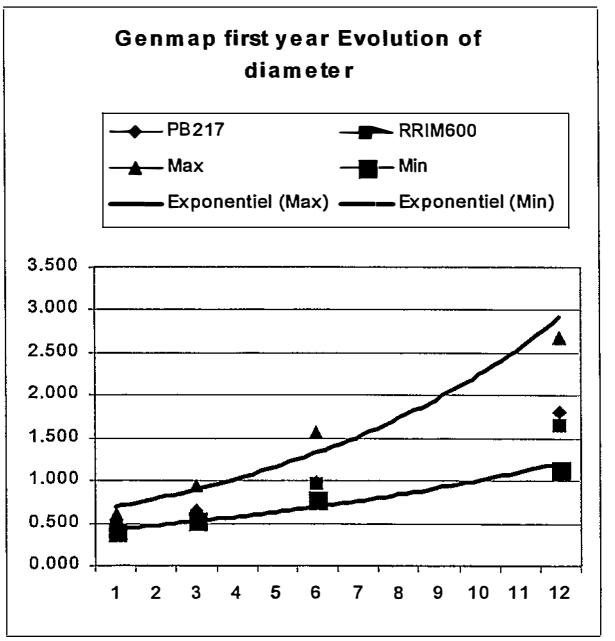


Table : Evolution of height in Genmap (cm)

Date	Age (months)	Mean	PB217m	RRIM600m	Min	Max
July 2002	1	32.7	25.24	29.23	21.44	41.96
September 2002	3	48.8	40.57	38.31	32.81	64.31
December 2002	6	95.8	75.31	72.37	60.00	143.8
June 2003	12	207.7	159.68	167.90	92.07	297.9

Table : Evolution of base diameter in Genmap (cm)

Date	Age (months)	Mean	PB217m	RRIM600m	Min	Max
July 2002	1	0.51	0.451	0.468	0.40	0.61
September 2002	3	0.74	0.644	0.573	0.53	0.94
December 2002	6	1.17	0.974	0.963	0.782	1.561
June 2003	12	2.04	1.808	1.661	1.143	2.684

Estimation of the variance components and large sense heritabilities

	V6T	V6Nb	V6Lm
Progenies	66.8	0.036	3.403
Replications	4.2	0.003	0.054
Blocks/rep	7.3	0.015	0.265
Error	317.5	0.276	12.293
Total variance	395.8	0.330	16.015
Heritability	**0.17**	**0.12**	**0.22**

	V6Ta	V6Nba	V6Lma	V6T1	V6Nb1	V6Lm1
Progenies	76.7	0.039	4.051	47.25	0.039	11.25
Replications	2.4	0.000	0.175	0.00	0.000	0.05
Blocks/rep	20.1	0.016	0.444	21.49	0.016	1.89
Error	327.9	0.162	13.042	280.01	0.162	44.08
Total variance	427.1	0.217	17712	348.75	0.217	57.27
Heritability	**0.19**	**0.19**	**0.24**	**0.14**	**0.19**	**0.20**

	V7	V8
Progenies	0.00592	98.8
Replications	0.00052	12.0
Blocks/rep	0.00420	40.4
Error	0.05116	535.8
Total variance	0.06180	687.0
Heritability	**0.10**	**0.16**

	V9	V10
Progenies	0.022	270.0
Replications	0.007	169.0
Blocks/rep	0.013	166.7
Error	0.178	2262.8
Total variance	0.220	2868.5
Heritability	**0.11**	**0.11**

Variation of V10 heritability depending on the number of replications and the number of trees per plot :

Nb rep	Nb trees/plot	Tot nb trees	H^2 V10
4	4	2991	0.1066
3	4	2241	0.0998
2	4	1484	0.0229
4	3	2255	0.0636
4	2	1504	0.0524
4	1	748	0.0364

The control is the combination with 4 replications and 4 trees per plot.

For each of the 5 other combinations, only one case was tested :
- 3 reps and 4 trees per plot : deletion of rep n° 4
- 2 reps and 4 trees per plot : deletion of reps n° 3 and 4
- 4 reps and 3 trees per plot : deletion of tree n° 4
- 4 reps and 2 trees per plot : deletion of trees n° 3 and 4
- 4 reps and 1 tree per plot : deletion of trees n° 2, 3, and 4.

The trial has 16 trees per genotype. A design with total randomization (16 replications of one tree per genotype) might have provided us with a higher heritability. But the plots with 4 trees per plot, in this trial, are helpful for visual observation of the genotypes (aspect and variability).

Evolution of heritability with the growth of the trees :

During the first year, heritabilities of genotype-adjusted means for diameter and height tend to decrease along time :

Heritabilities	1 month	3 months	6 months	12 months
Diameter	0.32	0.22	0.10	0.11
Height	0.43	0.23	0.16	0.11

This could be due to the effect of replacement of died trees all along the first year, which would emphasize the error variance along time. As a matter of fact, analysis carried out on the sample of trees with no replacement since July 2002 shows that heritability is not increased. Consequently, we can deduce that the impact of the environment (heterogeneity of the soil) increases when the trees get higher.

Correlations between the main variables of the first year, carried out with adjusted means of the genotypes, from a file of 2740 trees and a mean-data file of 797 plots.

	V2	V3	V4	V5	V6T	V6NB	V6LM	V7	V8	V9	V10
V2	1.00										
V3	0.67	1.00									
V4	0.67	0.53	1.00								
V5	0.53	0.68	0.78	1.00							
V6T	0.44	0.54	0.61	0.70	1.00						
V6NB	0.20	0.26	0.51	0.57	0.06	1.00					
V6LM	0.46	0.56	0.78	0.89	0.81	0.62	1.00				
V7	0.58	0.43	0.89	0.72	0.58	0.58	0.79	1.00			
V8	0.46	0.54	0.80	0.88	0.71	0.62	0.92	0.84	1.00		
V9	0.40	0.30	0.65	0.53	0.46	0.49	0.63	0.81	0.71	1.00	
V10	0.40	0.40	0.67	0.68	0.57	0.50	0.73	0.79	0.81	0.89	1.00

By discarding the trees with V10=0, and V8=0, and V5=0, and V3=0, and V10V8<=0, and V8V5<=0, and V5V3<=0, we obtain a file with 2528 trees which include no tree issued from replacement after planting. From this tree-data file we obtain a plot-data file of 796 plots and 200 genotypes.

	V2	V3	V4	V5	V5V3	V6LM	V6NB	V6T	V7	V8	V8V5	V9	V10	V10V8
V2	1.00													
V3	0.65	1.00												
V4	0.67	0.55	1.00											
V5	0.52	0.71	0.77	1.00										
V5V3	0.25	0.26	0.67	0.87	1.00									
V6LM	0.41	0.50	0.59	0.68	0.58	1.00								
V6NB	0.15	0.25	0.44	0.49	0.49	0.07	1.00							
V6T	0.42	0.56	0.75	0.86	0.79	0.82	0.58	1.00						
V7	0.56	0.44	0.86	0.70	0.64	0.58	0.50	0.76	1.00					
V8	0.46	0.56	0.79	0.86	0.78	0.72	0.55	0.90	0.83	1.00				
V8V5	0.32	0.34	0.65	0.59	0.56	0.61	0.49	0.76	0.77	0.92	1.00			
V9	0.35	0.25	0.62	0.48	0.49	0.49	0.37	0.60	0.81	0.69	0.73	1.00		
V10	0.37	0.36	0.64	0.64	0.62	0.60	0.38	0.72	0.78	0.80	0.77	0.88	1.00	
V10V8	0.19	0.08	0.32	0.26	0.30	0.33	0.12	0.34	0.51	0.39	0.41	0.76	0.86	1.00

Relative height

The ratio **V13** = V10/V9 (height / base diameter) , indicator of relative height, is often used in forestry studies as a stability factor of the trees (in case of wind risk). It can be analysed here.

Variance analysis :

	Genotype	Rep	Block	R^2	CV%	**Mean**	Min	Max	Std
V13	***	***	**	0.55	9.6	**98.1**	69.6	114.8	5.12

Values for the two parents (they appear lower than the means) :

	PB2171	PB2172	**PB217m**	RRIM6001	RRIM6002	**RRIM600m**
A13	86.8	91.4	**89.1**	87.3	92.8	**90.0**

The correlation coefficients between A9, A10, A11, and A13 are the followings :

	A9	A10	A11	A13
A9	1.00			
A10	0.92	1.00		
A11	0.95	0.97	1.00	
A13	0.37	0.67	0.52	1.00

The relative height A13 is positively correlated with the three other variables related with vigour (base diameter, height, dry weight of the trunk).

Genmap Appendix 16

Extraction of 25 trees out of the trial for indirect estimation of variables in Genmap

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File Sample25-June2003.xls.

In an outer part of the trial, 448 trees from the family RRIM600 x PB217 were planted in order to complete the land. In June 2003, there were 396 living trees there. In order to have a view on allometric relationships between parameters, and in order to estimate some parameters for each tree of Genmap, by use of other measured predicting variables, 25 trees from the outer part were extracted for being measured and weighed.

The 25 trees, coded from T1 to T25, were chosen with view to cover the range of variation of the sizes of the trees in the field (from Data1 in Sample25-June2003.xls). They belong to 15 different genotypes of the Genmap family.

Photos were taken of each tree in the field, then after extraction. Photos were taken of each taproot : these photos clearly showed for all the 25 trees that the taproot growth was impeded by some lateritic or other physical barrier of the soil at around 30-40 cm below ground level.

Following measurements and calculations were made on these 25 trees (at one year old) :

- Height in cm (from 70 to 340, mean : 236 cm)
- Heightleafy : height of the leafy part of the tree (from 39 to 245 cm, mean : 149 cm)
- Number of leaves in GU6 (from 1 to 27, mean : 14.3)
- Diam10 : base diameter (10 cm high) in cm (from 0.92 to 2.96 cm, mean : 2.24)
- Number of GUs (from 6 to 11, mean : 8.3)

- Amap description of the topology of each tree. For each GU : base diameter, length, Nb of lost ramifications, Nb of leaves, fresh weight, estimated volume (considering each GU as a cylinder). Cf Description in Sample25-June2003.xls.

- Estimation of the volume of the whole tree (from the estimated volumes of the Gus) ; (from 43 to 1500 cm3, mean : 762.8 cm3)
- Total number of leaves for each tree (from 28 to 149, mean : 62.8)
- Total number of leaflets with area measured for each tree (from 81 to 436, mean : 187.6)
- Fwt : fresh weight of the sum of all GU (without petioles and leaflets) then calculation of the weight of the trunk (from 39 to 1362 grams, mean : 607.1 grams); one common sample for estimating dry matter content for dry weight
- Fwpet : fresh weight of all the petioles ; (from 13 to 101 grams, mean : 43.7 grams) ; one common sample for estimating dry matter content for dry weight
- Fwfol : fresh weight of all the leaflets ; (from 69 to 446 grams, mean : 217.8 grams) ; one common sample for estimating dry matter content for dry weight (dry weight : from 22.3 to 144.0 grams, mean : 70.3 grams).
- Fwr : fresh weight of the taproot + main secondary roots ; (from 92 to 678 grams, mean : 373 grams) ; one common sample for estimating dry matter content for dry weight

- Tfw : total fresh weight of each tree (from 223 to 2461 grams, mean : 1241.6 grams)
- Ratfwf : ratio of leaflets / total tree, based on fresh weight (from 8 to 34 %)
- Ratfwp : ratio of petioles / total tree, based on fresh weight (from 1 to 7 %)
- Ratfwt : ratio of trunk / total tree, based on fresh weight (from 17 to 58 %)
- Ratfwr : ratio of roots / total tree, based on fresh weight (from 23 to 41 %)
- W1f : average fresh weight of one leaf (from 2.15 to 5.47 grams, mean : 3.44 grams)
- W1d : average dry weight of one leaf (from 0.694 to 1.766 grams, mean : 1.11 gram)
- SLA : Specific Leaf Area (from 132.3 to 192.4 cm^2/gram, mean : 154.7 grams)
- W1p : average fresh weight of one petiole (from 0.33 to 1.33 grams, mean : 0.70 grams)

- for each tree, measurement of the area of each leaflet, with Delta-T Image Analysis System DIAS device (from 4449 to 33486 cm^2, mean : 10666 cm^2) ; mean area of one leaf (from 116.9 to 269.4 cm^2, mean : 213.3 cm^2). We assume that there is no difference in area between the central leaflet and the two lateral leaflets (area of mean leaf = area of mean leaflet x 3).

The estimated dry matter contents were :
- leaflets : **32.3 %** (24.26 / 75.17 grams)
- petioles : **30.7 %** (26.31 / 85.75 grams)
- trunk : **56.4 %** (335 / 594 grams)
- roots : **52.3 %** (119 / 227 grams).

The average fresh weight of one leaf is 3.44 + 0.70 = 4.14 grams. The average dry weight of one leaf is 1.11 + 0.21 = 1.32 grams (the petiole weight makes around 16 % of the weight of one leaf).

Distribution of leaflets areas (cm^2) for each of the 25 trees :

Those distributions can be assimilated to normal distributions. Alim95 = threshold area under which 95 % of the leaflets areas are located.

Tree	N	A1leaflet	CV%	Alim95
10	260	39.88	56.3	76.92
1	111	42.09	39.0	69.88
23	81	43.04	52.8	88.23
12	100	44.49	54.4	86.04
4	295	46.73	51.1	84.31
7	150	47.95	46.3	93.21
11	436	49.02	72.1	119.31
20	191	49.45	49.3	89.59
21	144	49.75	47.9	91.37
14	138	52.41	55.4	105.47
2	107	53.45	52.8	99.36
3	195	54.00	48.2	98.17
9	310	54.06	35.9	87.65
17	183	55.99	54.3	123.19
13	203	56.61	53.3	104.52
19	226	57.28	40.5	95.85
16	198	60.77	54.5	120.98
24	166	63.95	59.7	140.42
18	133	64.00	60.8	142.53
8	195	64.22	53.7	128.77
15	115	65.01	44.2	108.99
5	256	70.93	50.2	136.62
22	124	72.52	46.1	127.34
25	145	76.01	38.5	129.20
6	229	89.43	43.5	151.51

A matrix of phenotypic correlation coefficients was drawn from the following data issued from measurement of the 25 trees :

Predictive variables D10 and H were measured over every tree of Genmap. Other variables such as Nbgu and Htlf could have been measured over every tree of Genmap for use as predictive variables. The base diameter is a factor of growth through the section, or through the volume of the tree; consequently, variables S10 ($D10^2$) and V10 (D10*H) were introduced in the correlation analysis. All these variables, D10, S10, V10, H, Nbgu, and Htlf can be considered as predictive variables.

- D10 Base diameter at 10 cm high (cm) * 100
- H Height
- S10 = $D10^2 / 100$
- V10 = $D10^2*H / 10000$
- Nbgu Number of growth units
- Htlf Height of trunk with leaves

Predictable variables :

- Vol — Total volume of the trunk (cm3)
- Nbl — Total number of leaves
- Nblgu — Average number of leaves per GU
- Dwl — Dry weight of all the leaves of one tree
- Dwp — Dry weight of all the petioles of one tree
- Dwt — Dry weight of the trunk
- Dwr — Dry weight of the roots of one tree
- Tdw — Total dry weight of one tree
- Dratl — Share of dry leaves weight, relative to total dry weight (Dwl/Tdw)
- Dratp — Share of dry petioles weight, relative to total dry weight (Dwp/Tdw)
- Dratt — Share of dry trunk weight, relative to total dry weight (Dwt/Tdw)
- Dratr — Share of dry roots weight, relative to total dry weight (Dwr/Tdw)
- Tla — Total leaf area of one tree
- Sla — Specific leaf area (Tla/Dwl)

Univariate statistics about the variables :

Variable	N	Mean	Std Dev	Minimum	Maximum	CV%
H	25	235.6	67.5	70.0	340.0	28.7
D10	25	224.0	53.1	92.0	296.0	23.7
S10	25	529	215	84	876	40.7
V10	25	1377	789	59	2979	57.3
NBGU	25	8.3	1.2	6.0	11.0	14.2
HTLF	25	149.1	56.9	39.0	245.5	38.2
VOL	25	762.6	414.6	43.0	1500.0	54.4
NBL	25	62.8	27.4	28.0	149.0	43.6
NBLGU	25	7.6	3.0	2.8	15.0	39.4
DWL	25	70.3	33.0	22.3	144.0	46.9
DWP	25	13.4	6.5	3.9	30.9	48.5
DWT	25	342.4	187.9	22.0	768.2	54.9
DWR	25	195.1	75.9	48.1	354.6	38.9
TDW	25	621.3	289.6	99.5	1238.5	46.6
DRATL	25	12.4	4.5	4.7	24.4	36.5
DRATP	25	2.4	1.0	0.8	5.2	43.3
DRATT	25	51.7	9.8	22.1	63.0	18.9
DRATR	25	33.5	5.9	24.1	48.3	17.6
TLA	25	10665.6	4708.6	3486.0	21372.0	44.1
SLA	25	154.7	12.7	132.3	192.4	8.2

Phenotypic correlation coefficients (ddl = 23) :

	H	D10	S10	V10	NBGU	HTLF	VOL	NBL	NBLGU
H	1.00								
D10	0.94	1.00							
S10	0.94	0.99	1.00						
V10	0.96	0.94	0.97	1.00					
NBGU	0.62	0.50	0.50	0.55	1.00				
HTLF	0.88	0.80	0.80	0.83	0.55	1.00			
VOL	0.96	0.90	0.92	0.96	0.55	0.83	1.00		
NBL	0.72	0.66	0.70	0.78	0.32	0.74	0.75	1.00	
NBLGU	0.57	0.56	0.59	0.65	0.00	0.62	0.62	0.94	1.00
DWL	0.69	0.68	0.69	0.72	0.22	0.66	0.72	0.87	0.85
DWP	0.56	0.59	0.59	0.58	0.13	0.51	0.57	0.65	0.65
DWT	0.96	0.91	0.94	0.99	0.59	0.83	0.97	0.80	0.66
DWR	0.91	0.84	0.85	0.88	0.61	0.83	0.93	0.67	0.52
TDW	0.95	0.90	0.93	0.97	0.57	0.84	0.96	0.81	0.68
DRATL	-0.70	-0.65	-0.61	-0.58	-0.72	-0.51	-0.60	-0.12	0.08
DRATP	-0.69	-0.62	-0.59	-0.58	-0.69	-0.55	-0.61	-0.22	-0.03
DRATT	0.90	0.90	0.87	0.83	0.61	0.71	0.80	0.48	0.35
DRATR	-0.83	-0.89	-0.87	-0.83	-0.34	-0.69	-0.76	-0.68	-0.63
TLA	0.65	0.63	0.65	0.68	0.17	0.63	0.69	0.86	0.85
SLA	-0.69	-0.74	-0.71	-0.64	-0.58	-0.59	-0.63	-0.43	-0.32

	DWL	DWP	DWT	DWR	TDW	DRATL	DRATP	DRATT	DRATR	TLA	SLA
H											
D10											
S10											
V10											
NBGU											
HTLF											
VOL											
NBL											
NBLGU											
DWL	1.00										
DWP	0.91	1.00									
DWT	0.76	0.62	1.00								
DWR	0.73	0.66	0.91	1.00							
TDW	0.82	0.70	0.99	0.95	1.00						
DRATL	-0.04	0.01	-0.58	-0.59	-0.53	1.00					
DRATP	-0.08	0.11	-0.58	-0.57	-0.53	0.93	1.00				
DRATT	0.45	0.35	0.82	0.71	0.78	-0.85	-0.80	1.00			
DRATR	-0.70	-0.61	-0.81	-0.62	-0.79	0.47	0.44	-0.87	1.00		
TLA	0.99	0.92	0.73	0.71	0.79	0.01	-0.02	0.40	-0.66	1.00	
SLA	-0.54	-0.45	-0.64	-0.62	-0.65	0.52	0.54	-0.70	0.67	-0.44	1.00

Remarks about these correlations :

- The correlation coefficients with D10 on one hand and with D1010 on the other hand have the same level

- V10 appears to be more predictive than D10 or S10 for the prediction of variables H (direct link due to calculation of V10), Nbgu, Htlf, Vol, Nbl, Nblgu, Dwl, Dwt, Dwr, Tdw, and Tla.

- Quite all variables are positively correlated, as they are indicative of global growth of the trees

- Only Dratl, Dratp, Dratt, and Sla are negatively correlated with the other variables.

- We can see that the relative share of the trunk is increasing along with the growth of the trees, and this increase is made to the expense of the shares of the other components : leaves, petioles and roots.

- Specific Leaf Area (Sla) is negatively correlated with growth parameters : this means that leaves become thicker, and so heavier, when trees get bigger.

Prediction of some variables

Dwt (dry weight of the trunk) :

The best determination coefficient (quality of prediction) that we can obtain, by using successively V10, S10, H, Nbgu, Htlf, and D10 is $R^2 = 0.981$. By using only V10, S10, and H, we obtain quite the same $\mathbf{R^2 = 0.980}$ with following regression equation :

Dwt = 5.6 + 0.27 V10 – 0.29 S10 + 0.5 H

Nbl (number of leaves) :

The best determination coefficient (quality of prediction) that we can obtain, by using successively V10, S10, Nbgu, Htlf, H, and D10 is $R^2 = 0.783$. By using only V10, S10, H, and D10, we obtain $\mathbf{R^2 = 0.741}$ with following regression equation :

Nbl = - 112 + 0.23 V10 – 1.28 S10 – 1.08 H + 3.5 D10

Tla (total leaf area) :

The best determination coefficient (quality of prediction) that we can obtain, by using successively V10, Nbgu, Htlf, S10, D10, and H is $R^2 = 0.591$. By using only V10, S10, D10, and H we obtain $\mathbf{R^2 = 0.519}$ with following regression equation :

Tla = - 18965 + 28.7 V10 – 171.2 S10 + 503.6 D10 – 136.5 H

We did not have the opportunity to validate these equations on another independant set of trees. Estimated values for Dwt, Nbl, and Tla are stored in Genmap4.xls. Dwt is also stored in Genmap3.xls under the name of V11 (values for 2989 trees).

We observe that the distribution of Dwt is not normal. In order to apply variance analysis to it, we transform it into Rdwt = Square-Root(Dwt).

Results from variance analyses are the followings :

	Genotype	Rep	Block(rep)	R^2	CV%	**Mean**	Min	Max	Std
Rdwt	***	***	**	0.50	24.9	**15.8**	8.28	22.39	2.15
Nbl	***	***	NS	0.50	36.0	**50.7**	8.93	98.89	9.95
Tla	***	***	NS	0.49	33.7	**9216**	946	16254	1691

Results for the parents are the followings :

	PB2171	PB2172	**PB217m**	RRIM6001	RRIM6002	**RRIM600m**
Rdwt	13.01	11.63	**12.32**	11.95	13.49	**12.72**
Nbl	46.96	42.24	**44.6**	25.97	43.85	**34.91**
Tla	8569	7512	**8041**	5062	7323	**6192**

Estimation of the variance components and large sense heritabilities

	Rdwt	Nbl	Tla
Progenies	1.908	59.75	1457711
Replications	0.827	14.13	479424
Blocks/rep	1.203	10.68	307703
Error	15.526	333.98	9663940
Total variance	19.464	418.54	11908778
Heritability	**0.11**	**0.15**	**0.13**

Although the estimations of Rdwt, Nbl, and Tla have varied accuracies, those heritabilities do not appear lower than those issued from original variables D10 = V9, and H = V10 (which were 0.11 and 0.11 respectively). But all these heritabilities (D10, H, Rdwt, Nbl, Tla) are low.

For assessing those results by comparing them with another type of variable, we did the same calculations for one estimated variable which was predicted with a very low accuracy : A1lt = average area of one leaflet, predicted from H, D10, S10, and V10 with $R^2 = 0.15$.

	Genotype	Rep	Block(rep)	R^2	CV%	**Mean**	Min	Max	Std
A1lt	*	*	NS	0.43	7.9	**55.2**	44.9	60.4	2.37

	A1lt
Progenies	1.168
Replications	0.167
Blocks/rep	0.397
Error	19.007
Total variance	20.739
Heritability	**0.06**

It must be recalled that the regression process used for estimating a new variable is reducing the actual variability of the studied variable.

From the analysis of 25 trees, we tried to estimate some variables which could not be measured over all the trees of Genmap. The limitations of this approach are the followings :

- small size of the sample (25 trees)
- no separation of the two different variance factors (within each genotype, and between the genotypes)
- no validation of the regression equations on an independant set of trees.

This method allowed us to estimate, for each tree of Genmap trial, and then for each genotype, biomass (dry weight of the trunk) with a rather good accuracy, and total leaf area with a much lower accuracy.

Genmap Appendix 17

Classification of the genotypes for vigour at one year old

Highest clonal V9 (base diameter)	:	2.68 cm
Highest clonal V10 (height)	:	298 cm
Highest clonal estimated dry weight of the trunk V11	:	501 grammes
Highest individual V9 (base diameter)	:	4.30 cm
Highest individual V10 (height)	:	420 cm
Highest individual estimated dry weight of the trunk V11	:	1639 grammes

Classification of the 200 genotypes according to A11 (adjusted estimated dry weight of the trunk, per tree) as a global indicator of vigour at one year old :

Rrit-Code	Codec	A11
1377	195	68.6
953	97	111.1
1337	181	115.3
1364	190	116.2
1165	141	125.9
977	102	129.0
737	33	130.2
859	75	130.6
935	94	130.6
1306	173	133.9
1119	131	134.1
2172	200	135.3
1192	147	136.4
6001	197	142.8
714	**24**	145.2
1174	143	146.2
1027	111	147.1
704	**18**	148.1
980	104	149.1
1215	153	150.1
1360	188	151.0
894	82	152.8
1353	186	154.5
1295	168	156.3
1289	166	156.5
728	31	157.5
1370	193	162.3
1320	178	162.6
1199	150	166.2
1409	196	168.7
2171	**199**	169.3
1314	177	169.8
1155	138	171.3
1020	109	175.0
1044	116	175.3
1249	159	176.9
699	**15**	178.0
675	**3**	179.6
1288	165	179.8
909	89	181.4
6002	**198**	182.0
735	32	182.3
824	65	182.8
722	**28**	185.0
1362	189	185.5
1259	160	186.6
845	71	186.9
1089	127	187.1
1347	184	188.5
974	101	189.9

Rrit-Code	Codec	A11
716	**25**	191.0
1209	151	193.5
1230	155	194.6
703	**17**	197.1
1292	167	199.4
1049	118	202.8
860	76	203.6
1130	133	204.8
770	45	205.9
798	57	207.6
1077	126	208.5
1335	180	209.7
1348	185	213.2
1164	140	213.5
680	**6**	214.3
1367	192	216.7
1296	169	217.9
897	85	219.6
858	74	223.2
827	66	224.1
1068	124	224.4
978	103	225.0
1375	194	225.3
1279	163	228.0
1310	176	228.3
689	**11**	229.2
752	38	229.2
796	55	230.4
816	63	232.3
724	**29**	232.6
1217	154	232.6
1356	187	233.2
1055	121	233.8
808	62	236.2
1270	161	240.9
1305	172	241.8
788	53	242.4
795	54	242.4
1138	134	242.7
1002	105	244.0
1245	158	244.6
677	**4**	245.5
1005	106	246.5
1123	132	246.5
1366	191	246.8
1019	108	249.0
1193	148	249.6
800	58	250.0
966	100	250.0
880	81	250.9

Rrit-Code	Codec	A11
785	51	251.9
839	69	252.2
1175	144	252.8
1095	128	253.8
707	**20**	255.7
720	**27**	256.0
708	**21**	256.3
686	**9**	257.3
702	**16**	258.2
1144	136	258.2
1302	171	259.5
1012	107	260.5
755	40	261.1
1066	123	261.1
1046	117	263.1
1058	122	263.1
948	96	267.3
1042	114	268.0
902	86	269.6
743	34	272.3
1273	162	272.3
1052	119	273.2
1195	149	273.9
688	**10**	274.9
758	43	277.2
924	92	277.6
1069	125	278.2
774	46	279.2
803	59	280.2
672	**1**	280.6
674	**2**	282.2
683	**7**	282.2
1308	175	282.6
1039	113	283.9
1140	135	283.9
939	95	284.3
1188	146	284.3
854	73	285.3
834	68	285.9
915	90	287.3
833	67	287.6
807	61	288.3
896	84	292.1
1034	112	292.1
1159	139	294.5
713	**23**	295.2
1285	164	295.5
1153	137	296.2
697	**14**	301.0
1344	183	303.5

Rrit-Code	Codec	A11
805	60	306.6
1024	110	311.5
1242	157	312.9
727	**30**	313.3
864	77	314.4
756	41	314.7
1300	170	315.1
1327	179	315.8
873	78	317.9
1053	120	319.7
1097	130	324.0
719	**26**	326.9
965	99	326.9
709	**22**	330.5
1185	145	331.6
1213	152	333.1
1343	182	334.2
1043	115	340.8
843	70	341.9
875	80	348.9
754	39	350.8
744	35	351.2
904	87	351.2
695	**13**	351.9
922	91	352.7
822	64	353.1
874	79	358.7
1233	156	358.7
693	**12**	361.4
757	42	362.9
964	98	363.7
769	44	364.8
679	**5**	377.5
846	72	377.9
780	49	379.1
778	47	379.9
1168	142	387.7
932	93	398.4
787	52	404.0
1096	129	404.0
783	50	410.1
907	88	414.5
705	**19**	418.6
684	**8**	428.5
895	83	443.9
749	37	445.6
797	56	462.3
748	36	475.2
779	48	484.4
1307	174	501.3

Classification of the 200 genotypes according to A9 (adjusted base diameter at one year old :

Rrit-Code	Code C	A9
1377	195	1.14
935	94	1.46
859	75	1.48
1360	188	1.50
1364	190	1.50
714	24	1.53
737	33	1.55
1337	181	1.56
1165	141	1.57
1306	173	1.57
6001	197	1.59
953	97	1.59
1215	153	1.61
977	102	1.61
1192	147	1.61
1289	166	1.62
1119	131	1.63
699	15	1.63
728	31	1.66
894	82	1.66
909	89	1.68
704	18	1.69
1044	116	1.69
1370	193	1.69
1155	138	1.70
1320	178	1.70
1027	111	1.70
1409	196	1.71
1295	168	1.71
675	3	1.71
1174	143	1.72
716	25	1.73
1249	159	1.73
722	28	1.73
6002	198	1.73
845	71	1.74
1353	186	1.75
2172	200	1.75
1199	150	1.80
689	11	1.81
1068	124	1.81
1089	127	1.81
1314	177	1.82
824	65	1.83
1130	133	1.83
1348	185	1.84
1335	180	1.84
1144	136	1.85
808	62	1.85
1164	140	1.85

Rrit-Code	Code C	A9
1020	109	1.86
980	104	1.86
2171	199	1.86
1362	189	1.87
724	29	1.87
680	6	1.88
974	101	1.88
1049	118	1.89
1055	121	1.89
735	32	1.90
978	103	1.90
1230	155	1.90
1347	184	1.90
703	17	1.91
1259	160	1.92
796	55	1.92
1375	194	1.92
1077	126	1.93
1209	151	1.94
1292	167	1.94
1217	154	1.94
948	96	1.94
1288	165	1.95
788	53	1.96
860	76	1.96
798	57	1.96
897	85	1.97
1066	123	1.97
1367	192	1.97
816	63	1.98
1138	134	1.98
1245	158	1.99
770	45	1.99
1012	107	1.99
1042	114	2.00
755	40	2.00
1002	105	2.00
702	16	2.00
1058	122	2.00
839	69	2.01
1123	132	2.01
1279	163	2.01
858	74	2.01
677	4	2.02
1193	148	2.02
688	10	2.03
686	9	2.04
1356	187	2.04
834	68	2.04
743	34	2.04

Rrit-Code	Code C	A9
1005	106	2.04
807	61	2.05
902	86	2.06
1095	128	2.06
708	21	2.06
1366	191	2.07
672	1	2.07
785	51	2.07
833	67	2.07
1305	172	2.08
758	43	2.08
800	58	2.08
1046	117	2.08
827	66	2.08
1302	171	2.09
1273	162	2.09
774	46	2.09
1296	169	2.09
752	38	2.10
1140	135	2.11
1019	108	2.11
1285	164	2.12
674	2	2.12
1024	110	2.12
707	20	2.13
1310	176	2.13
1308	175	2.13
720	27	2.13
795	54	2.13
966	100	2.15
1052	119	2.15
854	73	2.16
1175	144	2.16
805	60	2.17
1344	183	2.17
697	14	2.17
1195	149	2.18
1270	161	2.18
803	59	2.19
1034	112	2.19
1053	120	2.19
1188	146	2.20
915	90	2.21
1343	182	2.21
896	84	2.21
939	95	2.21
880	81	2.23
727	30	2.23
873	78	2.24
756	41	2.24

Rrit-Code	Code C	A9
1039	113	2.25
924	92	2.26
1185	145	2.26
1069	125	2.27
683	7	2.27
1159	139	2.27
709	22	2.27
705	19	2.28
769	44	2.28
1097	130	2.29
1213	152	2.29
904	87	2.29
964	98	2.29
1242	157	2.29
1153	137	2.30
1327	179	2.30
719	26	2.31
1043	115	2.32
757	42	2.32
843	70	2.33
713	23	2.33
693	12	2.34
1096	129	2.34
965	99	2.34
922	91	2.36
679	5	2.36
780	49	2.37
864	77	2.37
754	39	2.39
846	72	2.39
1300	170	2.40
822	64	2.40
907	88	2.40
875	80	2.42
778	47	2.42
744	35	2.43
695	13	2.45
1233	156	2.45
874	79	2.47
787	52	2.48
783	50	2.50
1168	142	2.52
932	93	2.54
895	83	2.56
1307	174	2.57
748	36	2.59
749	37	2.63
684	8	2.64
797	56	2.68
779	48	2.68

Classification of the 200 genotypes according to A10 (adjusted height at one year old :

Rrit-Code	Code C	A10	Rrit-Code	Code C	A10	Rrit-Code	Code C	A10	Rrit-Code	Code C	A10
1377	195	92.07	716	25	185.94	924	92	211.02	727	30	228.61
953	97	134.86	1292	167	185.97	702	16	211.09	896	84	228.65
1364	190	136.97	1230	155	186.84	1217	154	211.16	1213	152	228.73
1165	141	141.42	770	45	187.59	1273	162	211.32	834	68	228.82
1337	181	141.62	808	62	187.65	800	58	211.35	1140	135	229.08
859	75	143.42	1296	169	188.04	1019	108	211.36	864	77	229.95
2172	200	147.90	974	101	188.17	677	4	211.52	965	99	231.11
977	102	151.19	1068	124	189.56	1095	128	212.43	1344	183	231.84
1119	131	154.05	798	57	191.29	674	2	212.89	1053	120	233.25
894	82	154.39	1367	192	192.67	1123	132	213.00	709	22	233.51
1192	147	155.28	824	65	192.75	1042	114	213.21	843	70	234.65
1027	111	157.77	1164	140	193.06	1012	107	213.47	1242	157	235.29
935	94	158.09	1130	133	193.62	785	51	214.25	744	35	235.78
1353	186	158.12	897	85	193.66	839	69	214.35	1343	182	236.36
737	33	159.35	1335	180	193.67	1348	185	215.10	697	14	237.51
1306	173	160.15	1310	176	194.64	724	29	215.98	1034	112	238.08
735	32	161.39	752	38	194.73	939	95	216.09	875	80	238.42
980	104	163.20	909	89	195.20	803	59	216.24	757	42	239.52
714	24	164.35	1089	127	195.42	1144	136	216.30	1097	130	239.68
1020	109	164.62	858	74	197.24	1039	113	217.20	1327	179	240.20
6001	197	164.93	816	63	197.50	708	21	217.20	1233	156	240.63
1215	153	166.37	680	6	198.18	755	40	217.62	822	64	240.64
1409	196	166.85	1138	134	198.39	948	96	217.64	922	91	241.14
1289	166	166.85	827	66	200.07	1052	119	217.84	1043	115	242.06
1174	143	167.31	1077	126	200.28	1302	171	218.33	1185	145	242.86
1370	193	167.64	795	54	201.20	688	10	218.69	695	13	243.51
1314	177	167.86	1002	105	201.31	915	90	219.45	904	87	246.81
1360	188	168.19	1375	194	201.64	774	46	219.53	754	39	247.76
1320	178	168.98	1270	161	201.92	1069	125	219.89	778	47	248.44
728	31	170.14	743	34	202.95	807	61	220.17	874	79	249.30
1155	138	170.75	689	11	202.98	1058	122	220.25	787	52	250.92
675	3	170.83	1305	172	203.17	833	67	220.43	846	72	253.07
6002	198	170.86	1175	144	203.69	805	60	220.52	679	5	254.43
2171	199	171.46	966	100	203.92	1153	137	221.03	693	12	255.87
699	15	173.50	1055	121	204.31	713	23	221.04	769	44	256.71
704	18	175.17	1356	187	204.35	854	73	221.42	705	19	256.80
1044	116	176.25	720	27	204.43	873	78	221.61	932	93	257.61
1199	150	176.91	902	86	204.55	1188	146	221.69	1168	142	258.50
722	28	177.28	1046	117	205.25	1308	175	222.80	684	8	259.40
1295	168	177.65	1279	163	205.69	756	41	223.27	964	98	262.78
1259	160	178.03	796	55	205.92	788	53	223.55	749	37	263.15
845	71	178.04	686	9	206.21	758	43	224.17	895	83	265.55
1209	151	178.99	1366	191	206.83	1159	139	224.56	780	49	265.62
1362	189	179.77	880	81	206.88	683	7	224.57	783	50	269.51
703	17	180.32	978	103	207.02	1066	123	225.34	797	56	270.16
1347	184	181.26	1005	106	207.31	719	26	225.98	907	88	270.22
1288	165	181.46	707	20	208.95	1300	170	226.66	1096	129	271.29
1049	118	183.45	1193	148	209.33	672	1	226.83	779	48	277.48
1249	159	184.16	1245	158	210.12	1024	110	226.89	748	36	282.07
860	76	185.61	1195	149	210.35	1285	164	227.39	1307	174	297.85

Genmap Appendix 18

Description of architecture (topology) of 32 genotypes in January 2003 (7 months old)

(File Sample32-June2003.xls).

452 trees, distributed over genotypes 1 to 30 + RRIM600-2 (code 198) and PB217-1 (code 199) with 11 to 16 trees per genotype.

Measurements, countings and observations :

- Base diameter of each tree (at 10 cm high, on the first GU)
- Description of the topology of the GUs of axis 1 (T1, T2, ...) and of ramifications (R1, R2, ...)

For each GU :

- Base diameter
- Length
- Length from base to first leaf (if any)
- Number of lost axes (if any)
- Number of leaves (if any)
- Length of central leaflet of the first leaf (if any)
- Width of central leaflet of the first leaf (if any).

Overall, 2090 GUs were observed, including only 61 relays (R, ramification continuing axis A1 after the death of terminal bud). Secondary ramifications were counted and then pruned. There were 807 axillary ramifications A2 ditributed over 241 GUs, with following distribution :

Number of Gus with :

- 1 A2	67 GUs	67 A2
- 2 A2	40	80
- 3 A2	32	96
- 4 A2	34	136
- 5 A2	24	120
- 6 A2	21	126
- 7 A2	13	91
- 8 A2	4	32
- 9 A2	5	45
- 14 A2	1	14
	241	807

1195 GUs were bearing from 1 to 43 leaves. Number of Gus with :

- 1-5 leaves 212 GUs
- 6-10 259
- 11-15 299
- 16-20 262
- 21-25 123
- more than 25 : 40

Over 450 studied trees :
- 12 have 1 GU
- 17 have 2 GUs
- 50 have 3 GUs
- 103 have 4 GUs
- 197 have 5 GUs
- 61 have 6 GUs
- 10 have 7 GUs.

Average lengths are :
- GU1 : 27.5 (over 450 measures)
- GU2 : 7.7 (over 438 measures) = stress due to planting.
- GU3 : 14.0 (over 421 measures)
- GU4 : 31.4 (over 371 measures)
- GU5 : 32.4 (over 268 measures)
- GU6 : 29.6 (over 71 measures)
- GU7 : 22.6 (over 10 measures).

Analysis of central leaflet morphology

After elimination of abnormal data, we can analyse 1097 leaflets for the following variables :

- L length
- W width
- Wparl =W/L*100 (shape factor)
- Wfoisl =W*L (area indicator)

The four variables have a normal distribution.

	Mean	CV %
L	16.40	23.9
W	6.003	24.0
Wparl	36.98	14.3
Wfoisl	103.15	44.2

We observe that the variability of Wparl is relatively limited (genetic constraint on the shape of the leaflets). Conversely, the variability of the area indicator Wfoisl is higher, as it combines the variabilities of L and W as well as the influence of environment.

Correlation coefficients :

	L	W	Wparl	Wfoisl
L	1			
W	0.84	1		
Wparl	-0.29	0.26	1	
Wfoisl	0.95	0.95	**-0.02**	1

There is no relationship between Wparl and Wfoisl (shape and area).

Variance analysis

	Genotype	R^2	CV%	**Mean**	Min	Max	Std
L	***	0.10	23.0	**16.40**	12.67	18.37	0.65
W	***	0.14	22.6	**6.003**	4.852	7.420	0.234
Wparl	***	0.26	12.5	**36.98**	32.1	43.3	0.796
Wfoisl	***	0.11	42.4	**103.15**	64.6	139.0	7.55

Here, determination coefficients R^2 for the different variables L, W, Wparl, and Wfoisl, are quite equivalent to large sense heritabilities (which are, calculated with Sas-Varcomp, 0.08, 0.12, 0.25, and 0.08 respectively.

Main architectural characteristics of the 32 clones :

% GuL Percentage of GUs with Leaves
Nb L/GuL Number of leaves per leafy GU
NbL/Tree Number of leaves per tree
W/l Central leaflet shape indicator (width/length)
Vol/Gu Mean volume per Gu (considered as a cylinder)

Clo	NbTrees	Tot Nb Gu	%GuL	Nb L/GuL	NbGu/Tree	Nb L/Tree	w/l	Vol/Gu
1	15	74	56.8	13.5	4.93	37.9	0.376	29.1
2	14	61	54.1	13.5	4.36	31.9	0.377	40.8
3	14	65	55.4	11.9	4.64	30.5	0.381	22.2
4	16	78	48.7	11.9	4.87	28.2	0.387	23.8
5	14	71	59.2	17.6	5.07	52.9	0.370	42.2
6	14	64	46.9	10.5	4.57	22.4	0.372	26.8
7	15	70	54.3	12.1	4.67	30.6	0.357	33.0
8	16	76	57.9	13.3	4.75	36.7	0.389	45.1
9	14	62	53.2	15.9	4.43	37.5	0.358	34.3
10	13	58	50.0	13.9	4.46	31.1	0.373	36.3
11	13	59	55.9	12.3	4.54	31.3	0.388	36.3
12	13	73	56.2	12.0	5.62	37.9	0.382	31.1
13	16	82	59.8	10.2	5.12	31.1	0.378	34.6
14	15	72	52.8	14.4	4.80	36.5	0.380	32.6
15	12	57	57.9	11.8	4.75	32.3	0.368	23.9
16	15	71	46.5	13.8	4.73	30.2	0.363	35.1
17	13	49	57.1	12.7	3.77	27.4	0.343	26.3
18	15	64	48.4	12.5	4.27	26.0	0.372	29.1
19	13	68	57.4	13.9	5.23	41.7	0.373	46.3
20	12	55	58.2	10.9	4.58	29.1	0.363	24.6
21	15	63	55.6	12.8	4.20	29.9	0.366	32.3
22	15	70	55.7	14.3	4.67	37.2	0.384	40.2
23	16	70	58.6	12.0	4.37	30.8	0.364	26.6
24	14	64	53.1	11.4	4.57	27.6	0.380	25.9
25	16	68	52.9	12.8	4.25	28.8	0.364	28.4
26	15	67	59.7	12.6	4.47	33.7	0.370	35.0
27	16	69	47.8	13.6	4.31	28.0	0.370	30.5
28	11	58	51.7	12.8	5.27	35.0	0.380	28.5
29	15	74	54.1	11.8	4.93	31.3	0.364	24.7
30	14	68	50.0	12.8	4.86	31.2	0.366	29.3
198	11	42	54.8	10.8	3.82	22.6	0.392	23.4
199	12	48	62.5	11.1	4.00	27.8	0.358	17.3

Genmap Appendix 19

Difference in growth between Genmap and a neighbouring plot

In the plot neighbouring Genmap, there is a large scale clonal trial planted in the end of August 2002. The trees in this plot appear to be much smaller than those of Genmap, although there is a difference of only 2 months between the planting of the two trials. A sample of 19 trees of the neighbouring plot was fastly measured in June 2003 for base diameter (average = 1.62 cm) and height (average = 136 cm). For comparison, we obtain for 2991 trees of Genmap : 2.04 cm (base diameter) and 207 cm (height). Cf Plot-East in Sample25-June2003.xls.

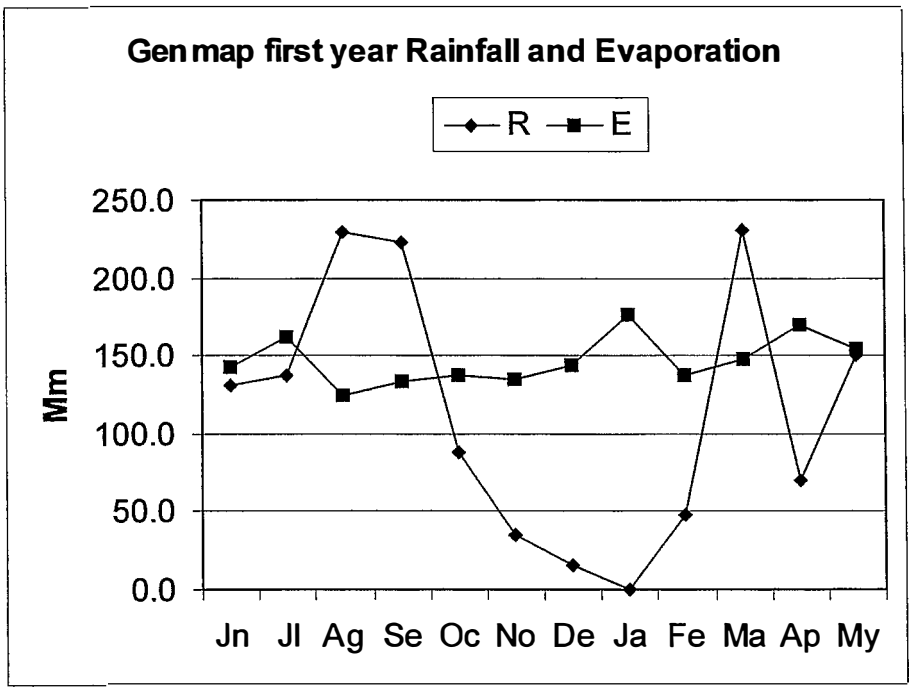
Genmap Appendix 20

Climate of the first year

	Decade	Tmax	Tmin	Rhmax	Rhmin	Rain	Cumrain	Evap	Cumevap	Sun	Cumsun
June 2002	1	35.3	24.1	97	45	6.5	7	53.9	54	83.5	84
	2	34.5	23.5	99	42	29.0	36	42.1	96	44.2	128
	3	33.6	23.2	99	47	95.7	131	46.2	142	42.2	170
July 2002	1	34.0	24.4	100	47	38.3	170	53.9	196	58.9	229
	2	34.8	23.5	100	42	44.0	214	47.5	244	62.5	291
	3	34.4	22.7	99	43	55.4	269	60.1	304	56.0	347
August 2002	1	33.9	23.6	100	54	72.6	342	41.0	345	23.6	371
	2	33.0	23.8	98	53	13.8	355	37.0	382	**16.1**	387
	3	33.2	22.5	100	53	**143.2**	499	45.8	428	50.4	437
September 2002	1	33.8	23.6	100	49	27.7	526	47.1	475	53.2	491
	2	33.5	22.9	100	50	76.2	602	50.6	525	47.4	538
	3	33.6	23.4	100	47	119.2	722	**35.2**	560	38.7	577
October 2002	1	34.4	20.5	99	35	27.4	749	43.4	604	75.2	652
	2	34.6	18.0	98	26	16.5	766	48.5	652	82.8	735
	3	34.3	22.8	100	55	43.6	809	45.2	698	67.5	802
November 2002	1	33.0	19.2	100	37	**0.0**	809	43.8	741	62.6	865
	2	35.5	22.7	100	37	7.6	817	43.0	784	78.8	944
	3	34.0	21.0	98	35	26.9	844	47.9	832	84.5	1028
December 2002	1	34.6	20.4	99	40	7.4	851	42.6	875	64.5	1093
	2	35.3	20.8	100	35	**0.0**	851	48.9	924	91.3	1184
	3	34.9	20.6	100	36	7.5	859	52.6	976	75.5	1259
January 2003	1	34.1	18.4	99	23	**0.0**	859	57.1	1033	91.0	1350
	2	33.0	**13.2**	98	14	**0.0**	859	57.9	1091	98.2	1449
	3	35.0	17.7	98	**9**	**0.0**	859	60.8	1152	**98.9**	1548
February 2003	1	36.2	15.9	99	**9**	**0.0**	859	**61.8**	1214	92.1	1640
	2	35.7	22.2	100	35	41.5	900	37.9	1252	63.1	1703
	3	35.1	22.8	99	39	6.5	907	38.1	1290	60.6	1763
March 2003	1	37.0	22.2	100	25	66.9	973	52.4	1342	68.6	1832
	2	35.4	20.8	100	34	45.2	1019	50.6	1393	67.4	1899
	3	34.4	22.5	99	41	118.2	1137	45.1	1438	47.0	1946
April 2003	1	37.2	23.7	100	38	7.6	1144	53.9	1492	80.5	2027
	2	37.4	23.7	100	33	1.5	1146	60.1	1552	84.6	2111
	3	36.8	22.2	99	32	61.0	1207	56.3	1608	71.8	2183
May 2003	1	**37.6**	23.8	100	38	56.8	1264	47.1	1655	80.4	2264
	2	35.0	24.0	100	34	52.3	1316	46.4	1702	60.4	2324
	3	34.5	23.2	100	40	40.9	1357	60.3	1762	75.6	2400

Maximum temperatures are quite stable all along the year. Minimum temperatures are a little colder in December-January-February. Maximum relative humidity reaches saturation all along the year (during the night). Minimum relative humidity varies between around 10 % in January (dry season) and 50 %in August (rainy season). Total rainfall in 2002-2003 was of 1357 mm, with four months below 50 mm (from November to February) ; although this quantity of water is not very high, the distribution aver the months is not too bad. Evaporation is rather stable all along the year, but a marked water deficit occurs during 5 months (from October to February). Insolation is high, with 2400 hours for the whole year, with a minimum in August (cloudy and rainy season)..

Evolution of rainfall and evaporation :



If we cumulate the water deficits from each decade, we find a cumulated deficit of 805 mm during the first 12 months of settlement of the trial, at a time when the root system cannot take water from lower levels of the soil. Water very probably was an important limiting factor for the growth of the trial during this first year.