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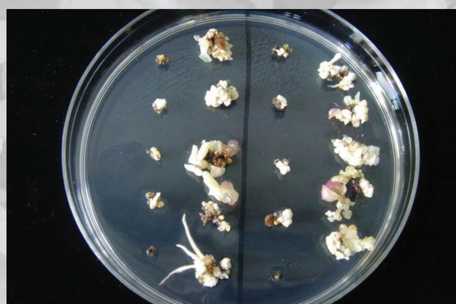
Supplying new cocoa planting material to farmers: A review of propagation methodologies

Authors and reviewers: Augusto Roberto Sena Gomes, George Andrade Sodré, Mark Guiltinan, Rob Lockwood and Siela Maximova

Editors: Brigitte Laliberté and Michelle End



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Citation: Laliberté, B., End, M., editors. 2015. Supplying new cocoa planting material to farmers: a review of propagation methodologies. Bioversity International, Rome, Italy. 200pp.

The English copy editing was done by Elizabeth O'Keefe (consultant copy-editor) and Vincent Johnson, the layout by Claudine Picq and cover design by Karen Lehrer of Bioversity International.

Cover illustrations: Background picture: G. Lockwood.
Small pictures (from top to bottom): A.R. Sena Gomes, A. Daymond, W. Solano, A.R. Sena Gomes.

ISBN: 978-92-9255-009-7

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Acknowledgements

This review document, coordinated by Bioversity International, is the product of expert opinions and discussions among the main authors and additional reviewers during international conferences and consultation workshops. The goal of this review is to present an impartial, evidence-based review of cacao propagation methods, which could serve as a basis for the assessment and implementation of strategies for providing farmers with quality planting materials that are adapted to current and future conditions and needs (cultural, institutional, technical, environmental and financial).

Although the term "cocoa" is generally used for the plant and its products in many English speaking countries, this document will refer to "cacao" for the plant and the unprocessed seeds of the species *Theobroma cacao*. Once the cacao seeds, commonly known as "beans", are harvested, fermented and dried, the product is known as cocoa.

Bioversity would like to thank the authors responsible for the substantial work developing chapters on their respective areas of expertise: Rob Lockwood, Consultant, London, UK (Chapter 2 – Propagation by Seeds), Augusto Roberto Sena Gomes, Consultant, and George Andrade Sodré, Comissão Executiva do Plano da Lavoura Cacaueira/ Centro de Pesquisas do Cacau (CEPLAC/CEPEC), both from Itabuna, Bahia, Brazil (Chapter 3 – Conventional Vegetative Propagation) and Siela Maximova and Mark Guiltinan from Penn State University, Philadelphia, USA (Chapter 4 – Tissue Culture) and the Editors Brigitte Laliberté, Bioversity International, Italy and Michelle End, CRA Ltd UK (Chapter - 1 General Introduction and Chapter 5 - Cross-cutting Issues and Conclusions). Bioversity would like to thank Aixa Del Greco for her involvement in the early stage of the review document and express gratitude to Elizabeth O'Keefe for the copy-editing of the review, to Vincent Johnson for the final English editing, Claudine Picq for the layout and Karen Lehrer for the graphic design of the front and back covers.

For Chapter 2: The author thanks the pioneers of large-scale production of hybrid cacao seed and especially the late Don Edwards for making possible today's methods that serve farmers so well. Many colleagues generously provided information about seed production in various countries, including Komlan Wegbe, Lee Ming Tong, Philippe Lachenaud, Roberto Augusto Sena Gomes, Smilja Lambert and Wilbert Phillips. Beatrice Owusua, from the library of the Cocoa Research Institute of Ghana, kindly provided copies of many papers.

For Chapter 3: The authors are indebted to Drs Uilson Lopes, José L. Pires and Wilson R. Monteiro for providing valuable insights into CEPLAC's cacao breeding programme. The appreciation is extended to Drs Rob Lockwood and Mark Guiltinan for all the important suggestions they made over the course of this study. The help of the following people in assisting and providing important information on cacao propagation technologies is sincerely appreciated: Basilio Leite (CEPLAC), Claudio S. Silva (Grupo Chaves, Lagoa Pequena Farm, Ilhéus, Bahia), David Butler (Cocoa Consulting), Ivan Garzón, Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP), Jackson de O. Cesar (Biofabrica), Sebastian A.F., Lopez (Cocoa Consulting) and Wanilson Serra (Bom Retiro Farm, Camacan, Bahia).

For Chapter 4: The authors would like to express their appreciation to all the organizations and individuals that contributed to the information presented in this chapter, including: Pierre Broun, Anne Buchwalder, Bernard Masseret and Vincent Petiard from Nestlé, France; Ivan Garzón and Freddy Amores from INIAP, Ecuador; the Indonesian Coffee and Cocoa Research Institute (ICCRI) team, and especially Agung Susilo; Ricardo Goenaga from United States Department of Agriculture (USDA), Puerto Rico; Andrew Wetten from the University of Reading, UK; and Jean-Philippe Marelli from Mars Center for Cocoa Science, Brazil.

Bioversity is also grateful for the expert feedback provided during the review workshop that took place in London UK in May 2013 and particularly the following people: Alison Branch (Mondelez International, , UK), Brigitte Laliberté (Bioversity International, Italy), Désiré Poko (Centre National de Recherche Agronomique (CNRA), Abidjan, Côte d'Ivoire), Kelvin Lamin (Malaysian Cocoa Board (MCB) Kota Kinabalu, Malaysia)), Martin Gilmour (Mars, UK), Michelle End (CRA Ltd, UK), Nicolas Cryer (Mondelez International, Reading, UK), Paul Hadley (University of Reading, UK), Philippe Bastide (Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) Montpellier, France), Pierre Broun (Nestlé, Tours, France), Roger Leakey (Consultant, Scotland, UK), Stephan Weise (Bioversity International, Italy), Stephen Yaw Opoku (Cocoa Research Institute of Ghana (CRIG) New Tafo-Akim, Ghana), Tony Lass (CRA Ltd, UK) and Wilbert Phillips (Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) San Jose, Costa Rica).

This review was funded by Bioversity International, the CGIAR Research Program (CRP) on Forests, Trees and Agroforestry (FTA), Mars Global Chocolate UK, Cocoa Research (UK) Ltd (CRUK Ltd.) and the Cocoa Research Association Ltd., UK (CRA Ltd.).

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Preface

When visiting cocoa farms in West Africa, it's common to see small farmer-run cacao seedling nurseries (often referred to as *'petit pépinière'* in Côte d'Ivoire) where seeds from just a few pods have been germinated and grown into small plants. The farmer's objective is to use the most vigorous and robust of these seedlings to plant new areas of the farm, or to replace dead, diseased or unproductive trees. The pods are likely to have come from the farmer's own trees or from those of a neighbour, relative, or friend. Farmers used to growing traditional varieties would rightly expect the seed to grow into trees very similar to those from which the pods were taken. However, in many cocoa-producing areas these traditional varieties have been replaced with types which will not 'breed true', meaning that there is no guarantee that the resulting plants (known as *'tout venant'* in Côte d'Ivoire) will be the same as, or will be higher yielding or more disease resistant than, the parent trees; in fact, they will often be very much inferior.

The need to replant with improved planting material is sometimes not a priority, and it seems that if there is an insufficient supply of improved planting materials from the relevant cocoa authorities, or a lack of knowledge about the benefits of using improved varieties that would justify any additional costs or effort in acquiring them, the default for those farmers wishing to replant is to use the material that they have close to hand, i.e. their own trees. For productivity and sustainability reasons it will be necessary to change the culture of making do with one's own or a neighbour's unimproved seed. In some cases there may be a lack of improved material to supply, but in most cocoa-producing countries officially approved planting materials will be an improvement on farmers' own selections. There can also be reluctance to use any form of new planting material at all; many examples can be found where trees are 'inherited', i.e. planted by a previous owner or grower and dutifully maintained over decades, even when giving poor and declining yields.

The global demand for cocoa has grown consistently over the years at around two to three percent per year. Predominantly consumed in the form of chocolate, levels of cocoa consumption in a particular country tend to follow advances in economic development, with marked increases being seen in several Asian markets in recent years. The potential for further increased demand for cocoa is clear and estimates have cited the need for an extra one million tonnes of cocoa by 2020 to keep pace with the increased demand and this would mean at least an additional one million tonnes by 2030. Production levels of cocoa in several countries are under threat for several reasons: ageing tree stocks; the use of non-improved planting materials, as described above; incidence of pests and diseases; and declining soil fertility. Increased use of better planting material lies at the heart of not only keeping pace with the increasing demand for cocoa, but also in the drive to make cocoa production more sustainable. Better varieties of cacao with improved yields and able to resist attacks from pests and diseases would enable a reduced overall environmental footprint for cocoa production, deforestation, and allow other tree crops to be grown in the same growing areas. A major obstacle in achieving this higher producing, more sustainable objective in cocoa production is the supply of significant volumes of improved planting material and the supply of better cacao plants to farmers.

This review describes the various methods currently available for the production and supply of large numbers of cacao plants to growers. Current global cocoa production is around four million tonnes per year, with average yields estimated at 400kg of cocoa beans per hectare, using an approximate planting density of around 1,100 trees per hectare. Assuming an annual ten percent tree replacement rate on farm (replanting to substitute old, dead, or diseased unproductive trees), a conservative estimate of new planting material needs would therefore be around one billionunits per year to be supplied to farmers. However, the current reality is very far removed from this. Although it is very hard to estimate the current supply of improved cacao planting material on a global scale, some studies have estimated the supply of improved plants to farmers in West Africa to be between ten and fifty percent of potential demand.

It seems that the cocoa farming sector, unlike some other crop systems, doesn't routinely replace older, less productive plants/trees. It's not clear if this is due to inertia, lack of knowledge and training, lack of supply (due to resource constraints), lack of significantly improved planting material, or low confidence in the planting material being offered or a combination of any or all of these. This review aims to fill some knowledge gaps on cacao propagation, detailing the various approaches to supplying large numbers of improved plants to farmers, and describing the advantages and challenges of each.

The review presented a real challenge mainly due to the lack of published materials available and with most published data dating back to the 1950s. The particular position of cocoa globally with different techniques, countries, environments, economies, pests and diseases, industry and consumers' requirements means that pulling together all existing knowledge and formulating recommendations proved to be a difficult task. But it is hoped that the result of the efforts of the key authors provides a basis to build on for case-specific recommendations.

All cocoa-producing countries need a policy for developing or acquiring better planting material, and a strategy on how to propagate it and supply plants to farmers. This principle is repeatedly highlighted at international conferences, workshops and project meetings, and in discussions with cocoa-sector stakeholders. Regardless of production volumes or status of producing countries, it is not sustainable to have farms with old, poorly producing trees. We hope that this review will be useful to both new countries and regions just starting to grow cacao, and those established cocoa-producing countries looking to update and modernize their planting-material production systems. This review is not aimed at smallholder farmers, although it has been written with their continued profitable involvement with growing cacao very much in mind. The information in the review is aimed at policymakers, cocoa sector boards, countries new to cocoa, larger landowners contemplating growing cacao, and producing-country public-private partnerships. As the supply of new improved planting material to farmers is at the heart of improving cocoa productivity and modernizing the crop, we hope that the information in the review will make its way into national cocoa plans, and help to make cocoa farming more attractive and more sustainable.

Martin Gilmour, R&D Director, Cocoa Sustainability, Mars Global Chocolate

Stephan Weise, Deputy Director General - Research – Research, Bioversity International

Chapter 1 – General introduction

1.1 Background

High performance planting material is the cornerstone of a sustainable cocoa economy. Cacao competes with other crops for suitable land and labour, and the sector must ensure that farmers have access to good cacao planting materials as part of a package of measures to increase productivity and thereby improve the overall economy of their farms. Many recent initiatives, including donor-funded projects and industry-supported farmer training programmes, have largely concentrated on short-term interventions, such as managing pests and diseases, maintaining and improving soil fertility, managing shade, and pruning existing trees into optimal architecture. The apparent slow decline in output from several cocoa-producing countries with aging cacao farms means that the time is now right to make a substantial effort in the development and supply of better planting material for farmers as part of an overall effort to improve the sustainability of cacao production.

Although some improved planting materials have been developed, or are under development, through national breeding programmes and collaborative efforts such as the projects "Cocoa Germplasm Utilization and Conservation: a Global Approach (1998-2004)" and "Cocoa Productivity and quality improvement: a Participatory Approach (2004-2010)" supported by the Common Fund for Commodities (CFC), the International Cocoa Organization (ICCO) and coordinated by Bioversity International (Eskes 2006, 2011), farmers are often unable to access supplies of these materials, either directly themselves or via national or donor-supported rehabilitation/farmer training programmes. There is a clear need for a status report on the current technologies used to propagate improved planting materials, and the prospects for improving and scaling up these mechanisms to meet the increasing demand.

The current techniques used to propagate cacao-planting materials include:

- Seed propagation: although many farmers plant seeds from pods harvested from their own trees or obtained from neighbouring farmers, improved seedling varieties have been developed and are being produced and distributed in large numbers from national and/or commercial seed gardens. Some farmers prefer to plant the seed directly in their fields (also known as 'planting at stake'), whilst others sow the seed in a nursery, or obtain young seedlings for planting out at three- to six-months old, often depending on weather conditions.
- Conventional vegetative propagation: various techniques may be used to bud or graft materials from improved varieties onto seedling rootstocks in the nursery, or onto plants already growing in the field. The latter can include side grafting onto the trunk, lateral branches or upright suckers ('chupons') of mature trees. Once the bud or graft has established ('takes'), the aerial part of the rootstock/older part of the tree is cut away. Another technique involves producing rooted cuttings of improved materials. Usually these operations are carried out professionally, with the establishment of a clonal garden to supply budwood/graftwood or cuttings of the improved types, and nursery facilities in order to supply farmers with young container-grown plants. Graftwood can be obtained from clonal gardens for use in professional field-grafting operations, and, occasionally, for use by the farmers themselves.
- Tissue culture: this technique involves the regeneration of somatic embryos from floral tissues and the maturation of these embryos, *in vitro* through tissue culture, into plantlets. Laboratory-based procedures are used to multiply large numbers of selected, improved varieties, which are supplied to farmers as young plants.

- Coppicing: this method entails cutting the main trunk of the old tree and allowing new chupons to grow; it is not really a technique that involves new planting materials, but it can be used to rejuvenate ageing cocoa trees and cocoa farms.

Although the term "cocoa" is generally used for the plant and its products in many English speaking countries, this document will refer to "cacao" for the plant and the unprocessed seeds of the species *Theobroma cacao*. Once the cacao seeds, commonly known as "beans", are harvested, fermented and dried, the product is known as cocoa.

1.2 Objectives and approach to this review

This review of cacao propagation methods is based on three technical reports that were developed by the authors in the areas of their expertise *vis-a-vis* the following main propagation techniques:

- Seed propagation, with particular emphasis on West Africa – Rob Lockwood
- Conventional vegetative propagation, with particular emphasis on the situation in the state of Bahia in Brazil – Augusto R. Sena Gomes and George A. Sodré
- Tissue culture, particularly the evaluation of the somatic embryogenesis (SE) technique and its application in Latin America and Indonesia – Siela Maximova and Mark Guiltinan.

The technical reports were consolidated into one overarching, multi-institutional, multi-authored expert report on cacao propagation methods. A broader group of experts reviewed the consolidated report, and a workshop was organized to discuss and review the technical findings and provide input to the final report. The workshop took place from 13-15 May 2013, in London, UK and involved additional experts (see Annex 1, List of participants). Preliminary findings were also presented and discussed at the INGENIC meeting in Cameroon in October 2012 and during the WCF Partnership meeting in Washington in June 2013.

Chapter 1 introduces the context of cacao production and its propagation. It then presents the objectives and approach for this review. It provides an overview of aspects of cacao morphology and biology which may have some impact on the propagation methods used. Finally it introduces the key issues that constrain availability of improved cacao planting materials.

Chapter 2 covers cacao propagation by seeds. It outlines seed-garden and farmer-nursery management and use, including articulating their current status at a county-specific level and more widely. The chapter concludes by examining seed-propagation costs by highlighting key challenges to seed propagation and by offering recommendations for improvements.

Chapter 3 examines conventional vegetative propagation in cacao, including an overview of the main methods, current status, advantages and constraints, costings based on experience in Brazil, risk management and future perspectives and research. The aspects covered include: rootstock production, rooted cuttings; grafting; budding; marcotting or air layering; and management of clonal gardens.

Chapter 4 looks at tissue culture as a means of cacao propagation, mainly via somatic embryogenesis. It examines current status, costs, advantages and constraints of tissue culture, the possibilities of combining somatic embryogenesis with conventional propagation techniques in an integrated system and future perspectives.

Chapter 5 articulates cross-cutting issues and offers overall conclusions. Issues relate to: the availability of recommended varieties/clones proven for each technique; technologies and facilities for propagation; labour, skill requirements and farmer training; phytosanitary aspects; predicting demand for planting materials; choosing propagation methods; production objectives, scales and timeframes; and legal, technical, institutional, and financial considerations.

The Annexes provide more information, including lists of the key experts involved in the review, acronyms and abbreviations and references. It also includes detailed information linked to Chapters 3, 4 and 5. For the Chapter 3, seed production capacity is summarized for the principal cocoa-producing countries, with an account of each country provided, including the genetic background to cacao cultivation and, where available, the estimated uptake of improved planting material. For Chapter 4, more information on cacao propagation planning and management for conventional vegetative propagation is provided Annex 5 includes a summary of the status of application of methodologies and a summary of the main characteristics of each of the propagation techniques together with notes on their advantages, and points for consideration, challenges and opportunities.

The purpose of this publication is to present an impartial, evidence-based review of cacao propagation methods, which could serve as a basis for the assessment and implementation of strategies for providing farmers with quality planting materials that are adapted to current and future conditions and needs (cultural, institutional, technical, environmental and financial). In summary, the review sets out to: (1) compile relevant scientific background information on each of the propagation methods (technical, genetic, plant health, economic, situational); and (2) analyse the advantages and constraints, including current supply limitations and costings for each of the propagation methods.

The aim of the review is to enhance our understanding regarding the current supply of planting materials and the possibilities available to increase the supply of affordable, high quality materials, and thus achieve one of the main objectives of the Global Cocoa Agenda developed by the International Cocoa Organization (ICCO) during the First World Cocoa Conference (WCC1), in November 2012 in Abidjan and re-enforced during the Second World Cocoa Conference (WCC2), in June 2014 in Amsterdam. The target audiences of this review include ministries of agriculture, cocoa boards and relevant authorities in current and prospective cocoa-producing countries, policy-makers, donors, investors, researchers, farmers and other decision-makers.

1.3 Cacao morphology and development

Detailed descriptions of the morphology of cacao are available from a number of sources, such as Wood and Lass (1985), Niemenak et al. (2010). We provide a summary of these morphological descriptions to clarify some of the terms used in this review, and to highlight the impact that cacao's morphology and physiology have had on the techniques used to propagate it, either by seed (sexual) or through vegetative propagation (asexual, clonal) techniques, including somatic embryogenesis.

Cacao is characterized by its dimorphic architecture, with both orthotropic (upright) and plagiotropic (lateral) branching. Germinating cacao seeds (often referred to as 'beans') first produce a root. Then the hypocotyl elongates to raise the cotyledons above ground level. Because there are no dormant buds on the stem of the hypocotyl, it is possible to graft a bud from the desired clone onto a seedling chosen to be the rootstock without the risk of unwanted shoots developing from the seedling rootstock, providing the graft is made below the cotyledon scar and the rootstock stem is removed immediately above the bud from the clone. Once the cotyledons have opened out, the plumule usually produces four leaves with very short internodes. Subsequent leaves on the orthotropic stem are arranged spirally and each has a bud in its axil. When the plant reaches a height of between 1m and 2m, vertical growth ceases with the formation of a number of buds (commonly five) at its apex, each with a very short internode; this is known as a jorquette, where the buds grow out laterally and simultaneously to form a fan, or plagiotropic sylleptic branches. The leaves on plagiotropic branches are arranged alternately. If the apical bud is damaged before jorquetting, or the young plant is bent, buds lower down the stem will develop to form chupons (proleptic branches), all with the orthotropic growth habit (see Figure 1.1).

After several years of growth, at least one bud will emerge just below the jorquette, developing into a chupon, and vertical growth recommences. This pattern may be repeated several times, with trees reaching heights of up to 20m in the forest, and while under cultivation they should be pruned to a more manageable height. There is considerable genetic variation in tree architecture, for example in the height of the primary jorquette, chupon formation and branch angle, though this is also greatly influenced by environmental and agronomic factors. As described in detail later in this review, cacao can be vegetatively propagated using various techniques from both orthotropic and plagiotropic source-materials, and the technique used will also influence the architecture of the resulting tree. Moreover, the propagation technique used appears to affect the vegetative vigour of the resulting plant. Unlike most tropical trees, where the normal response following vegetative propagation is an increase in vigour (Roger Leakey, personal communication to Rob Lockwood, 14 May 2013), in cocoa breeding trials conventional vegetative propagation has been shown to reduce vegetative vigour and so yield at the optimum planting density of the corresponding seedling population (Pang and Lockwood, 2008, Nsiah and Lockwood, 2012.). The underlying mechanisms have yet to be elucidated, but Lockwood (personal communication, 2014) suggests that since several studies have found little difference between the performance of cuttings and budded material, the reduction in vigour appears to be associated with all conventional vegetative propagation of cacao. The vigour of the seedling population is restored when the conventionally propagated material is propagated by the somatic embryogenesis method (Masseret et al. 2006, Paragraph 4.2.1 of this review).

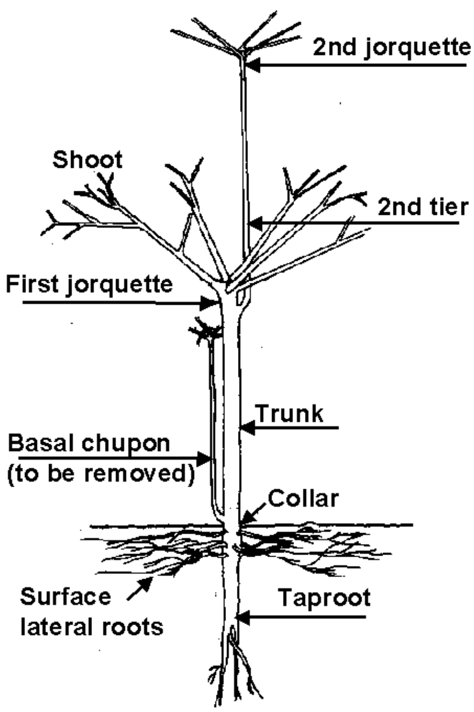

Figure 1.1 General structure of a seedling cacao tree.
Courtesy EcoPort (http://ecoport.org) : Mossu G. (image ID 3187)

Cacao plants are cauliflorous, producing flowers and fruits on the trunk and branches from 12-months after planting, depending on the genotype and growing conditions. The inflorescence is a highly compressed dichasial cyme, emerging from a leaf axil. Many flowers develop in the same positions; these sites eventually become enlarged due to secondary thickening and are known as 'cushions'. Flower development takes around 45 days from differentiation to anthesis, with the rapid expansion of the flower bud, from the size of a pinhead to fully grown, taking place in the last 10-12 days before anthesis.

Under certain conditions cacao trees can produce flowers and fruit all year round, but in most places this is markedly seasonal. Flowering intensity and the growth rhythms of the inflorescences are affected by environmental factors, particularly rainfall/water availability, light intensity, existing fruit load, and genetic factors (further details are provided in Lockwood, Chapter 2, in this document).

The structure of the cacao flower is shown in Figure 1.2. The flower has a ring of conspicuous staminodes, often dark red in colour, which surround the ovary like a crown. The fertile stamens are much less obvious, and are positioned lower down inside the flower with the anthers hooked inside the pouches of the translucent petal hood. The petal hood forms a physical barrier between the gynoecium and the anther, so that without disturbance or insect visitation, pollen from within the same flower is unlikely to cause accidental self-pollination.

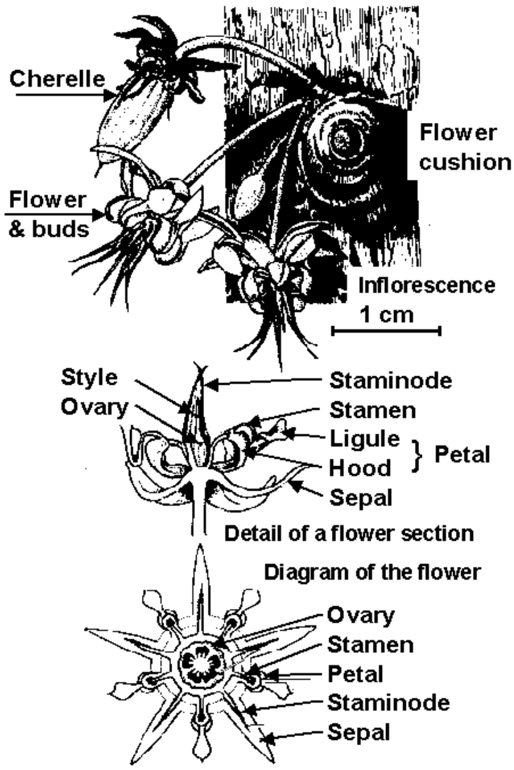

Figure 1.2 Inflorescence and flower of the cacao tree
Courtesy: EcoPort (http://ecoport.org) : Halle N. (image ID 3188)

Pollination is carried out mainly by ceratopogonid flying midges but other small flying and crawling insects may also be involved (Posnette 1950, Glendinning 1972); however, since the latter are likely to be restricted to the flowers of a single tree, they would only be successful pollinators on self-compatible genotypes.

Cacao has an unusual and complex sexual incompatibility system, which is affected by genetic and environmental factors. Classically, the compatibility reaction was considered to be absolute: a tree would either set flowers with its own pollen (self-compatible) or it would not (self-incompatible); and self-incompatible trees would only set flowers on cross-pollination with another tree expressing a different compatibility allele; over 20 of such alleles have been identified to date (see for example, Glendinning 1966). However, from the early 1970s it became apparent that some alleles, particularly allele 2 as identified by Knight and Rogers (1955), are quantitative in effect and thus the incompatibility mechanism can break down under some circumstances. Bartley and Cope (1973) carried out an exhaustive examination of the genetic relationships between incompatibility alleles in cacao, and suggested that a mechanism had evolved that could permit normal self-incompatibility to be overcome in situations where cross-pollination is rare, thus allowing the occasional selfing of an isolated self-incompatible tree. Furthermore, it seems that the alleles vary in effectiveness. Lanaud et al. (1987) provide quantitative estimates of the extent of self-fertilization in several genotypes grown in environments with natural pollination.

Although the genetic and physiological basis of the mechanism has not yet been fully understood, data from extensive cross-compatibility and inheritance studies suggest that three independent genes are involved, along with two levels of control, occurring at the sporophytic and gametophytic levels (Cope, 1962). More recent physiological and microscopical investigations have not yet fully elucidated the mechanism, but it is likely that several factors are involved, including gametic nuclear fusion/non-fusion and peduncle thickening/floral abscission (Ford 2004, Royaert et al. 2011). It seems that the system can be manipulated to increase the chances of obtaining seed arising from the self-pollination of normally self-incompatible clones, or seed from normally cross-incompatible parents, using mixtures of compatible and incompatible/mentor pollen (Lanaud et al. 1987, Opeke and Jacob 1969, Posnette 1940), though Lockwood (Personal Communication 2014) comments that the incompatibility reactions of many of the clones and crosses in these studies are known to be liable to breakdown. The incompatibility reaction can also be manipulated by increasing the carbon dioxide levels around the flower for six hours following pollination (Aneja et al. 1994), although the underlying mechanisms have yet to be determined.

1.4 Availability of improved planting materials: key issues

Detailed descriptions of cacao genetic resources, including the genetic diversity of the crop, its history and nomenclature, and the current status of the types grown, can be found in a number of sources including Bartley (2005), Dias (2001), and Wood and Lass (1985), and are summarized in the Global Strategy for the Conservation and Use of Cacao Genetic Resources, as the Foundation for a Sustainable Cocoa Economy (CacaoNet, 2012). Information on the names of varieties and clones mentioned in this report can also be found in the International Cocoa Germplasm Database (ICGD), which is accessible at: www.icgd.rdg.ac.uk. A range of formats for clone names are used in the literature, such that the name may appear with or without hyphens, or with different use of capital letters (for example, "LCT-EEN 37/A" or "Lcteen 37A"). The formats used here are those suggested by the authors but the ICGD can be used to resolve any synonymy.

It has been estimated that currently only about 25% of all cacao plantings consist of improved varieties. If farmers cannot access improved materials, or cannot afford to do so, which is the case to a greater or lesser extent in many cacao-growing regions, particularly in West Africa and Indonesia, the farmers use their own materials, or those of neighbouring farmers, for propagation. Where farmers cultivate traditional varieties, such as the homozygous and self-compatible West African Amelonado, Brazilian Comum, Ecuadorian Nacional or Venezuelan Criollo, it is likely that the material will be remain genetically uniform even when propagated by seed. However, poor husbandry and susceptibility to one or more pests and/or diseases can result in poor yields. Where the source material is non-true breeding, such as the Trinitario hybrid complex, improved bi-parental varieties and other heterozygous types, the farmers are likely to experience problems with even greater variability in the performance of their tree stocks when they propagate by seed. This has been a particular problem in West Africa where farmers continued their traditional practice of propagating using seed from their own trees following the release of the open-pollinated mixed Amazonian variety (see Rogers 1955, Hammond 1958), and subsequently following the distribution of bi-parental crosses from seed gardens. Some farmers try to identify their best trees for yield (sometimes referred to as 'super trees') and either harvest seed from them (e.g. in West Africa), or graft them onto those trees that are considered less productive (e.g. in Latin America) in an attempt to improve their tree stocks. However, there are constraints to attempting to use the yield of a tree as a simple indicator of its potential to contribute its yield characteristics to its offspring, as described by Lockwood, Owusu-Ansah and Adu-Ampomah (2007).

The use of cacao germplasm for selecting varieties with improved agronomic performance and/or quality characteristics has a long history, and many cocoa-producing countries have ongoing breeding programmes with objectives tailored to their own farmers' needs and the market requirements for their cacao beans. Whilst yield, tree architecture and adaptation to local growing conditions continue to be important selection criteria, many programmes also focus on improving resistance/tolerance to endemic pests and diseases, and incorporating resistance to threats that have devastated cacao production in other regions. Bean quality characteristics, including weight, cocoa butter content and flavour potential must also be addressed to ensure that new releases meet industry expectations. The production of improved types is a very long and expensive process requiring many years of careful management, meticulous data collection and analysis, and a thorough testing of promising materials under different farm conditions, before they can be considered as proven and suitable for release to farmers.

This review does not set out to provide fully detailed information on cacao breeding programmes and their outputs, nor does it make recommendations on planting materials for use in any particular area. However, the individual chapters on method provide some information on the recommended materials that have been, or are being propagated by seed, vegetative or tissue-culture techniques. In addition, an extensive review of the history of seed variety development and the implementation of recommendations in seed gardens, as well as information on recommended clones in some of the main cocoa-producing countries, is provided in Annex 3.

The availability of improved types, proven suitable for a particular region, is of overarching importance when considering the most appropriate propagation method to use for supplying farmers with new planting materials. In Latin America and the Caribbean, much of the recent breeding work has been targeted at the identification of clones that have resistance to the prevalent diseases (mainly witches' broom, and/or frosty pod and black pod diseases), and which can produce cocoa with the desired quality characteristics (often fine-flavour profiles). Most farmers in West Africa currently use seedling planting materials. The breeding programmes in the region have been largely focused on the development of high yielding seedling varieties with resistance to black pod disease and, in some cases, cocoa swollen shoot virus (CSSV) disease; and on the quality characteristics required for mainstream cocoa (good cocoa flavour and high butter-fat content). However, several recent initiatives focus on developing clones suitable for use in West Africa; though it will be many years before the first outputs from these initiatives will have been thoroughly proven. In South-east Asia, breeding for both seedling varieties and clones has been carried out, though most of the large breeding programmes set up by plantation companies in Malaysia in the 1970s/1980s are no longer in existence. Successful clones produced from these programmes are still in use in the region today.

Although farmers may seek uniformity in tree architecture and production within their individual fields, extensive use of very closely related or clonal material across a wide area increases the vulnerability of the crop to changes in local growing conditions and to threats of pests and diseases - whether due to the evolution of more virulent strains of native diseases, or to the accidental or natural introduction of new pest species into cacao-growing areas. It is therefore important to ensure that farmers are provided with an appropriate mixture of clones or seed varieties that have been confirmed as suitable for planting together, taking into account agronomic and genetic factors such as tree architecture and vigour, nutritional requirements, period of flowering, and pollination compatibility issues. It is also essential that the planting materials that are provided produce cocoa of the quality required by the manufacturers. Since farmers have no easy means of assessing characteristics such as cocoa butter content and flavour potential of their cacao, it is vital that the types distributed have been thoroughly evaluated to ensure that their quality characteristics meet industry expectations and that they are stable across different growing environments.

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Chapter 2 – Propagation by seeds

Rob Lockwood [1]

2.1 Description of the method

2.1.1 Background

Seeds are the products of sexual reproduction where fertilization of a female gamete by a male gamete is necessary to produce a zygote (seed). As outlined in Chapter 1.3, the flower morphology and incompatibility system of cacao favour out-breeding, leading to high levels of heterozygosity in some cacao types. However, there are other types/populations that are highly homozygous, either due to self-fertilization, or cross-fertilization by a genetically closely related type over many generations by natural means, or through a breeding programme using controlled self-fertilization or a double haploid approach (pure or inbred lines). The levels of variability of any seed-propagated populations will depend on the levels of heterozygosity of the parents and environmental interactions. If a highly homozygous type is self-fertilized, the seedling progeny produced may be genetically uniform (sometimes known as 'clonal seed'), though they may not have desirable yield or other characteristics due to being intrinsically poor genotypes or the effects of inbreeding depression. If two clones are crossed, then the resulting seed is known as 'hybrid' (strictly speaking it should be referred to as a 'bi-parental cross' unless at least one clone is homozygous) and will give rise to a genetically uniform population, which will possess good characteristics providing the parental types have been selected well. However, even genetically uniform populations are not necessarily uniform for yield, as experience with clone trials in Ghana and in Trinidad shows.

There are very few inbred clones available in cacao, and most breeding work has focused on the selection of parental clones which, though they might be heterozygous at least to some extent, when cross-fertilized produce a progeny with the desired yield characteristics and acceptable levels of variability. Bi-parental crosses account for the vast majority of the output of the current cacao seed gardens.

In this review, the topic of seed gardens is discussed mainly in the context of West Africa. This region produces 73% of the world's cocoa (See ICCO, 2013/2014 estimates), thus driving the global cocoa economy, and will remain the principal producer for the foreseeable future.

Selection of self-compatible trees to produce clonal seed by natural pollination within a mono-block was underway in the 1920s, with the ineffectiveness of single plant selection for yield already recognized (Harland 1928), even if largely ignored since. Posnette's (1951) pursuit of clonal seed led to the establishment of blocks of clone E1 (which turned out to be self-incompatible) in Ghana in the 1950s (Hammond 1955, Glendinning and Edwards 1962) but they were superseded by bi-parental crosses (referred to in the literature as hybrids, although they did not involve highly inbred parents) and were never used. Clonal seed was also an early breeding objective in Papua New Guinea (Bridgland 1960), Cameroon (Liabeuf 1960) and Nigeria (Toxopeus 1969a); but as in Ghana, efforts in this area were discontinued when interest turned to the development of bi-parental crosses.

The precocity and high early yields of the first bi-parental crosses between Pound's (1938) Upper Amazon material and local selections in Ghana (McKelvie 1957) and Trinidad (Montserin, de Verteuil and Freeman 1957), prompted a rapid development of the capacity to reproduce them in bulk. Indeed, distribution of the parental clones needed to establish the

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seed gardens in Ghana, the former British Cameroon now Province of West Cameroon and Nigeria (and later in Sierra Leone) began in 1956, only four years after the first trial was planted.

2.1.2 The first seed gardens

The layout of the first bi-clonal seed gardens in Ghana was described by Glendinning and Edwards (1962). Similarly designed seed gardens followed through the rest of West Africa, and in Brazil, Costa Rica, Ecuador, India, Indonesia, Malaysia, Papua New Guinea and the Philippines. The polyclonal seed gardens described by Edwards (1969) and by Toxopeus (1969b) worked to the same principles. Choices of isolation distances and proportions of the two parental clones were based on:

- The self-incompatibility of at least one parent, usually the Upper Amazon, although in one case in West Africa the Trinitario was the self-incompatible clone, and in others both parents were self-incompatible. Self-incompatibility was believed to be effective in preventing self-fertilization.
- Extensive studies of pollen movement by insects, reviewed by Glendinning (1972).

Edwards (1975) described how the yields of seed gardens set up in the 1950s/1960s in Ghana had been limited by the differences in establishment ability, growth and flowering characteristics of the parental clones. Furthermore, the biggest demand for seed was in the June to mid-October period for direct sowing in the field, but the natural peak of cropping was in November and December. In typical cacao-growing areas with a bi-modal rainfall pattern, harvesting peaks occur about six months after the main rains, when rainfall is insufficient to allow the establishment of cacao by sowing directly in the field without irrigation.

Later, Lanaud et al. (1987) showed that in seed gardens that relied upon self-incompatibility and natural pollination in West Africa, 20-97% of seeds were produced by self-fertilization rather than cross-fertilization, and were not bi-parental crosses. This may explain why in the late 1970s Malaysian planters preferred seed that was produced by manual pollination of freshly opened flowers (David Lim Hong Kee, personal communication, 1982) to the naturally pollinated alternative. Indeed, Ooi Ling Hoak (1981) reported from Malaysia that for the same crosses, manually pollinated materials out-yielded seed garden materials by 7-19%. Later, N'Goran, Clement and Sounigo (2000) estimated that seeds produced by manual pollination out-yielded open-pollinated seed by about 40% in Côte d'Ivoire. The high frequency of failure of the incompatibility mechanism to prevent self-fertilization may go a long way towards explaining farmers' disappointment with "hybrid" seed produced by natural pollination.

2.1.3 Mass manual pollination of freshly opened flowers

Edwards (1973) investigated the use of mass manual pollination of freshly opened flowers (Figure 2.1) as an alternative to natural pollination and for use in Ghana's original seed gardens, which were in effect monoclone blocks of (nominally) self-incompatible clones. Manual pollination is carried out by touching the anther anywhere on the stigma or style, which is receptive along its full length. The pollen and stigma/style are sticky.

Figure 2.1. Manual pollination of freshly opened flowers.
Photo credit: Andrew Daymond

Using the dominant morphological marker gene 'axil spot', Edwards (1972a, 1972b*)* showed that when flowers were plentiful and pollinations were completed over a short period of time, ideally on one day, contamination by extraneous pollen was usually less than 5%, which many people consider to be acceptable. Bastide and Sounigo (1993) made a similar estimate of contamination. Skilled pollinators averaged about 125 pollinations/hour (Edwards 1972a), with about two thirds of manual pollinations recovered as ripe pods (Edwards 1972b).

Seed production at more than 95% purity by mass manual pollination of freshly opened flowers depends on the following three features of cacao and its interaction with its natural pollinators:

- Freshly opened cacao flowers are easily recognized because their anthers are creamy-white in colour when viewed through the translucent anther hoods. The anthers turn brown within 24 hours of anthesis.
- When cacao flowers are plentiful, only a small proportion - less than 5% - will be visited by pollinating insects.
- During the main flowering seasons, rigorous removal of all natural sets stimulates flowering.
-

Monoclonal blocks of nominally self-incompatible clones used as pod-bearing parents are usually used for seed production by mass manual pollination of freshly opened flowers. Such blocks offer some protection against successful self-fertilization due to natural pollination (even by a neighbouring tree) and are easier to manage than polyclonal blocks. If isolation is required, the larger the blocks the better.

Pollen parents are grown in nearby blocks, close to the female blocks so that the manual pollinators do not have to travel far when collecting flowers that will provide pollen. It is not known what ratio of pod-bearing trees to pollen parent trees is appropriate, perhaps 33:1, depending on how many pollen parents are used for each pod-bearing clone. Both pod-bearing and pollen parents should be stripped of all natural sets at all times, in order to stimulate flowering and minimize the risk (and temptation) to harvest natural sets as seed pods. The training of manual pollinators is key to success. The suggested standards are:

- Minimum 125 pollinations/hour
- Minimum 75% setting measured ten days after manual pollination.

High work rates and high setting rates are required partly because purity of seed production depends on saturating the population of flowers with the intended pollen, and partly to minimize costs.

On pollinating days, a trusted person should collect freshly opened flowers at first light. They can be placed in plastic pots. A skilled pollinator will need about 150 flowers/day, bearing in mind that each anther is used only once, although Edwards (1972a) showed that individual anthers can be used up to five times, which is useful when pollen is scarce.

Manual pollination should start as early in the day as is possible, in order to minimize the time in which natural pollination can occur. If the trees have been prepared properly, at the right times of the year, there will be more than enough flowers to be able to set a full crop by pollinating flowers that are within reach of the ground.

Pollinations on a single tree should be done on one day. Under good conditions, the target crop is equivalent to about two tonnes of dry bean equivalent per hectare, approximately 50,000 pods/ha. The average number of pollinations per tree should be about 50% more than the average number of pods expected, so at 1,000 trees/ha, approximately 75 flowers per tree, but this will be adjusted up and down according to the size and condition of the individual trees.

Synchronized mass manual pollination produces cherelles of uniform age with little competition between them, which circumvents the normal crop-limiting mechanism, cherelle wilt. Trees are weakened if over-cropped, the first signs being reduced flushing and smaller pods. If the signs are ignored and over-cropping continues, the pods get even smaller and tend to ripen prematurely. If this too is ignored, flushing ceases and the tree dies, even if manual pollination has stopped.

Figure 2.2. Clone C42 bearing seed pods in Jamasi seed garden, Ghana.
Photo credit: Rob Lockwood 2012

If the trees are properly prepared, all the pollinations can be done within reach of the ground, even when there is a single stem. The procedure is to remove all set and stale flowers, and to pollinate all freshly opened ones, cushion by cushion, up to the maximum number of pollinations required. When flowers on a cushion are pollinated, the unopened buds should be removed; all unused, freshly opened flowers should also be removed. There is no need to mark the flowers that have been pollinated.

A week after manual pollination, any new flower buds are removed from the cushions with sets, and any sets from cushions that were not pollinated manually. A week later natural sets are removed again, and then at monthly intervals.

This procedure for seed production by mass manual pollination of freshly opened flowers is easy to describe and to understand, but implementing it successfully in the field, to the standards that are required day in, day out; year in, year out depends on good planning and skilled management. As Bastide and Sounigo (1993) reported, the process is not as easy as it appears when carried out by experienced teams such as Ghana's Seed Production Unit. If multiple, village-level seed gardens are established, a management system will be required to ensure that they are managed well enough and consistently enough over their 40-year life to ensure the high standard of seed purity that farmers need and deserve.

2.1.4 Farmers' nurseries

Seeds may also be sown 'at stake' rather than in a nursery, i.e. directly at their final growing positions, as was traditionally carried out in West Africa and in Brazil, and there is no cash expenditure involved. In Ghana, Oppong et al. (1999) obtained satisfactory results from planting seed at stake during the main rains, but not the later minor rains, with a good survival rate through the first dry season provided that there was adequate temporary shade. This may well be the cheapest possible way of establishing a cacao farm and as such may persist in West Africa, with major implications on both the capacity to produce improved seeds and the timeliness of their availability.

In West Africa, the technologies used by farmers in cacao nurseries range from sowing seeds from their own trees in the ground near a water source, and transplanting bare-root plants into the field; to planting high potential seeds purchased from seed gardens, in polybags under controlled amounts of shade, and tending to the plants at permanent sites until both they and the field conditions favour planting in their final position. As much of the cacao planting is new and often in remote places, and since planting may be completed in one season, the nurseries are temporary with minimal costs involved. For example, discarded drinking water sachets are used as containers.

2.1.5 Seed gardens: genetic base and utilization

Invaluable insights into the genetic base and its utilization in supplying cacao farmers globally are available from the inventory of parents (or grandparents) that have been planted for seed production in Brazil, Cameroon, Costa Rica, Côte d'Ivoire (incomplete information), Dominican Republic (incomplete information), Ghana, Indonesia, Malaysia (incomplete information), Nigeria, Papua New Guinea and Togo (see Annex 3). The main breeding populations, and the number of representatives from each that have been used, are shown in Table 2.1. Details on these populations can be found in the International Cocoa Germplasm Database (ICGD) (www.icgd.rdg.ac.uk).

The first feature of an analysis of the detailed information on parents used in West Africa, and especially from Nigeria in the East to Côte d'Ivoire in the West, is that it seems that only a relatively narrow genetic base has been exploited, though in Côte d'Ivoire and Ghana a much broader base is being evaluated, and soon a wider range of parents will be used for seed production.

Table 2.1. Breeding populations used in the parental lines of seed gardens in the major cocoa-producing countries.

Population	Number of representatives	Notes
Be	4	
Catongo	1	
CAS	2	
CC	3	
EET	8	
GC	2	
Gu	2	
ICS	15	Includes UIT1 and UIT2 which are ICS clones of Nicaraguan Criollo type and 'Pa35'
IMC	9	
Javilla	1	An Ecuadorian Refractario type
Locally selected Trinitarios	21	
Ma	5	
Mocorongo	1	
Nanay	6	
Parinari	24	
Pound	4	
Sca	3	
SIAL	6	
SPa	1	
UF	8	
West African Amelonado	1	

Globally, given the geographic and phenotypic diversity of the material that has been used to produce new seedling varieties, the genetic base of cacao cultivation appears to be relatively rich, in contrast to say bananas, Arabica coffee, oil palm and rubber, other major tropical perennial crops in which the breeders have made much greater progress. Clones such as CCN51 represent a further broadening of the base.

The second feature of the analysis of the parents that have been used is that many of them were either collected from uncultivated cacao growing in and around the area of the centre of diversity of the species, or are first or second generation descendants from them. This cannot be described as 'population improvement' or recurrent selection, as almost invariably the individual trees that were used to produce the next generation were selected at random, or at best on the basis of single tree selection for yield, which is known to be ineffective (e.g. Harland 1928, see also Lockwood, Owusu-Ansah and Adu-Ampomah 2007).

As Bartley (1981, p. 520) observed in his discussion of attempts to exploit genetic variation 'a large proportion of these selections have never reached the stage of evaluation or have been utilized. Among the remainder, the majority, as would be expected, are scarcely an improvement on the average of the population from which they were derived'.

In some instances, the candidate parents were evaluated as clones prior to use – for example, the Keravat Trinitarios and of course W.E. Freeman's TSA and TSH selections in Trinidad, but these were the exception rather than the rule. In one programme, the clones were evaluated after their use as parents: sentence first, verdict afterwards (Lewis Carroll 1865). As Nsiah and Lockwood (2012) observed, clone evaluation is the single plant selection phase of a recurrent selection programme, and seedling breeding will not achieve its potential until it is supported by very large-scale and rigorous clone selection programmes. The problem in cacao breeding is less a lack of genetic resources, more a failure to utilize them effectively using proven breeding methods. The intermediate quarantine system, supported at various times by the governments of Britain, France, the Netherlands and the United States, and by the confectionery industry, has done a superlative job in safely distributing a large amount of germplasm.

The third feature is that several clones have become favourites across continents and indeed over continents. Sometimes this may have been because they moved through intermediate quarantine relatively early, for example IMC67, but this does not explain why clones that were distributed more recently, such as Pa150 and Pound7, are universally important parents. Furthermore, some of the more recently introduced populations, such as Be and Ma, which have been used for seed production in Brazil, have shown promise in West Africa, as Adomako, Allen and Adu-Ampomah's (1999) data show. Indeed, while working in Sabah, Rob Lockwood and Joe Pang Thau Yin found that clones that were good parents in Ghana usually performed well as parents unless they were unduly susceptible to infection with vascular streak dieback disease (caused by infection with *Ceratobasidium (*formerly *Oncobasidium) theobromae*).

2.2 Current status of propagation by seeds

2.2.1 Farmers' nurseries

In West Africa, on occasion, both individual entrepreneurs and communities operate nurseries of a more permanent nature. Freud et al. (2000) surveyed planting practices in Ghana and Côte d'Ivoire in 1994 (Table 2.2.).

Table 2.2. Planting practices in Ghana and Côte d'Ivoire, 1992-1994.

Planting practice	Farmers engaged in the practice in Ghana (%)	Farmers engaged in the practice in Côte d'Ivoire (%)
Directly sown seed	77	46
Bare-root seedlings	4	15
Polybag seedlings	11	22
Mixture	8	16

Gyamfi (1982) estimated that in the late 1970s, 40% of Ghanaian farmers used their own nurseries, 8% used seedlings from central nurseries, and the balance either sowed seed at stake or raised seedlings in nursery beds. Aguilar et al. (2003) estimated that between 1995 and 2002 the majority of farmers in Côte d'Ivoire were using polybag seedlings, with just a few using bare-root seedlings. The use of polybag seedlings is the norm in Sulawesi (Freud et al. 2000).

One of the earlier reports on different planting methods was that of Benstead and Wickens (1954). The yield of plants raised from 'basket' seedlings (i.e. seeds sown in a woven basket in the nursery in the days before polybags became available) was three times greater than those raised from seeds sown at stake, or transplanted as bare-root seedlings. In Ghana and Côte d'Ivoire there was a possible positive economic effect on yield of trees raised from polybag seedlings, while in Ghana a negative effect was noted when bare-root seedlings were used (Freud et al. 1999).

Quartey-Papafio surveyed nursery containers when polybags were just entering into use, discussing polybag seedlings, and seedlings grown in beds and lifted with a ball of soil. He pointed out that planting materials that have not been given a good start achieve poor subsequent growth until they overcome initial weakness, and that 'efficient nursery work is apt to be less expensive than inefficient work' (Quartey-Papafio 1964).

The logistical problems posed by the weight and bulk of seedlings grown in containers have stimulated interest in improved methods of planting bare-root seedlings. Hunter and Camacho (1960) cited Schenk (1935) as having investigated transplanting practices at the time and concluded that the least laborious, least expensive and most successful way of transplanting 15-month-old seedlings was to cut them off at 30cm height and to plant them using the bare-root method.

In experiments in which the plants were dug up with minimum root disturbance, covering the parts above ground with polybags was the most efficient but not the cheapest method of transplanting, and was also dependent on there being adequate shade in the field. Cutting back in the nursery a week before planting was the most cost-effective method (Hunter and Camacho 1960). Esan (1982) found that wrapping bare roots in damp newspaper and covering the aerial parts with a polybag gave the best results, and is attractive because it avoids the problem of transporting seedlings with balls of soil. However, in a four-year experiment in Ghana, Amoah et al. (1999) showed that transplanting seedlings with a ball of soil gave better results than other methods of packaging bare roots, with or without the application of clay slurry.

In Ghana, it was noted that bare-root seedlings transplanted two to three months after sowing could give good establishment provided that there was adequate shade and good rain (Amoah et al. 2000). Subsequently, the same authors found that six-month-old seedlings, whether polybag or bare root, grew faster than younger bare-root seedlings, and that the polybag seedlings were more precocious (Amoah et al. 2003).

O'Rourke (1954) reported that spraying plants with a liquid plastic resin greatly improved the establishment of both seedling and clonal plants. Alvim, Pinheiro Lima and Alfonso (1982) showed that the combined reduction of leaf number and use of anti-transpirants greatly improved the establishment of bare-root seedlings, whereas Souza et al. (1982a, 1982b) obtained best results by transplanting 2- to 6-month-old seedlings with a ball of soil, rather than by transplanting bare-root seedlings, direct sowing, or using anti-transpirants. Both Olofinboba and Fawole (1976), and Filho and Alvim (1978) found that the application of anti-transpirants in the greenhouse reduced water use.

While working in Nigeria, Freeman (1965) kept seedlings in the nursery for five to nine months under three shade regimes, using four sizes of polybags, and measured establishment and early growth. Freeman noted that there was no advantage in heavier shade, or in the use of bags larger than 15.7 x 25cm lay flat, and that smaller bags (12 x 25cm, or 12 x 30cm) produced inferior results. Similarly, no advantage was seen in keeping seedlings in the nursery for more than five to six months. Sowing should be avoided during 'Harmattan' conditions (associated with the dry and dusty West African trade wind that blows between the end of November and the middle of March) with attendant low night temperatures because this compromises germination (as noted by the author in Ghana). In West Africa, rodents are considered to be the principal pest in the nursery because they eat seeds and dig up seedlings (Everard 1964).

In Malaysia, 2- to 6-month-old polybag seedlings produced better results than younger or older ones, with the work confirming the unreliability of sowing directly in the field (Teoh and Shepherd 1972).

As would be expected, results from transplanting investigations were variable, perhaps because the general experience is that good shade in the field is the most important factor, and that reliable rainfall is required before, and for several weeks after, planting bare-root seedlings. The broad horticultural experience is that container-grown plants establish and grow better than bare-root seedlings so they are preferred whenever they are both affordable and practicable. The transition to polybag seedlings seems set to continue in West Africa. However, when estimating seed demand and hence seed garden capacity, less efficient seed utilization should be assumed, with the exact efficiency varying from country to country, and not readily quantifiable in some.

In a paper presented in 1981, Niamke (1982) reported that in Côte d'Ivoire, the extension services assisted farmers in creating nurseries, and supplied 45 seed pods from seed gardens per planned hectare of cacao, and later five further pods to raise replacement plants. The seed gardens appear to have fallen into disuse in the late 1980s. Freud et al. (1999) reported that based on a 1994 survey in Côte d'Ivoire, and computed on the basis of cacao area, farmers' planting materials comprised 25% Amelonado, 62% varieties from selected trees and 13% hybrids. Variety had no effect on yield. Between 1995 and 2002, the use of on-farm seed became generalized (Aguilar et al. 2003), with the proportion of farmers accessing improved hybrids falling from 7.5% in 1998, to 4.5% in 2005. Results from a 2001-2003 survey of planting material on 280 farms in Côte d'Ivoire led Pokou et al. (2009) to estimate that less than 10% of farms were planted with Amelonado, much of the remainder being Upper Amazon derivatives; 71% of farms were established with seed taken from their own or a neighbouring farm; 23% of farmers said that they had received seed from extension services. Many farms were established with 2,000 or more trees/ha, a practice that has implications for seed garden capacity.

As described in Chapter 3, major advances in clonal nursery practices have taken place in South America, especially in Brazil and Ecuador. By contrast, nursery practices for seedlings in West Africa have seen little advance since the introduction of polybags. Large quantities of topsoil are used for filling bags, which are heavy to handle and carry. There may well be scope to adapt technologies that are commonplace in forestry practices, which could result in sharp reductions in both cost and environmental impact.

2.2.2 Country-specific information on seed gardens

Seed gardens are known to exist, or to have existed, in at least 17 cocoa-producing countries. Table 2.3, below, summarizes the global status of cacao seed gardens; it should be noted that this is a work in progress and susceptible to considerable refinement. Further details are available in Annex 3.

Ghana has by far the largest seed garden capacity, operating at 28 sites and with a professional management team forming the Seed Production Unit of the Ghana Cocoa Board (Cocobod). It appears that each year Ghana produces more cacao seed pods than the rest of the world combined.

In many respects Ghana is the global model for seed garden development. However, only 18% of capacity is in the western part of the country, which has seen the most cacao development in recent years.

Table 2.3. Summary of information on seed gardens.

Country	Year/decade	Area (ha)	Design	Pollination	Comment
			Africa		
Cameroon	1960-70s	90	Biclonal	Natural	800,000 pods annually
	N/A	24	N/A	Natural and manual	185,000 pods annually; manual pollination introduced in 2005
	2010s	25	N/A	N/A	Under development
	2010s	4 x 0.5	Polyclonal	Natural and manual	Village seed gardens, little interest in manual pollination
Côte d'Ivoire	1960-70s	100	Biclonal	Natural	100 ha of seed gardens in 1975; 750,000 pods in 1974; ceased operation in late 1980s
	N/A	6	Multiclonal	Manual	92 ha of seed gardens in 2012, producing sufficient seed pods to plant 25,000 ha annually
Ghana	1960s	192	Biclonal	Natural	Mass manual pollination introduced in 1971
	1971 onwards	~350	Monoclonal	Manual	6.9 million seed pods produced in 2010/11
Nigeria	1960-80s	>166	Biclonal and mixed Amazons	Natural	In 2010, over 40 ha of seed gardens effective but data incomplete; excludes plantings at Cocoa Research Institute of Nigeria Some manual pollination in 2010
	2008 onwards	24	Multiclonal	Natural	One 2-hectare seed garden being planted in each of 14 states
	2011	1	Multiclonal	Unstated	Community seed gardens
Sierra Leone	1970	6.5	Biclonal	Natural	1 ha seed garden survives, negligible production
Togo	1971-75	16	Biclonal	Natural	
	N/A	4	N/A	N/A	
			Americas		
Brazil	1960-80s	177	Biclonal	Natural	100 million seeds produced annually by 1979 in Bahia
	1970-80s	N/A	N/A	Natural and manual	71 million seeds distributed to smallholder farmers in Ouro Preto, Rondonia, Amazon, 1976-98
Costa Rica	1970-80s	N/A	N/A	N/A	26 million seeds of 32 crosses, involving 19 parents, distributed to 19 countries
Dominican Republic	N/A	N/A	N/A	N/A	Referred to by Batista (1982) but no detailed information available
Ecuador	1950s	4	Biclonal	Natural	Scavina crosses
Nicaragua	1980s	N/A	N/A	N/A	40 bi-parental crosses
Peru	N/A	N/A	N/A	N/A	Referred to by Evans et al. (1998), no details available

Key: N/A –not available; > - more than; ~ - approximately

Asia

Country	Year/decade	Area (ha)	Design	Pollination	Comment
India	N/A	N/A	Biclonal	Natural	Seven crosses produced
Indonesia	estimated to be in the 1970s	8	Multiclonal	Natural	Indonesian Coffee and Cocoa Research Institute (ICCRI). In 1986, all pods were used, even from self-compatible clones
	N/A	N/A	Multiclonal	N/A	2012 seed production estimated at about 12 million seeds
	1980s	~10	Biclonal	Natural	PT Hasfarm Products. Same crosses as earlier crosses at BAL Plantations Sdn. Bhd. in Sabah. Manual pollination introduced in 1986
	N/A	N/A	Multiclonal	N/A	
	1980s	17	Biclonal	Natural	PTPII. 10 million seeds sold in 1986
	N/A	N/A	Multiclonal	N/A	
	1980s	49	Semi-synthetic	Natural	PTPVI
		3 x 10	Bi-parental	Natural	
	N/A	N/A	Multiclonal	N/A	PTPVII
	N/A	N/A	Amelonado	N/A	PTPIX
	N/A	N/A	Multiclonal	N/A	PTPNXII PT Glenmore
	N/A	N/A	Multiclonal	N/A	PT Inang Sari
	1980s	4x1	Multiclonal	Natural	PTPP London Sumatra Plantations
Malaysia - Sabah	1969 1970s 1980s	0.4 10.6 20.1	Multiclonal Monoclonal	Natural with some manual	BAL Plantations Sdn. Bhd. (Sabah)
	1960s	10.7	Multiclonal	Natural	Sabah Department of Agriculture produce Sabah first series hybrids
	1970s	12.1	Multiclonal	Natural	
	1980s	26.3	Multiclonal	Natural	Last planting in 1982
	1970s	12	Multiclonal	Natural	Produce Sabah first series hybrids
	1970s	N/A	Multiclonal	Natural	Produce Sabah third series hybrids
Philippines	Late 1970s	20.0	Multiclonal	Natural	Material from Sabah
			Pacific Islands		
Papua New Guinea	1979	9.6	Multiclonal	Natural	Kerevat
	N/A	~1.0	Multiclonal	Manual	Lejo Cocoa Station was used to produce cacao seeds for the Commonwealth Development Corporation (CDC) up until 1992
	1985	N/A	Multiclonal	Natural	Tavilo Plantation

Key: N/A –not available; > - more than; ~ - approximately.

2.2.3 Seed garden capacity versus actual demand

A review of seed garden operations in the five larger cacao-producing countries in West Africa shows that Ghana has by far the largest capacity, but anecdotal evidence suggests that even in Ghana farmers are not always able to get seed pods when they need them. Côte d'Ivoire has the second largest active capacity. There are no official estimates of the proportion of potential demand that can be met in different countries; the shortfall in the other five countries is much greater than it is in Ghana. The factors that have to be considered when modelling the demand for planting materials include:

- Area to be planted with cacao, which will increasingly comprise replanting, with an average 25-year cycle suggested
- Number of trees to be planted per hectare
- Number of propagules required for each method of planting (seed-at-stake, bare-root plants, polybag plants; whether seedlings, clones obtained conventionally or by somatic embryogenesis), including replacement planting points.
-

When estimating the requirements for seed gardens, the following factors should be taken into account, and some suggestions are given on the starting assumptions to be used based on the author's experience:

- The proportion of total plantings that will be made with seed
- The proportion of the seed component that will be improved seed from seed gardens
- The proportions of polybag, bare-root and seed-at-stake plantings
- The out-turn of seed use, with 66.7%, 40% and 20% suggested for polybag, bare-root and seed-at-stake planting respectively (in other words, 100 seeds will give 66.7 trees growing in the field, with 3.3 times more seed being required for seed-at-stake planting than polybag planting)
- The yield of the seed garden, with the equivalent of 800 kg of dry bean equivalent/ha and a pod value of 24 (pod values are calculated as the number of pods required to give 1 kg of dry cocoa, i.e. 38 beans/pod, with a dry bean weight of 1.096 g)
- A ratio of pod-bearing to pollen-providing trees of 100:15
- Seed utilization efficiency, i.e. the ratio of seeds produced to plants established.
-

Comparisons between seed production data and areas of cacao established give an indication of what proportion of seeds actually become productive trees. The estimate for Ghana is that perhaps only 50% of seeds are used. Partial figures for seed sales in Sabah (Malaysia), over a seven-year period suggest that once supplies from the Sabah Department of Agriculture are taken into account, seed utilization efficiency may have been no higher than in Ghana, despite a much higher price for the seeds.

For example, the 2011/12 annual report of the Ghana Cocoa Swollen Shoot Disease Control Unit states that in September 2012, hybrid cacao (0-7- and 8-15-year-age groups) was being cultivated on 468,217 ha of land, based on data from the 2006-12 survey, which accounts for 1,518,189 ha of cacao. From 1991 to 2010 the Seed Production Unit produced 64,779,504 pods. An additional 228,194 ha were classified as 'mixed'. If it is assumed that this cacao was 50% hybrid, then each hectare of hybrid cacao used 111.2 seed pods. This is a slight over-estimate because the survey began in 2006 and some cacao would have been planted before the survey began. Assuming that 65 pods are required to establish one hectare of cacao then seed utilization efficiency may be as low as 50%, and it could be less given that many observers estimate that perhaps only half of Ghana's cacao farmers can access improved seed.

In Sabah, 139,350 ha of cacao were established between 1981 and 1987 (Malaysian Cocoa Board, 2004). Much of the hybrid seed used for these plantings would have come from two sources: BAL Plantations Sdn. Bhd., which sold 124,433,380 seeds from 1980 to 1986; and the Sabah Department of Agriculture, which sold 79,346,000 seeds (though no data are available from their seed gardens at Tenom). If all the seeds sold were used in these plantings, it would equate to 1,462 seeds per hectare planted (though in fact some of the seeds were exported from Sabah). Since the planting density was lower than in Ghana, (typically 1,111 seeds/ha), and by no means all of the plantings were with hybrids; and since the number of seeds sold from Tenom was likely to have been greater than the number of seeds exported out of Sabah, it is highly likely that the seed utilization efficiency was well below 100%, perhaps less than 50%. The Sabah experience suggests that increasing the price of seed does not improve utilization efficiency. Through the 1981-86 period, the selling price of open-pollinated seeds from BAL's bi-parental seed gardens was about US$ 35,000/t dry bean equivalent, with a 50% premium for seeds produced by mass manual pollination of freshly opened flowers, and an 87.5% premium for seed produced by capped manual pollinations.

In conclusion, although predicting demand for improved seed is complicated by the number of factors involved and uncertainties surrounding the estimates for some of these variables, based on the series of assumptions described above, models suggest that a great deal more seed garden capacity is required in Africa and arguably in Indonesia too.

2.3 Costs of seed propagation

The literature provides no guidance as to the costs involved in establishing cacao seed gardens, but as modern seed gardens are no more than monoclonal or polyclonal blocks, no special costs are incurred. There are no special nursery requirements for seed garden establishment and operation. General agronomic practices in seed gardens correspond to those for commercial clonal cacao, though irrigation may be beneficial in West Africa at least. There would, however, be differences in costs between a farm nursery and a central community nursery.

The ideal cacao seed production system would have the following features:

- Full integration with a long-term practical breeding programme
- Limited number of seed production sites in favourable agro-ecological conditions
- Professional management, with investment in, for example, training and irrigation
- An efficient seed distribution system, probably based on seed storage
- A supporting, independent seed certification system.

A partial budget for producing budded material in Africa is presented below. It was developed by the author from a zero base (i.e. built up from first principles rather than by modification of an existing budget) using multiple sources, realistic estimates of labour productivity and costs of materials. The seed for rootstocks and budwood are assumed to be available locally at nominal cost. The budget itemizes direct costs only, i.e. materials, labour and expenses directly related to production. Other costs, such as depreciation or administrative expenses, are more difficult to assign to a specific product, and therefore are considered indirect costs. Such expenses have been accounted for in the partial budgets by adding 20% to all labour costs for training and supervision.

2.3.1 Nursery costs

For the purposes of this budget, it has been assumed that the nursery would be established close to a water supply, that does not run dry, and that an area sufficient to sow 5,000 seedlings as rootstocks for budding can be created for US$ 915, including the purchase of shade netting and equipment, such as wheelbarrows and simple tools (costs estimated by the author for West Africa in 2012). The price for topsoil is estimated to be approximately US$ 40/m^3, delivered to the nursery. US$ 350 should be allowed for budding knives, budding tape and labels, polythene sheets onto which the budded plants can be placed standing (it stops them rooting through), and fertilizers and agrochemicals. The labour force that conducts the watering would also undertake minor maintenance tasks, including pruning, staking and weeding. The rootstocks should be usable after seven months – in Ghana, sowing takes place in late December, with budding in late July - and the budded plants will require tending for 39 weeks until they are planted in May-June of the following year.

Labour costs are assumed to be the total costs of employment. US$ 5 per day is currently a realistic rate for casual labour in rural West Africa. Such labour is fully capable of undertaking all the tasks that are required in the nursery and field, including budding and manual pollination, if training and supervision are provided. Permanent employment, especially in government service, makes labour costs much more expensive, with US$ 25/day realistic for the lowest grade, up to a possible US$ 45/day for field staff. The estimated costs of producing 2,025 plantable plants from 5,000 seeds, and key assumptions, are summarized in Table 2.4 (see next page).

2.3.2 Field costs

Relevant assumptions used to estimate nursery costs can also be applied to estimate field costs (Table 2.5 below). It is difficult to estimate labour requirements precisely since they depend on the current use of the land. An estimate has been provided here based on the requirements for land that has light shrub cover with sufficient forest trees to provide suitable permanent shade. The materials required during the pre-planting year are plantain suckers. The planting density of 1,111 plants/ha is normal for cacao seed gardens in West Africa, with a 20% loss in the first dry season expected with clonal plantings. Fertilizer inputs are expected to be 0 kg, 125 kg and 250 kg of sulphate of ammonia ($(NH_4)_2SO_4$) in the years 0, 1 and 2; and then 250 kg/ha of sulphate of ammonia, 250 kg of muriate of potash (KCl), and 125 kg of triple super phosphate ($Ca(H_2PO_4)_2.H_2O$) annually. Fertilizer costs are based on local prices in West Africa in 2012-13.

Table 2.5. Partial budget for planting clonal cacao and its upkeep – cost/ha.

	No. of days of manual labour	Plants required/ha	Transport (US$)	Materials (US$)	Estimated cost (US$), at different labour rates		
					US$ 5 per day	US$ 25 per day	US$ 45 per day
Land preparation (year -1)	90		50	250	840	3,000	5,160
Planting year	48	1,111	200	50	2,282[2]	6,300[3]	10,319[4]
Planting year + 1	63	222[1]	50	144	921	3,005	5,090
Planting year + 2	60			371	731	2,171	3,611
Planting year + 3 *et seq.*	69			626	1,040	2,696	4,352

[1] assumes 20% of the original plantings will need to be replaced

[2] assumes cost per plant is US$ 1.57 (based on calculations in Table 2.4 – nursery budget)

[3] assumes cost per plant is US$ 4.15 (from Table 2.4)

[4] assumes cost per plant is US$ 6.73 (from Table 2.4).

Table 2.4. Partial budget for producing 2,025 plantable budded plants, based on the nursery costs for raising and budding 5,000 seedling rootstocks, at three different labour costs.

General materials & equipment	Quantity	Estimated cost (US$), at different labour rates		
		US$ 5 per day	US$ 25 per day	US$ 45 per day
Shade net (bundle)	1			
Polythene bags - 18x25cm in size	5,000			
Wheelbarrow	1			
Watering cans	1			
Mattock	1			
Pickaxe	1			
Shovel	1			
Spade	1			
Empty drums	1			
Total		**915**	**915**	**915**
Materials required to raise and bud rootstocks				
Top soil (m^3)	15	600	600	600
Budding tools, tape, agrochemicals and polythene sheets		350	350	350
Labour required to raise and bud rootstocks				
Filling and arranging of bags, sowing of seeds (number of days of manual labour required/costs)	17 days	85	425	765
Watering for 30 weeks (number of days of manual labour required/costs)	60 days	300	1,500	2,700
Budding, including budwood collection (number of days of manual labour required/costs) Assumptions: - Budwood collection accounts for 25% of budding costs - Budding helpers account for 50% of budding costs - 125 buds per day	63 days	315	1,575	2,835
Watering for 39 weeks post-budding (number of days of manual labour required/costs)	78 days	390	1,950	3,510
Additional days of manual labour for supervision and training (calculated as 20% of total days of manual labour)	43.6 days	218	1,090	1,962
Total costs of manual labour in US$		1,308	6,540	11,772
Total costs		**3,173**	**8,405**	**13,637**
Number of plantable plants produced based on the following assumptions: - 125 buddings made per day of manual labour - 90% of seedlings are usable - 75% of buddings are successful - 60% of those successful buddings are suitable for field planting	2,025			
Unit cost per plantable budded plant in US$		**1.57**	**4.15**	**6.73**

2.3.3 Costs of seed production by mass manual pollination

The assumptions used to estimate the cost of seed production by mass manual pollination of freshly opened flowers are set out in Table 2.6. A sustained yield equivalent to 800 kg/ha and 38 usable beans/pod are considered to be realistic in West Africa, except under very high rainfall conditions where the actual yield will depend on the success of black pod control.

The number of pollinations per day has been set low at 450. This allows for time to be spent collecting flowers that will provide the pollen, and also leaves time to revisit trees and remove flowers/natural sets after manual pollination. The recovery of pollinations to ripe pods is a cautious 40%. The maintenance costs are taken from Table 2.5 (field costs), with 20 days of manual labour per hectare added for the removal of natural sets and for tending to the pods obtained by manual pollination; in addition, US$ 200/ha is added for black pod control. The Table indicates that on the assumptions used, the cost difference between producing a seed by mass manual pollination and by reliance on natural pollination ranges from US cents 0.088 – 0.786 depending on the cost of labour.

Table 2.6. Variable costs of producing cacao seeds by mass manual pollination.

Assumptions	Activity Cost of manual labour per day in US$		Estimated cost (US$) at different labour rates US$ 5/day	US$ 25/day	US$/day
	Pods/ha	19,875			
	Pollinations per day	450			
	Recovery	40%			
	Seeds/pod	38			
	Pods/t dry bean equivalent	25,000			
Costs	Labour cost in US$ (for 110 days of manual labour plus 20% additional days for training/supervision)		660	3,300	5,940
	Variable cost in US cents/seed[1]		0.088	0.437	0.786
	Add maintenance cost US$/ha[2]		2,022	6,808	11,594
	Cost/pod in US$		0.102	0.343	0.583
	Cost/seed (US cents)		0.268	0.901	1.535
	Direct cost in US$ /t dry bean equivalent		2,543	8,564	14,584

[1] Calculated as labour/No. of pods/seed per pod to estimate the additional cost of using mass manual pollination compared to using natural pollination.

[2] Calculated from Table 2.5, plus additional labour and black pod control, as detailed in text.

2.3.4 Further remarks on costs

The cacao nursery costs estimated above are based on a particular set of assumptions and especially a permanent nursery site. When establishing a cacao farm in West Africa, the farmer will probably choose a site on or close to the main farm itself, the principal consideration being availability of water. In the event that extra shade is needed, a temporary structure would be erected using whatever materials are to hand, probably bamboo for the frame, palm fronds for the shade, and natural materials to hold the frame together. Soil would be collected nearby. There may be no need to purchase any materials - discarded water sachets can be used as polybags and seeds can be taken from existing trees. In other words, the costs in this study are not indicative of farmers' costs, but relate instead to the formal sector.

The impact of labour costs on nursery, field and seed production costs is strikingly large, reflecting how labour intensive these activities are. At the lowest of the three labour rates used, US$ 5/day, the additional cost of using mass manual pollination to produce the seed rather than relying on natural pollination can be estimated at US cents 0.088 per seed (based on Table 2.6); therefore the marginal cost of establishing one hectare of cacao, planted at stake, at 2.5 m^2 spacing (1,600 trees/ha, 90% germination, 75% nursery outturn), using seed of known parentage, is about US$ 2.08 more than that of seed from open-pollination (in both cases, there would be similar costs involved in harvesting and transporting the seed pods, and the depreciation and management costs of the seed garden). Even at a labour cost of US$ 45/day for manual labour, the equivalent additional seed cost is as little as US$ 18.71/ha. Where the seeds are raised in the nurseries before planting, the cost of a 6-month-old seedling produced by labour costing US$ 5/day is estimated at US$ 0.45 plus the cost of the seed, giving a cacao establishment cost of US$ 792/ha (assuming 10% mortality) for planting material. The equivalent cost for budded cacao, produced by labour at the same cost and assuming 20% mortality, is US$ 2,899/ha.

The zero-base estimates of the maintenance and operational costs are markedly lower than US$ 7,787/ha (Cameroon, 2012), US$ 5,662 (Ghana) and US$ 5,912 (Nigeria), as cited by Asare et al. (2010), partly because much higher labour productivity is assumed for the manual pollination work.

2.4 Challenges and recommendations

High performance seedling varieties were identified in West Africa and Indonesia fifty or more years ago, yet capacity to produce them in bulk is still limited in several important cocoa-producing countries. As the frontiers of cacao development move in countries like Côte d'Ivoire and Ghana, it has been difficult for seed garden development to keep up. This is the principal reason why relatively small amounts of improved seed have been used in western Ghana - there were few cacao stations and low seed production capacity when it was at the frontier of cacao development.

For the seven West African countries that together produce over 70% of the world's cocoa, it is hard to estimate what proportion of the theoretical demand for seed can be met from the seed gardens, because many of the assumptions in the calculations are themselves estimates. However, there is undoubtedly a large shortfall. A seed garden development campaign should span several years, during which the estimate of required capacity can be refined. In West Africa, the momentum for seed garden development that built up during the sixties was largely lost, except in Ghana where Cocobod invested continuously over a period of many years. Moreover, the possibilities for improving the outputs of existing seed gardens through the development of new pollen parents for the existing pod-bearing trees are under-exploited. The development and continual improvement of seed gardens has sometimes been a low priority for researchers due to the long-term nature of cacao breeding and the consequent lack of opportunities for the academic publications that are valued by their institutions. More broadly, cocoa breeders should pay greater attention to how their proposed seedling crosses can be produced in bulk, quickly, efficiently and economically: the breeding and seed production operations must be closely coupled.

Though seed gardens can be regarded as long-term assets, it can take time to build new capacity. The timescale will depend on three principal factors:

- ***Availability of parental material.*** In Liberia, where there is no starting material, it is estimated that it would take six years from the introduction of budwood to the harvesting of the first seed pods. However, in Sierra Leone there is sufficient budwood of one clone to allow a doubling of the seed garden area within twelve months, if a decision is made to go ahead with the development. Somatic embryogenesis is a means of rapid multiplication of parents. In many situations it would be quicker to establish seed gardens to produce older bi-parental crosses where the supply of materials of the more recently recommended parents is limited, and this may be worthwhile if the only other alternative planting material available to farmers is open-pollinated seed.

- ***Growing conditions.*** Multiplication rates and growth in the field are much faster under the near ideal conditions of BAL Plantations in Sabah than they are anywhere in West Africa.
- ***Skill.*** It may take time to train workers in the necessary propagation and manual pollination techniques.
-

The basic technology for mass production of high quality seed is understood, and easily applied. There is, however, scope for technological development in two key areas:

- ***Seed purity monitoring***. Seed garden managers need methods of monitoring seed purity during routine production, which is easily achievable through the use of molecular markers, although at a high price. If seed purity falls below the acceptable standard, say at least 95% true-to-type, more attention should be paid to pollinating only when female flowers are plentiful, because the number of flowers that are pollinated naturally appears to be a function of the population of pollinating insects; and to ensuring that all natural sets are removed from the trees so that they do not ripen as pods that are passed on to farmers.
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- ***Timing of seed availability***. The natural seasonality of cropping does not coincide with the period of maximum demand in West Africa with its bi-modal rainfall pattern in the main production areas. Farmers who use polybag nurseries want seed pods shortly after they finish harvesting, when they have time to prepare for planting during the main rains, or a little later for bare-root nurseries. If seeds are to be sown at stake, then they are required once the rains have set in, by which time road access to planting sites may be difficult. This constraint could be addressed by two approaches:
  - *Store seeds for up to eight months*. Seed storage would help meet demand for seeds in West Africa, in particular at times when they are not available in substantial quantities. Storage would also help with the logistics of seed distribution as a typical pod containing 40 fresh seeds weighs over 400g (i.e. each pod comprises more than 75% 'packaging'), and many new farms are geographically remote and difficult to access (see, for example, Grimaldi 1981). Furthermore, it would undoubtedly help to improve the efficiency of seed utilization, because it would reduce wastage. Conventional storage of cacao seeds was reviewed by, among others, King and Roberts (1982), and Mumford and Brett (1982). As the seeds withstand neither desiccation nor low temperatures, only warm, moisture-retaining packing media can be used, typically maintaining viability for up to three weeks, occasionally longer, which helps with the logistics of seed distribution (e.g. Duris 1989) but is insufficient to ensure seeds will be available at the time when they are needed. Knowledge of seed storage practices is therefore desirable, as affordable short-term storage could make seed more accessible to many cacao farmers. Mumford and Brett (1982) peeled seeds and removed the mucilage under aseptic conditions and stored them for 175 days in polyethylene glycol at a concentration of 400 g/litre in the dark, and at a temperature of 25°C, obtaining complete albeit slow germination. The principal challenge is to prevent fungal contamination. Figueiredo (1986) reported that seed washed in distilled water,

dried with sterilized towels and stored in 40% methyl cellulose at 25°C in the dark, showed 92% germination after 210 days. No fungal contamination problems were reported. Duris' (1989) work on the use of pectolytic enzymes is relevant to the preparation of large quantities of seed for six months storage.

– *Manipulate the seasonality of cropping*. This entails preventing a crop from setting until several months after the usual time, which can be done by frequent manual stripping of cherelles. This will promote some flowering but in some clones at least flowering will be suppressed by the cooler and duller weather that normally follows crop set. The crop will develop and mature through the dry season, so irrigation may be required.

Leaving aside the technical challenges and the costs involved, storing the seeds may be the more attractive option for three reasons:

- The logistical problems associated with delivering pods to remote areas would be largely solved
- The operational difficulties, extra investment and operational costs (e.g. irrigation), and likely yield loss resulting from changed seasonality of cropping, would be avoided
- Seed utilization efficiency would improve.

2.4.3 Concluding remarks

A seed garden system can be an efficient and economical way to supply high quality planting materials in the large quantities needed by the farmers. Developing seed supply systems to the same or better standard as exists in Ghana is a matter of political and scientific will, cash investment, availability of suitable land in a favourable agro-climatic zone, and the development of management and operational expertise. Seed production is sometimes seen as an opportunity for partial or even complete privatization. The indicative data on the establishment, maintenance and operational costs of seed gardens show that this would require sharp rises in the cost of seeds. The additional cost involved in producing seed using manual pollination rather than natural pollination is estimated to be US$ 0.033 per pod, which equates to an additional cost of around US$ 833 per tonne of dry cocoa beans (assuming 25,000 pods per tonne), plus processing costs. In Ghana, seed pods are sold for approximately US$ 0.033, and labour for manual pollination is in reality often both more expensive and less productive than the general estimates provided in this analysis, so the seed pods are heavily subsidized. The argument that farmers would place greater value on higher priced seed is not borne out by the Malaysian experience. Furthermore, the readiness of Ghanaian farmers, and others in West Africa, to use on-farm seed should act as a deterrent against pricing at full economic cost. The tradition of farmers using their own seed, based on the true-breeding West African Amelonado variety, is a strong one.

2.5 Acknowledgements

The author thanks the pioneers of large-scale production of hybrid cacao seed and especially the late Don Edwards for making possible today's methods that serve farmers so well. Many colleagues generously provided information about seed production in various countries, including: Komlan Wegbe, Lee Ming Tong, Philippe Lachenaud, Roberto Augusto Sena Gomes, Smilja Lambert and Wilbert Phillips. Beatrice Owusua, from the library of the Cocoa Research Institute of Ghana, kindly provided copies of many papers.

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Chapter 3 – Conventional vegetative propagation

Augusto R. Sena Gomes[2] and George A. Sodré[3]

3.1 Description of the method

Various asexual techniques for propagating cacao plants have been developed since the late 1890s. Cheesman (1934) credits the first successful attempt to propagate cacao asexually to J. H. Hart, who presented the Agricultural Society of Trinidad and Tobago, in 1898, with several cacao plants that had been produced by approach grafting (inarching) onto seedling stocks. According to Harris (1903), and as reported by Mooleedhar (2000), cacao was first budded successfully in Jamaica in 1902 by the patch-bud method, which involves inserting buds into vigorous sucker shoots growing from the base of the tree (chupons). The use of such propagation techniques spread throughout the Caribbean region at the Dominica Botanical Station in 1909, and budding was successfully carried out on very young trees in Haiti (Casse 1910) and further afield; for example, grafting techniques were used on cacao in Ghana in 1906 (Evans 1907). The application of various grafting techniques, including budding, in other cocoa-producing regions in the 1930s, such as Java in Indonesia, has also been attested to (Van Hall 1932). Pyke (1933) elaborated a technique for producing rooted cuttings in Trinidad, which was further developed in the 1950s (see Evans 1951, Alvim 1953, Alvim et al. 1954, Murray 1954). Additional improvements were made by the Inter-American Institute for Cooperation on Agriculture (IICA) and the Organization of American States (OAS) at Turrialba in Costa Rica, and the Pichilingue experimental station of the Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP) in Ecuador (Alvim 2000). Important contributions to vegetative propagation methods in cacao have been made in West Africa, especially in Ghana, by McKelvie (1957), Richards (1948), Archibald (1955), and Hall (1963). Asexual propagation in cacao has also been extensively studied and used in Malaysia (Ramadasan 1978, Lee 2000), and Indonesia (Wood and Lass 1985).

The scientific and technical basis of most important methods of asexual propagation of woody plants - including extensive aspects of plant anatomy, physiology and management procedures – have been discussed by Hartmann et al. (2002). Technical details for successful cacao propagation methods have been published by Sena Gomes et al. (2000), Rosa (1998), Marrocos et al. (2003), and Sodré and Marrocos (2009) for southern Bahia; Alvarez (1996) for Ecuador; Ramadasan and Ahmad (1986), Yow and Lim (1994), and Lee (2000), for Malaysia; and Wood and Lass (1985) for Indonesia.

The most important vegetative propagation methods are briefly described in the following sections, with emphasis on the propagation technologies utilized in the region of Bahia, in Brazil. These include: 1) Rootstocks; 2) Rooted cuttings; 3) Grafting; 4) Budding; 5) Marcotting or air layering; and 6) Clonal gardens. Some selected photos, showing details of various cacao propagation facilities, and conventional vegetative methods utilized in several cocoa-producing countries, are provided in Annex 4, Figures 1 through 13.

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3.1.1 Rootstocks

Rootstocks are required for budding or grafting techniques and can include nursery- or field-grown seedlings produced specifically for this purpose, as well as trunks, branches and chupons of mature field plantings, where the intention is to replace all or part of the existing canopy with improved varieties.

Seedling rootstocks are obtained by planting and maintaining pre-germinated selected seeds (half- or full-sibs, or in some cases populations) in plastic bags, in a greenhouse facility, for a variable time (from just a few days or weeks, up to six or eight months). The varieties used for rootstocks and management practices vary according to the propagation technique to be used and the country/region involved. Table A5.1 in Annex 5 outlines cacao propagation methods in use according to country/region.

Budding and grafting techniques have long been used to propagate many tree crops, and the use of rootstocks has been found useful in minimizing the effects of constraints such as soil compaction and infection by soil pathogens on root systems, and consequently on scion growth. In addition, in many species the rootstock can affect fruit quality and tree sizes (Hartmann et al. 2002). This has been observed in many fruit trees, such as apple, pear, cherry, plum and walnut (Westwood 1993); as well as orange (Azevêdo 2003), mango (Smith et al. 2008) and avocado (Duran 2012). Westwood (1993) emphasized that rootstocks profoundly affect the field performance of the scion in most pome species; affecting not only yield per tree, but also yield efficiency, which varies significantly depending on the variety of rootstock used. However, a review of rootstock experiments in cacao suggests that although rootstocks can be selected to confer resistance to certain diseases, and may offer prospects for improving the ability of the plant to achieve establishment, there is little evidence as yet that the rootstock selected will improve yield efficiency (Lockwood and Nsiah, in preparation). For example, results of experiences in Trinidad and Malaysia showed no evidence of grafting incompatibility caused by rootstock-scion interaction (Mooleedhar 2000 and Pang 2004 respectively). The results demonstrated that the effects of rootstock on yield were correlated with those on vigour, and that there was no effect on the ratio of yield to continuing vegetative growth. Rootstock did not influence bean weight, or number of beans per pod, or the uniformity of the trees; and there was no indication of an interaction between rootstock and scion for any of the traits that were studied.

In contrast, Cheesman (1946) found that rootstocks consistently reduced the average yield in all six ICS clones tested (being ICS1, ICS3, ICS4, ICS5, ICS6, ICS8) at a conventional planting density over a five-year study period. On the other hand, Efron, Tade and Epaina (2005) reported the most significant effects of grafting onto rootstocks of MJ12226 – mutant cacao with abnormal growth characteristics – such as reduced stem elongation, short internodes, multiple stems, and reduced root and leaf size in the scion. Although the rootstock significantly reduced tree vigour in the early years, with arrested growth of the scion in the nursery or soon after planting in the field, this effect was of short duration and the grafted plants reverted to a normal growth pattern after some time (Tade et al. 2009). Cacao clones, bud grafted onto these mutant rootstocks, have been shown to sustainably produce significantly greater yields at higher planting density compared with lower densities, over a two-year period (Tade 2009). Moreover, Pang (2004) examined 12 cacao seedling families as rootstocks in Sabah, Malaysia using three commercial cacao clones as scions. Results showed that yields from a pure Scavina rootstock were about 10% above average, while those from pure West African Amelonado were about 10% below average. Rootstock did not influence bean weight, number of beans per pod or the uniformity of the trees. There was no indication of an interaction between rootstock and scion for any of the traits that were studied, corroborating results reported by Mooleedhar (2000). Irizarry and Goenaga (2000) carried out a six-year study in Puerto Rico on the effects of budding 45 clones onto EET400 open-pollinated seedlings. Results indicated that on average only 45% of the initial cacao yield was recovered after grafting. The 55% difference was attributed to environmental effects, and possibly to a certain degree of scion-rootstock incompatibility. The authors point out that the close relationship between the scion and the rootstock in selections of the family EET400 x SCA12 did not favour a substantial overall yield recovery increase as compared with selections in other families.

According to Kelvin Lamin (personal communication, 2013) there have been some reports from Malaysian cacao growers of lower yields from certain field grafted stock/scion combinations, possibly due to stock/scion interactions, but in general, any rootstock effect can be overcome by using superior scions/clones which are high yielding and disease tolerant. In addition, rootstock age significantly influences the rates of take of budded scions in Nigeria (Are 1969). Are's greenhouse studies show that older rootstocks (e.g. 18-month-old F3 Amazon seedlings) improved leaf area, dry matter content, and height of budded C10 and C57 scion clones. The genotype of the cacao rootstock may influence the survival rate of the grafted plant as reported by González, González and Ventura (2006). Results of their studies indicate that rootstocks of ICS39, ML4, Pound4, Pound7, Pound12, IMC67, IML44, IML89, UF296, UF613, EET103, Catongo, and Native (from half-sib seeds), grafted with scions of UF677, altered differentially the survival rates of grafted cacao seedlings under greenhouse conditions. The highest percentage survival rate was registered using Pound4 and IMC67 rootstocks. The authors conclude that the rates of grafted plant survival were influenced by many factors including genotype of the rootstock and scion, type of grafting, management practices and environmental conditions. Conversely, Marinato et al. (2006), testing four-month-old seedling rootstocks, grafted with a single scion clone using two grafting modalities, under greenhouse conditions at an agricultural station of the Executive Commission for Cacao Farm Planning (CEPLAC) in the state of Espirito Santo in Brazil, found that the rates of survival of grafted plants were not influenced by grafting methods. However, it was noted that a specific rootstock (ESFIP02) significantly enhanced vegetative growth of TSH1188 when split grafted compared with splice-grafted plants. Other effects of the rootstock on the scion of cacao plants related to nutrient transport from the roots.

In many species, rootstocks can be propagated by a variety of methods involving the use of seeds (sexually) or apomitic embryos (asexually), air layering, division, stem cuttings, grafting, budding and tissue culture, as detailed by Westwood (1993). However, in the case of cacao, sexual reproduction, usually from open-pollinated seeds, is the method favoured for the preparation of nursery-grafted or -budded commercial clones in all cocoa-producing countries (See Figures 3-6, and Figure 9 in Annex 4). This method is preferred because it is easy and cheap to obtain seeds from a seed garden or the farm, and grow them to a stage at which they are ready for budding or grafting. While there have been a limited number of experimental trials using clonal rootstocks (e. g. Cope and Murray 1952, Murray and Cope 1959), no published information is available concerning asexually propagated commercial cacao rootstocks. Further research is being carried out to investigate the use of rooted cuttings of several clones as rootstocks, at CEPLAC facilities in Bahia, Brazil, under the supervision of Dr George A. Sodré.

In the 1990s, farmers in Bahia were provided with rooted cuttings to propagate cacao clones (TSH516, TSH1188, TSH565, CEPEC42 and EET49) and increase planting density. By late 1990s, some of these farmers decided to graft the cuttings using self-compatible new clones due to the poor performance of the original material and its susceptibility to witches' broom (field observation by the authors of this Chapter in the late 1990s).

It is well known that some sexually propagated cacao seedlings are genetically variable in many important agronomic traits, and it is possible that the genetic variability of seedling rootstocks may also contribute to tree variability in the performance of grafted/budded clones. Although there are many published studies on rootstock-scion interactions, and on the ways in which rootstocks influence scion vigour and fruit quality in other tree crops, the mechanisms are not yet fully understood. However it is likely that changes in hydraulic resistance and the flow of stem-sap across the union between stock and scion are involved, as suggested by Simons (1986). According to Beakbane and Thompson (1947), root systems that inhibit shoot growth have a low xylem to phloem ratio, while the opposite is true for root systems that promote shoot growth, in which the xylem tissue has more and larger vessel elements. The consistency of this ratio has enabled it to be successfully used at the seedling stage in selection procedures for apple rootstock (clonally-produced root system) breeding programmes (Beakbane and Thompson 1947, Rogers and Beakbane 1957, Miller et al. 1961). A comprehensive review on the subject was published by Atkinson and Else (2003) for pome species. The authors focus on whole aspects and mechanisms that alter plant growth and agronomic performance, and conclude that rootstocks increase harvest index, and dwarfing further increases yield efficiency. In a recent review on cacao rootstocks, Lockwood and Nsiah (in preparation) point out that dwarfing effects have attracted the most attention in cacao, for two main reasons: firstly, when associated with improved yield efficiency, and sustained or increased assimilate production, dwarfing can be used to increase commercial yields; and secondly, dwarfing can make the crop 'pedestrian friendly', with all parts of the tree and especially the fruit within easy sight and reach, greatly simplifying pest and disease management. However, based on the findings to date and the current lack of dwarfing rootstocks, which exert a long-term effect in improving yield efficiency, there may be better prospects for selecting clones for use as scions that already have the desirable characteristics for architecture and yield (Lockwood and Nsiah, in preparation).

The supplies, parameters, facilities, and infrastructure needed for the production of seedling rootstocks are outlined in Annex 4 (A4.1 and A4.2); the basic protocols for production are also provided in Annex 4 (A4.3). Farmers may alternatively order seedling rootstocks from small to medium or large scale commercial nurseries found in most regions of cocoa-producing countries, particularly in the Americas.

Seedling rootstock management in the nursery includes the control and/or monitoring of:

- Timing, volume, intensity, and quality of irrigation water
- Timing and dosage of fertilizer application (foliar and substrate mixtures)
- Shade – to alter (increase or decrease) the quantity of overhead light interception, according to time of year, age, and varietal requirements
- Seedling growth, incidence of pests and diseases, and efficiency of applied control
- Minimizing of competition between seedlings by altering the space between plants
- Logistics and timing for selecting seedlings to be grafted or budded
- Selection of cloned plants to be released for field planting.

3.1.2 Rooted cuttings

Rooted cutting technology is universally utilized to clonally propagate a great number of plant species, including cacao. Pyke first demonstrated the success of this technique to vegetatively propagate cacao in Trinidad in 1933. Facilities and logistics to propagate cacao clones through this technology vary from simple to highly sophisticated, but the physiological principle involved in the process is common for all of them: to promote the growth of adventitious roots from the base of a stem cut, in an environment of high humidity. Adventitious roots are developed from the pericycle (vascular cylinder) region at the base of the stem, just above the cut (See Figure 11 in Annex 4). The rooting process is accelerated by plant growth regulators (hormones) that are applied exogenously. For cacao clones, rooting can be attained by the utilization of synthetic auxins applied to the cut base. The anatomy, physiology, and environmental factors involved in rooting of cuttings of a number of cultivated species have been studied by, among others, Hartmann et al. (2002) and Leakey (2004).

Since 1933, rooting technologies have been advancing in terms of facilities and logistics utilized to propagate cacao clones. Methods progressed from using closed rooting bin facilities, with sand forming the rooting substrate (Pyke 1933, Alvim 1953, Alvim et al. 1954); to planting cuttings in coir baskets or pots containing potting mixture, and covering the baskets with a polythene sheet (Murray 1954, McKelvie 1957, Burlé, 1958, Hardy 1960). These methods have also been utilized by commercial nurseries in Ecuador (Ivan Garzón, personal communication, 2012). A similar propagation technique that utilizes redesigned and improved high humidity bins covered with polythene, and cuttings of forest species, has been successfully tested in Kenya, Cameroon, Costa Rica, and Britain (Leakey et al. 1990). Recently, a very simple, non-mist closed system, known as a polybag propagator, was effectively tested for propagating tropical species (see Figure 12 in Annex 4) (Roger Leakey, personal communication, 2013). A further improvement involves the rooting of cacao cuttings in plastic tubes filled with commercial rooting mixture (compost, plus shredded coconut fibre), processed in a high humidity chamber equipped with an automated, intermittent fertigation system (Sena Gomes et al. 2000).

Research has been carried out to investigate factors affecting success rates for the rooting of cuttings of many species, including fruit and ornamentals (Hartmann et al. 2002) and forest species (Leakey 2004). Wood and Lass (1985) list a number of factors affecting the rooting of cuttings in cacao, which include: cacao type; nursery management; age of flushes; hormones; environmental factors (temperature, light and humidity); and rooting medium. Archibald (1953) found that light, temperature, air humidity in the bin, and rooting medium are very important factors in the closed-bin propagators utilized in Trinidad. It was noted that low light intensities normally cause ultimate starvation of the cutting, although cuttings with leaves can produce roots in darkness. High light intensity was seen to cause leaf damage. Temperature was not considered critical, and it was shown that rooting medium normally influences the amount of callus produced, as well as the quality of the root system formed. The author also found considerable variation in rooting behaviour between cuttings from different clones, different trees of the same clone, different parts of the same tree and different parts of the same shoot, due to internal factors, with photosynthetic efficiency of the leaf being a key determinant in the survival of the cutting.

Hall (1963) found significant differences between clones in rooting ability, and identified a positive correlation between this trait and semi-hardwood cutting production. These results were also observed in Nigeria by Toxopeus (1964). According to Hall's findings, Upper Amazon and Trinitario clones perform better than Amelonado, in rooting response and cutting production; while Toxopeus (1970) emphasized the seasonal differences in rooting ability among clones. Ahmad (2006) points out the differences in rooting performance in cacao stem cuttings, in relation to the position of the cutting on the stock plant. Edwards and Thomas (1980) indicated that the presence or absence of sclerenchyma cells on tree branches may determine rooting success in most plant species. A significant contribution to the advancement of rooting of cacao clones in Ghana was published by Amoah (2006a, 2006b), who reported on the influence of various physiological, technical, and environmental factors, and their interactions on the rooting success of cacao. Among the relevant factors, the author highlighted the importance of anatomical components and the nutritional status of the cuttings in relation to the nodal position on the stock plant. Results suggested that the rooting of cacao cuttings is enhanced by a well-differentiated region of the vascular cambium (a thin, sheathing lateral meristem, higher levels of non-reducing sugars, and a moderate carbon to nitrogen (C:N) ratio. They also indicated that rooting response was directly related to both the plant's anatomy and to carbohydrate levels, as reported earlier by Archibald in 1953. Studies on 15-year-old Amelonado trees at the Cocoa Research Institute of Ghana (CRIG) demonstrated that rooting was significantly affected by the nodal positions of the cutting, with the best rooting achieved in cuttings from nodal positions 4-6, from the apex of the shoot. Cuttings from nodal positions 1-3 had relatively large pith and a poorly developed vascular region; while cuttings from nodal positions 7-10 had an almost continuous ring of sclerenchymatous cells that arrested rooting.

These findings can be linked to the physiological analysis of the vegetative reproduction of forest species presented by Leakey (2004). According to Leakey's stem-cutting form of classification, in leafy cuttings (which depend on photosynthesis produced while in the rooting bed) such as cacao cuttings, rooting ability is widely influenced by plant factors, interactions between environmental conditions, and management practices. Within the shoots, Leakey focused on the effect of age on many plant processes, such as size, water status, carbon balance and senescence of leaves, from the top to the bottom of a shoot. Other factors include: internode diameter, stem lignification, nutrient and carbohydrate content, and respiration. These factors should be taken into account in the selection and management of cacao cuttings from stock gardens during the multiplication of clones by rooted cuttings.

The difference in architecture between plants raised from plagiotropic (fan) cuttings, and those raised from seedling trees, has been noted as a potential concern, and interest in developing true-to-type cacao trees with the typical dimorphic architecture has been increasing since vegetative propagation techniques were first developed for cacao in Trinidad by Pyke (1933), Cheesman (1935, 1948), and Evans (1951). Cuttings taken from fan branches of some, but by no means all, cacao varieties will develop a prostrate, dense, bush-like canopy, which requires repeated pruning for easier management and harvest. Although some farmers have considered this to be a negative aspect of planting the plagiotropic rooted cacao available to them, most have recognized that adopting the plagiotropic growth habit is a key step towards the domestication of cacao, especially where breeders have been able to select varieties with a satisfactory growth habit, which exhibit a balance between crop, continuing vegetative growth and planting density that does not necessitate continuous pruning.

Miller (2009) cites the experiences reported by Jagoret, Bertrand and Jadin (1992), and by Mooleedhar (2000), whereby they showed that plagiotropic rooted cuttings succumb more easily to lodging, and are susceptible to periods of soil moisture stress during establishment, and this is probably due to the combination of unbalanced canopy formation and inconsistent development of an orthogeotropic adventitious framework root system, which has been documented following exhaustive excavations of the root systems of established production trees (van Himme 1959, Charrier 1969, Fordham 1973). Lee (2000) indicates that the lack of taproots in rooted cutting trees is considered a disadvantage, especially during the dry season in Malaysia; growers recognize that plagiotropic rooted cuttings are not as effectively anchored as seedling material, which is seen as a serious shortcoming in many cocoa-producing areas, particularly where hillside plantings are common. It is well known that a shallow system of fibrous roots may limit the growth, production, and/or survival of cacao plants under water stress conditions, since the amount of water absorbed by roots during the dry period is very often not enough to supply leaf transpiration demands. However, it is possible that the lack of a good orthogeotropic framework is related more to poor rooting technology than to whether the source material is plagiotropic or orthotropic (Roger Leakey, personal communication, 2013). Lockwood (2012) cites studies of Murray (1961) in Trinidad, who noted that the root systems of field-grown cacao plants raised from cuttings were similar to those of plants raised from seed. Murray studied the performance of the two treatments in a small-scale experiment, in which two plants of ICS95 (rooted cuttings) and two of ICS1 x Sca12 (seedlings) were grown in shallow (230mm) and deep (1.07m) boxes of different surface areas to give the same soil volume. He observed, 15 months later, that the average fresh weights of the clonal and seedling plants were about the same, but the clonal plants had a greater proportion of roots and leaves. The main roots of clonal and seedling plants in the shallow boxes formed roughly the same proportion of the fresh weight, but the roots of the seedlings in the deeper boxes made up a much higher proportion of the total weight than those of the clonal plants. In the absence of taproots, two or three leading roots developed from which lateral roots emerged.

It is possible to produce rooted cuttings from orthotropic materials (e.g. chupons); trees originating from this material will develop the same dimorphic framework and taproots (e.g. Evans 1951, Glicenstein et al. 1990), with potential advantages including better establishment, resilience to water stress, and a reduced need for shape pruning as discussed above. However, despite the interest in this technique during early cacao vegetative propagation efforts, it did not advance as expected, mainly owing to the well-documented shortage of orthotropic material for large-scale production. Nevertheless, some initiatives to overcome the short supply of orthotropic scions were initiated in 1984 by the Institut de Recherches du Café et Cacao (IRCC) in Togo (Amefia et al. 1985). Bertrand and Agbodjan (1989) describe various methods designed to increase the number of orthotropic shoots, including pollarding, pruning, coppicing the tree trunk, and arching after notching. They observed a successful production of orthotropic shoots after five months of either coppicing, or arching after notching. In this same line of work, Glicenstein et al. (1990) demonstrated that bending young cacao seedlings into an arch, by tying the tops to the ground, in a greenhouse at Penn State University, USA, quickly promoted the production of a large number of orthotropic shoots per bud or flower cushion, on the top or side of the arched stem. Results of recent studies on rooted cuttings have been published by Miller (2009), and more details are provided in Chapter 4 of this document.

Materials, facilities, supplies and parameters required to plan and manage cloned trees through the use of rooting cutting technologies; and basic procedures for the production of rooted clones, based on experiences in Brazil and Ecuador, are described in Annex 4 (A4.8).

It is also possible to design very simple and cheap propagation units, without misting capabilities, for use by farmers (see Annex 4, Figure 12) (Leakey et al. 1990). Since such facilities do not require running water or electricity they are appropriate for remote locations. Leakey et al. (1990) claim that this simple method is effective and less prone to problems than misting and other more sophisticated systems. Burlé (1958) provides other examples of very simple propagation methods for cacao.

3.1.3 Grafting

Grafting refers to a conventional propagation technology in which a scion stick containing several buds is appropriately inserted into a top split (top grafting), or laterally under the bark (splice grafting) of a rootstock - field-planted or nursery seedling, basal chupon, stem, or high branch of a young tree. Similarly to budding, the basic physiological principles for achieving a successful take also apply to all the modalities of grafting: intimate contact of cambial tissues of the scion and rootstock, and external protection against union tissue dehydration. The intimate contact of cambial tissues favours the development of functional connections within the xylem and phloem tissues of the scion and stock. But, as pointed out by Hartmann et al. (2002), the physiological processes of cell recognition, callus formation, as well as vascular and parenchymal tissue differentiation are critical processes in graft development. Although there are many modalities of clonal propagation through grafting, only the methods of commercially important types will be discussed in this review. Examples of grafted seedlings are shown in Figures 2 through 10, in Annex 4. Details concerning the supplies and parameters required to plan and manage nurseries for the production of grafted seedlings; as well as basic protocols for production, are provided in Annex 4 (A4.4 and A4.5 respectively).

Top or split grafting

Top or split-grafting onto basal chupons of adult trees or seedlings in the field (generally between six and eight months old), or seedling rootstocks in the nursery (four to six month-old), is a very simple, quick, and easy method of propagating cacao clones, which has a high rate of success. Generally, a semi-hardwood scion (about 15cm long) taken from a fan branch or chupon of a selected variety, is cut to give a long v-shape gradually tapering into a wedge at the base, which is then carefully inserted into a vertical cut made in the stem of the seedling rootstock (diameter of around 1cm), or into a basal chupon of a tree in the field (two or more scions may be required for chupons with a diameter greater than 2.5cm). The graft is taped and covered with a plastic bag, or a special biodegradable grafting tape may be used to protect the graft until it has formed a union, generally after two to three weeks.

The conventional method used in the main cocoa-producing countries for the propagation of cacao by grafting rootstocks grown in plastic bags under greenhouse condition (see Figures 1-4 in Annex 4) is similar to the techniques commercially utilized to multiply forest fruit trees and ornamental plants (Hartmann et al. 2002). The split-grafting method has also been successfully utilized on a small scale to propagate cacao clones using ten to twelve-week-old rootstocks grown in plastic tubes under greenhouse conditions at farm level. The average success rate of summer grafting in the nursery is between 80% and 90% (see Table A5.2 in Annex 5). Supplies and parameters required to plan and manage nurseries for the production of grafted seedlings, as well as the basic protocols for production, are provided in Annex 4 (A4.4 and A4.5).

Side grafting

Side grafting, also referred to as lateral or splice grafting, is an important cacao propagation technique in which the scion is cut across obliquely (laterally) to form a slender wedge, and is pushed down inside the stem or bark of the stock. The cut side of the scion has to be set in intimate contact with the cambial tissues of the rootstock, and externally protected against tissue dehydration at the union of the graft. The rootstocks can be nursery- or field-planted seedlings, stems and high branches of young or mature tree. Materials and parameters required to plan and manage nurseries in the production of grafted seedling are provided in Annex 4 (A4.6), along with details of the basic protocols for production. Some visual details are shown in Figures 6 and 7, Annex 4.

Crown grafting

Crown grafting, which consists of grafting onto the main branches, above the jorquette, is commonly known as canopy replacement and is also a technique to quickly restore yield in cacao. The crown-grafting method has been well accepted by cacao farmers in Bahia, as being the fastest way to increase cacao production in farms of unproductive trees that are less than twenty years old. This method can also be commercially appropriate for the replacement of the tree canopy in situations where, for example, the trees suffer from certain diseases; or where unproductive trees do not develop basal chupons, thereby excluding the possibility of conducting side grafting. In general, the technique follows the same procedure described above for side grafting, and also detailed by Yow and Lim (1994) for lateral grafting, utilizing two to four branches per tree. Supplies and parameters required to plan and manage cloned trees through the crown-grafting technique; as well as the basic protocols for this method, are provided in Annex 4 (A4.7).

Cleft grafting and nurse-seed grafting

There has been a tendency to develop technologies to speed up the production of cloned plants, especially by grafting green scions onto very young seedling rootstocks. Ramadasan and Ahmad (1986) assert that the use of green buds seems to confer a tremendous advantage in bud emergence. They also point out that scion vigour is superior to that derived from older material from semi-hard budwood. Grafting techniques involving young rootstocks have shown encouraging results, though they require a mist humidification system for approximately one month after grafting to facilitate the healing of the graft union. For example, studies in Malaysia showed that cleft grafting and nurse-seed grafting produced an acceptably high level of end success, varying from 70% to 80% (Ramadasan and Ahmad 1986). Nurse-seed grafting is performed on five- to nine-day-old seedlings. After germination in greenhouse sand beds, seedlings are washed and placed on tissue paper to dry. A sliver of tissue is then removed from one side of the hypocotyl, after which a trimmed two-leaf softwood or semi-hardwood cutting is cut similarly on one side, brought into contact with the cut surface of the hypocotyl, and bound firmly with parafilm (see Figure 3 in Annex 4, which illustrates a similar technique in use in Java, Indonesia, for propagating clones by applying green-patch budding to two-week-old seedlings).

The latter methods are especially promising since they involve a drastic reduction in rootstock ages to a mere five to nine days, or two weeks. Ramadasan and Ahmad (1986) also highlight the fact that the cotyledon nutrient reserves available in the rootstock allow earlier bud burst and more vigorous scion development. However, according to Lockwood (personal communication, 2013), the technique is rarely used since it is difficult to implement on a commercial scale.

It has not yet been possible to successfully adapt some methods for use with very young seedlings. For example, studies by Ramadasan and Ahmad (1986) in Malaysia, on split or lateral grafting using two- to four-week-old rootstocks, showed disappointing rates of take; split grafting produced particularly low success rates (about 2%). Binding proved very difficult as the wedge tended to slip out due to the presence of a slimy mucilaginous fluid. Callus development along the slit tended to squeeze out the graft. In addition, a major drawback noted was the tendency for the auxiliary buds of the cotyledons to develop, despite regular excision (Ramadasan and Ahmad 1986). Although results of preliminary trials with lateral grafting using two- to four-week-old rootstocks were quite satisfactory (about 55%), no further work was recommended because it did not appear to be practical and convenient for large-scale propagation. These results were similarly reported by Giesenberger and Coester (1976), in studies performed in Amsterdam; and by Rosa (1998) in Bahia, Brazil, who registered rates of success of 35%.

3.1.4 Budding

Budding refers to the transfer of a single plant bud from a stock plant (of the variety selected to be replicated) to a rootstock such as a seedling, or the basal chupon, stem, or branch of an older plant (Annex 4, Figures 1 through 4). The budding method, which is one of the most important methods of conventional cacao propagation, varies according to the maturity of the budwood and the rootstock. For example, chip budding, or patch budding in its various formats, may be conducted using very young plants (green budding) or older plants (brown budding), and are considered conventional methods in most cocoa-producing countries. In any case, for successful propagation the cambial tissues of the bud and the rootstock must be placed in intimate contact to allow the development of functional connections within the xylem and phloem tissues, and to ensure external protection against dehydration. As discussed by Hartmann et al. (2002), physiological processes of cell recognition, callus formation, as well as vascular and parenchyma tissue differentiations are critical steps in graft formation. Budding can be performed using rootstocks from cotyledon to older seedling stages, basal chupons of various ages, tree trunks and high branches.

Despite the apparent advantages of savings in cost and budding materials, including bud sticks, conventional brown budding was gradually replaced by split-grafting techniques, both in the greenhouse and in the field in Bahia. Usually the method requires a certain level of skill, good sanitation and suitable nutritional conditions for the buds. Rates of take usually vary from 60% to 70% (Table A4.1, Annex 4), and rates of initial scion growth are normally very low. It is important to mention that budwood deterioration, very often caused by a complex of fungi, may arrest rates of successful budding in cacao. In a transfer of clonal accessions to the Miami Quarantine Station in Florida, USA, in 1989, five fungi associated with cacao bud sticks were identified as: *Botryodiplodia theobromea, Fusarium decemcellulare, Fusarium oxysporum, Pestalotiopsis* spp., and *Phomopsis* spp., a complex of causal agents that often trigger cacao dieback in budded seedlings (Purdy 1989). Since bud sticks were infected, it is equally important to mention that cacao dieback would reduce rates of take independently of the propagation method utilized.

A list of materials, supplies, and facilities required, as well as a basic protocol for the production and management of conventional brown-budded seedling rootstocks in nursery conditions is provided in Annex 4 (A4.4).

3.1.5 Marcotting or air layering

Marcotting, also known as air layering, is a vegetative propagation method that was developed to promote the formation of roots on attached branches of many species (Hartmann 2002). Normally, the method is confined to species that layer naturally, and is used to multiply ontogenetically mature shoots that cannot be propagated by other techniques (Leakey 2004). However, these restrictions do not apply to cacao since it can be propagated by not only by marcotting but by many other asexual methods, as well as sexually. There are several variations on this method of plant propagation, such as tip layering, simple layering, compound or serpentine layering, but in all these methods 'the induction of root development is usually done by wounding the part of the plant to be rooted' (Bareja 2010). The basic procedure utilized to root cacao branches is illustrated in Figure 13, Annex 4.

3.1.6 Clonal gardens

Most farmers acquire their initial supply of clonal scions from an institutional clonal garden or from neighbouring farms, although caution is advised in virus-infected areas. Once the initial grafted or budded plants have become established, either on farm or in a clonal garden, they are used as stock plants to further generate supplies of scion wood for the next generations of grafts. According to Phillips et al. (2009), CATIE established 36 hectares of clonal gardens with high-yielding and good quality clones resistant to frosty pod rot (*Moniliophthora roreri*) and black pod disease (*Phytophthora palmivora*), on six hectares in each of the following countries: Belize Costa Rica, Guatemala, Honduras, Nicaragua and Panama.

Clonal gardens established for the production of cuttings are normally shaded to allow between 20% and 50% of sunlight interception, a strategy that is in line with Leakey's (2004) suggestion to optimize light conditions to develop cuttings for rooting. According to Leakey, both low irradiance and the ratio of red to far-red light independently enhance rooting ability. Usually the gardens are maintained under an intensive management regime, which includes weeding, fertilizer application, disease and pest control, irrigation (in some projects) and pruning; these procedures vary according to local conditions and recommendations. For successful rooting, the shoots must be at the half-woody stage when the cuttings are taken. This keeps the stock material in the juvenile growth phase. As part of the Biofabrica project, which is currently being implemented in the state of Bahia, in Brazil, both semi-woody cuttings for rooting (from the terminal sections of the fan branches), as well as light brown sticks for grafting, are being produced in clonal gardens. However, as the stock trees age the cuttings will lose vigour and so the clonal gardens will need to be replanted every six years.

The production of cuttings varies according to projects. In Ghana, for example, the number of cuttings taken from a selection series planted in a clonal garden with 2.6 x 1.3m spacing was 100 to 160 cuttings per plant/year for Amazon selections, but only 20-40 cuttings/year for Amelonado selections, as described by Hall (1963), and Wood and Lass (1985). In contrast, the number of cuttings harvested in clonal gardens of the Biofabrica project in Brazil, with spacing of 3.0 x 3.0m, varies according to clone, from 56 (IP01), 63 (CEPEC2002) and 74 (SJ01), to 112 (CCN51) cuttings per plant, per year (Jackson O. Cesar, personal communication, 2012); most of these clones are crosses of *Trinitarios* and *Forasteros*. Guidelines for the establishment and maintenance of the clonal garden are presented in A4.10, Annex 4. A list of materials and labour requirements for establishing clonal gardens is provided in Table A4.2, Annex 4.

3.2 Current status of conventional vegetative propagation

3.2.1 General overview

Vegetative propagation technologies are used in many cacao-growing countries to produce clonal material either for use in seed gardens or to supply directly to farmers, though the latter is far more common in the Americas and in some parts of South-east Asia than in West Africa. Some examples below demonstrate how vegetative propagation technologies have been used in efforts to rehabilitate cacao plantings in several cocoa-producing regions, and the factors that are being taken into account when implementing the technologies.

Vegetative propagation has played a very important role in efforts to rehabilitate cacao farms in Brazil, particularly in the state of Bahia, following an outbreak of witches' broom disease in the early 1990s. The 2011 Brazilian cacao crop was produced in six Brazilian states, but more than 64% of the total production came from the state of Bahia, where farms extend across the Atlantic Forest. The remaining cacao is produced in the states of Pará, Rondônia, Espírito Santo, Amazonas and Mato Grosso, which contributed 24.3%, 7.4%, 3.4%, 0.5% and 0.2% respectively (IBGE/SIDRA 2011).

Economic pressures and the demand for high technology and innovation have been stimulating the development of cacao clonal varieties resistant to witches' broom since the late 1990s. CEPLAC's breeders have been working with farmers to develop improved varieties, and many of the clonal varieties that have been recommended by CEPLAC recently were selected through the participatory work of farmers in southern Bahia. From 1995 to 2006, CEPLAC's breeding programme made 39 recommendations of clonal varieties (as shown in Table 3.1) and a new edition of more advanced selections is expected to be released shortly.

Table 3.1 List of planting material released by CEPLAC (1994-1995) after the outbreak of witches' broom disease in cacao farms in southern Bahia in 1989.

1995	TSH516, TSH565, TSH1188, EET397 and CEPEC42
1998	TSA654, TSA656, TSA792 and TSH774
2001	CEPEC2001 (VB-900)
2002	CEPEC2002 (VB1151), CEPEC2003, CEPEC2004, CEPEC2005, CEPEC2006, CEPEC2007, CEPEC2008, CEPEC2009, CEPEC2010 and CEPEC2011
2003	CCN10, CCN51, CP06, CP38, CP39, CP40, CP49, CP53, PH16, VB276, VB515, VB679
2004	RVID08, LCT-EEN37A, SJ02, and PS1319
2006	IP01, CCN16 and PH09

Source: Milton Conceição, extension agent (CEPLAC), personal communication, 2012.

Field data from areas in which these varieties have been used in grafting procedures have shown very promising results, with a comparatively low rate of pod loss caused by witches' broom or black pod disease (*Phytophthora* spp.). Average production of some substitute clones is higher than that of the old cacao varieties. However, cacao tree planting density is generally low throughout the cocoa-producing region of Bahia, except where trees are established on a 3.0 x 3.0m, regularly spaced design. Regional planting densities rarely exceed 500 plants/ha and are normally around 350 plants/ha. Hence, the cacao rehabilitation programme for southern Bahia emphasizes the replacement of old cacao trees with high yielding, disease-resistant clonal varieties, while at the same time increasing stand density with grafted seedlings. CEPLAC extension agents estimate that over the last decade approximately 137,500 ha of cacao trees, covering a total area of 550,000 ha in southern Bahia, have been grafted to date; whilst selected clonal varieties are being cultivated in the open spaces between existing plants in just 40,000 ha.

In the above case, a rough estimation for planting 400 clonal plants/ha to raise planting density to about 900 plants/ha, would require a total of 40 million grafted seedlings or rooted cuttings to be planted in the remaining 97,500 ha of the total area covered by grafted cacao. Nevertheless, this is an ongoing process and the programme is utilizing several methods of plant propagation to achieve the goal.

In the south of the state of Pará, in the Amazon, the cacao planting area is expanding, especially in Altamira, Novo Repartimento, Uruará, and Medicilândia. Cacao growers in these regions are planting hybrid seedlings of improved selections supplied by CEPLAC. The cacao seedlings are established in the field in 3.0 x 3.0m regular spacing, and under temporary shade of banana trees that are normally removed after two to three years. Therefore, most of the productive areas in this region are managed without any overhead shade or windbreaks. Growers follow CEPLAC's recommended practices, which include the control of witches' broom and pests. Currently, the propagation of clonal varieties through vegetative technologies is not being utilized in this region.

Several other cocoa-producing countries in South America, especially Ecuador, have faced a similar situation in the reduction of cacao productivity and planting areas due to local climatic variations; infection by aggressive biological agents, such as witches' broom, black pod, *Moniliophthora* (*M. roreri*) and *Ceratocystis* wilt (*Ceratocystis cacaofunesta)*; damage by insects; market constraints; and the lack of investment in rehabilitation programmes. However, government and/or private collaborative initiatives based on research programmes have resulted in some new cacao varieties that are more resistant to the diseases mentioned and efforts are underway to provide them to farmers.

Improved technical assistance in the rehabilitation of old farms in countries such as Ecuador (Ivan Garzon, personal communication 2012), Colombia (Florez and Calderon 2000), and Peru (Gardini et al. 2004) is reversing the decline. Updated information on the current situation in Ecuador shows that most of the new cacao farm areas are being planted with the clone CCN51, which has been reproduced by the rooted cutting method. A small portion of the tree stocks have been propagated by grafting, particularly of the clones recommended by the Instituto Nacional Autónomo de Investigaciones Agropecuarias in Ecuador (INIAP) (Garzon et al. 2011a). Productivity levels vary, but selected farms or community groups have reported yields from 2,000 to 2,500kg of cacao beans per ha/year, mostly where the planting material has been grafted with CCN51 (Campo and Andía 1997, Espinosa et al. 2008). In addition, cacao production in the Caribbean region has also shown trends of some increase over the past three to four years, particularly in Jamaica and the Dominican Republic, according to data published by the Food and Agriculture Organization (FAO) of the United Nations (FAOSTAT 2012). Trends of increases in production were also observed for the same period in Brazil and Ecuador. FAOSTAT data show that the volume of cacao harvested in Ecuador jumped from

85,800 tonnes (t) in 2007, to 132,100t in 2010; while Brazilian production increased from 201,100t to 235,389t in the same period.

However, cocoa-producing countries in other parts of the world, for example Côte d'Ivoire, Nigeria and Indonesia in the eastern hemisphere, also faced productivity limitations associated with devastating viruses and/or fungal diseases. For instance, cocoa swollen shoot virus (CSSV), which is transmitted by a mealy bug, occurs especially in Ghana, but effects many the main cacao-growing countries of West Africa, where the disease has caused serious crop losses. Another example to illustrate this point is the vascular streak dieback (VSD) disease. This disease is caused by the fungal pathogen *Ceratobasidium* (formerly *Oncobasidium*) *theobromae*. VSD was first reported infecting cacao farms in Malaysia and Papua New Guinea in the mid-1960s. It became the most destructive cacao disease in Malaysia, and by the early 1980s it had spread widely into Indonesia. At the time, smallholder farmers in Sulawesi, Indonesia, were planting Amelonado seedlings, which are highly susceptible to the disease. As pointed out by Bloomfield and Lass (1992), different races of the pathogen in Indonesia, Malaysia and Papua New Guinea means that separate breeding programmes would be required to select cacao varieties resistant to the particular strains of each country.

For any of the cacao diseases mentioned above, the main long-term measure of control is that of planting resistant varieties, as discussed by Lockwood (2012). Breeding strategies used for the development of advanced varieties of cacao with the aim of rehabilitating crop production in southern Bahia follows Lockwood's argument. The breeding strategy in Bahia focuses on a continuous process involving scientific and technical planning and work, as recently discussed by Pires et al. (1996), Lopes et al. (2011), and Monteiro et al. (2012). CEPLAC's updated approaches to the production of improved cacao varieties are also in line with the recommendations of Bartley (1994) and Van der Vossen (1996). Breeding methods, such as reciprocal recurrent selection (RRS) schemes with distinct sub-populations, have also been successfully applied to crops such as oil palm and Robusta coffee, as outlined by Van der Vossen (1974) and Charrier and Berthaud (1988) respectively. This breeding strategy is presently being utilized for the improvement of cacao varieties in Brazil and elsewhere. In addition, integrating the knowledge of farmers and breeders in the selection of new varieties has shown to be successful in Brazil, dealing with an emergency situation aimed at finding varieties with good resistance to witches' broom. Most of the clonal varieties recommended by CEPLAC were selected in a participatory process involving farmers in southern Bahia.

Most farmers in West Africa use seed to propagate their cacao, but there is increasing interest in vegetatively propagated cacao in the region. For example, the Cocoa Research Institute of Nigeria (CRIN) recently recommended eight clones for use by Nigerian farmers (Adewale and Aikpokpodion 2012). CRIG is implementing a project to rehabilitate some farms in Ghana using clones known for their fine flavour characteristics (Stephen Opoku, personal communication, 2013), and there are initiatives in Côte d'Ivoire such as the Mars/World Agroforestry Centre Vision for Change (V4C) project in Soubré, where about 30% of the local production is the result of successful grafting programme (Désiré Pokou, personal communication, 2013). Since much of the Soubré region has been affected by CSSV, which can be transmitted through grafting and budding, extreme care must be taken to ensure that the stock material used for any conventional vegetative propagation techniques is free from the virus to prevent its further spread.

3.2.2 Rootstocks

Cocoa-producing countries in the Americas primarily utilize rootstocks to control soil pathogens. For example, rootstocks of the TSH1188 clone and of the Comum variety are utilized by Brazilian growers to control *Ceratocystis* wilt. Ecuadorian growers control different strains of the same disease through the use of open-pollinated CCN51 rootstocks, and other selected rootstocks that are recommended by INIAP for the control of *Ceratocystis* and the *Rosellinia/Lasiodiplodia* complex (Ivan Garzón, personal communication, 2012). Growers in Colombia use PA46, PA121, PA150, IMC67, and hybrid seeds of PA46 and EET62 x IMC67, to control root rot caused by *Phytophthora* spp. and *Rosellinia* (Florez and Calderon 2000). EET400 was the rootstock of choice for Izarry and Goenaga (2000) due to its consistent yield capacity. The relative tolerance of ICS60 to heavy soil made it the preferred genotype for rootstock establishment for Murray and Cope (1955). However, Nigeria also utilizes selected seedling rootstocks to escape the threat of disease. According to Adewale et al. (2012), F3 Amazon has been the most commonly utilized cacao rootstock establishment in Nigeria.

3.2.3 Rooted cuttings

The rooted cuttings technique, initially developed for research purposes, was used for the commercial propagation of cacao during the 1950s in Grenada, Trinidad and some other Caribbean islands; it was also used on a smaller scale in Central and South America (Wood and Lass 1985). The technique has more recently been used in large-scale operations to provide planting materials for rehabilitation projects in Brazil and Ecuador. Examples of recent activities in Brazil involving the production of rooted cuttings are provided below.

Rooted cuttings in Bahia, Brazil

Rooted cuttings had not been used on a commercial scale in the cacao farms of Bahia before the outbreak of witches' broom disease in early 1994. However, with the urgent need to provide large amounts of planting materials for the rehabilitation efforts, a new mass propagation facility, known as Biofabrica de Cacau, was initiated in 2000 with an annual capacity to produce up to eight million rooted fan cuttings ready for field planting. These rooted cuttings have been utilized to increase the planting density of the rehabilitated areas grafted with disease-resistant clone varieties. In addition, the rooted cuttings have also been planted on flat, deep soils and areas managed with a fertigation system, full sunlight, and windbreaks. This new system of planting is being used in southern and western regions of the state of Bahia.

The Biofabrica production facility (see Figure 11 in Annex 4) is a mass-propagating unit located 60 km north of CEPLAC's headquarters in Ilhéus, Bahia. CEPLAC provides scientific and technical support for the rooted cuttings project and supplies the recommended clone cultivars (listed above in Table 3.1) for propagation. The facility is well equipped with 20 rooting units measuring 2,500m^2 each; the tops and sides of the units are covered by a protective screen of saran or polypropylene tissues (50% light interception). Each unit comprises metal benches and an overhead, automated irrigation system, and currently holds 216,000 rooting cuttings in 0.288 litre plastic tubes for a six-month period (approximately 86 rooting cuttings per m^2/six-month-period). A new initiative is now under way to increase the capacity of each unit to approximately 700,000 (over 280 rooting cuttings per m^2/six-month period) by reducing the size of plastic tubes (Jackson O. Cesar, personal communication, 2013). The project has 80,000 stock plants to supply cuttings and materials to farmers for grafting.

The efficiency of rooting varies depending on the genotype and time of the year, but on average success rates range from 60% to 75% (see Table A4.1, Annex 4); some rooted cutting plants in production are shown in Figure 6, Annex 4. The rooting method used by Biofabrica is fairly similar to that used in the large-scale production of rooted cuttings of *Eucalyptus* spp. and several other species of trees (Hartmann et al. 2002), as described in Annex 4 (A4.8) for the use of the technology in Brazil and Ecuador.

Cacao rehabilitation projects in Brazil

Production data collected from a clonal planting trial conducted in a non-traditional cocoa-producing region in Nova Redenção, in western Bahia, Brazil, has shown impressive preliminary results for productivity. The region is characterized by its semi-arid climate with an average annual temperature of 23ºC (maximum 27ºC, minimum 18ºC); annual rainfall of 600mm, with irregular distribution; and relative humidity of 40%. In March 2003, rooted cacao cuttings that had been prepared in the Biofabrica facility in Ilhéus, were transported to Nova Redenção (450 km west of Ilhéus), and transplanted into deep, flat soil in the field, under the temporary shade of banana trees. They were managed with drip fertigation and were regularly pruned. According to Leite et al. (2012), the average productivity of the clones PH16 and CCN51 at 52 months, after field planting, reached 1,770 kg/ha of dry beans annually, with an outstanding performance from CCN51 in terms of the most agronomic traits, especially vigorous growth, resistance to pests and diseases, and high yield, with productivity at 2,260 kg/ha annually.

The establishment of some large-scale cacao holdings in southern Bahia, in areas of declining papaya farms, has been quite successful to date. An example can be seen in the project Lembrance, a clonal farm initiative established on 250 ha under the moderate shading of papaya trees, partially exposed to full sunlight. Field planting began in 2006 and continued annually up to 2009, with one to two rows per clone, in multiclonal blocks, and spacing of 3.8m or 4.0m x 2.0m. Rooted cuttings of 32 recommended clones, propagated at the Biofabrica facilities, were established in a semi-mechanized system (weeding, open planting holes, pesticide spraying, and drip fertigation). The project site is located about 260 km south of the main cocoa-producing zone in Bahia, in an area of deep, flat and low, naturally fertile, sandy soils. Currently, the project only works with the following clones: CCN10 and CCN51; CEPEC2002, CEPEC2004, CEPEC2005 and CEPEC2006; CP49; PH16; PS1319; and SJ02 (see Figure 11, Annex 4), all arranged in monoclonal blocks. The 22 remaining clones initially planted were eliminated due to low agronomic field performance, especially low pod yield. Updated yield results for the first 60 ha initially established are described in Table 3.2:

Table 3.2 Project Lembrance, Eunapolis, Bahia. Field planting conducted in 2006-2007.

Harvesting year	Average yield of clones / kg of dry beans/ha	Remarks
2009	1,200	Initial pod harvest
2010	1,950	First significant harvest
2011	2,505	Best yield: CEPEC2002; 3,540 kg of dry beans/ha/year; five year-old trees
2012	3,750	Forecasted

Source: Basilio Leite – CEPLAC/CEPEC, Personal communication, 2012.

Rooted cuttings in Ecuador

The cacao rehabilitation and expansion programme in Ecuador is unique since it is based on the production of rooted cuttings in massive farms using a single clonal variety. The clone CCN51 has for many years been the preferred cacao variety of Ecuadorian cocoa producers for both small- and large-scale commercial farms in the country due to its high yielding characteristics. The first farms established using rooted cuttings of this variety, were set up in the mid-1960s, initially at Sofia Farm in the Naranjal zone and expanding thereafter to Rancho San Jacinto in 1984, and other areas, such as Los Cañas (Campo and Andía 1997). However, 80% of Ecuadorian cocoa producers use Nacional varieties owing to their high quality and flavour ('sabor arriba'), while the remaining 20% use other varieties, especially CCN51.

Progress in the planting of CCN51 has now slowed down in Ecuador, and clones recommended by INIAP - such as EET19, EET48, EET62, EET95, EET96, EET103, EET544, EET558, EET575 and EET576 - are being grafted in areas traditionally planted with the old seedling-derived *Nacional* varieties (Ivan Garzon, personal communication 2012). These recommended clones are improved selections of the series. The clone EET111 (ICS95) is still recommended for specific regions; six of the aforementioned clones recommended by INIAP were released during the 1970s (EET19, EET48, EET62, EET95, EET96 and EET103) and are still being planted, particularly EET19, EET95, EET96 and EET103. The more recent releases and clones currently being developed combine some of the yield characteristics of CCN51, with the flavour characteristics of the Nacional varieties (Freddy Amores, personal communication, 2012).

Management protocol for the Caribbean region

Besides the choice of propagation method, it is also important to identify a protocol of agronomic procedures that can be utilized in programmes aimed at improving productivity. One example can be seen in the cacao rehabilitation productivity programme implemented in the Caribbean region in the 2000s (Maharaj and Rammath 2010). Basically, the programme focused on the production of fine cocoa in the smallholder farms, using the following agronomic procedures, which can also be applied elsewhere in cocoa-producing countries:

- Utilization of superior, improved clonal varieties (grafted seedlings or rooted cuttings) or hybrid seedlings (such as TSH1102 x TSH1095)
- Higher ratio of self-compatible/-incompatible trees
- Measures to improve cacao pollinator populations (e.g. by placing a layer of leaf litter, banana pseudostems, and harvested pod shells at the base of the tree)
- Annual pruning (to open the canopy)
- Optimal fertilizer application
- Appropriate shade management
- Strict field sanitation.

3.2.4 Grafting

Various grafting methods have been used both at a farm level and in commercial nurseries, particularly in the Americas. Top or split grafting of chupons or seedlings, in the field or the nursery, are the most popular ways used by the cacao farmers to propagate clones in Bahia, Brazil, and elsewhere since they are quick and easy methods, and generally present high rates of success. At the onset of a cacao rehabilitation programme implemented in commercial farms, in which cacao seedlings were top grafted onto basal chupons in farms in Bahia, Sena Gomes et al. (2000) registered the rates of take for commercial planting as ranging from 55% to 92%, depending on clone, location and time of the year. In Bahia, successful grafting is normally achieved between September and March, in years of typical climatic conditions. The utilization of the top-grafting technique on basal chupons of old trees, especially on trees with damaged bark, as part of a rehabilitation package, has also become very popular in Sulawesi, the largest cocoa-producing area in Indonesia. Many small nurseries in Sulawesi are also successfully top grafting Asian clones onto two- to three-month-old rootstocks of the M01 clone (Smilja Lambert, personal communication, 2013) (see Figure 3, Annex 4). Similar success has also been observed in several projects in the Philippines (as illustrated in Figure 2, Annex 4), as well as in Malaysia (Kelvin Lamin, personal communication, 2013). In Brazil, the top grafting of seedlings of a similar age, grown in plastic tubes in the nursery, achieves success rates of between 80% and 90% (see Table A4.1 in Annex 4). The Bom Retiro farm project in Camacan, Bahia, has been operating five greenhouse units for almost three years, with a production capacity of 200,000 grafted seedlings per year. Some details of this project are illustrated in Figure 5, Annex 4. This procedure is now becoming very attractive for several other projects but with the utilization of simpler infrastructures.

The lateral- or side-grafting method performed on the trunk of mature trees, as described by Yow and Lim (1994), was also used at the beginning of the clonal programme in Bahia (Sena Gomes et al. 2000, Pinto 2000) to replace trees infected with witches' broom with new disease-resistant clones, or to propagate them in clonal gardens. At that time, the rates of take were low when applied to mature tree trunks (less than 30%), but higher (more than 70%) when applied to young basal chupons or to high branches (crown grafting) of 15- to 20-year-old trees, as outlined in Table A4.1, Annex 4. Rosa (1998) recommended the utilization of two side grafts (one on each side of the tree trunk), claiming success rates of take of 70% in Bahia. Whilst this technique is no longer recommended for grafting onto the trunks of very old cacao trees in the region, it is still important for grafting onto young cacao plants (less than 20-year-old trees) in Bahia and in several other cocoa-producing countries, especially in Malaysia (Kelvin Lamin, personal communication, 2013), and in many rehabilitation projects in Sulawesi, Indonesia, as well as in the Philippines, Ghana, and Nigeria.

A variation of the conventional method has been used to propagate recommended INIAP clones in Ecuador. Seedling rootstocks are prepared with recommended seeds, resistant to major soil pathogens, such as *Ceratocystis* spp., *Rosellinia* and *Lasiodiplodia* complex. The scion of a Nacional clone, with a two-sided wedge, is inserted laterally into a cleft of a three- to four-month-old seedling rootstock, and is tied with a plastic strip. The scion normally has two or three size-reduced leaves (and three buds), and the grafted area is immediately enclosed with a transparent plastic bag for about three weeks to avoid tissue dehydration.

According to Rob Lockwood (personal communication, 2013), the side-grafting technique has been successfully utilized, though not extensively, in Indonesia, Malaysia and the Philippines, especially with mature cacao, using the method described by Yow and Lim (1994), with guidance from the Malaysian Department of Agriculture on the rehabilitation of mature cacao (Department of Agriculture Malaysia, 1993). Green-patch budding in the nursery on the hypocotyl stem of three-week-old rootstocks, and side grafting in the field to convert mature cacao plantings, has been conducted in Malaysia for the large-scale multiplication of commercial clonal cultivars. In Indonesia, vegetative propagation has been used as part of rehabilitation programmes to address the steep decline in smallholder yield, which fell from about 1,200kg/ha/year (1998-2003) to 120-240kg/ha/year in 2011. Several factors were associated with this decline, especially tree age (over 20 years) and poor crop management in relation to weed control, fertilization, pruning, infrequent/irregular harvesting, and the presence of pests and diseases such as VSD, stem canker (*Phytophthora palmivora*), anthracnose (*Colletotricum gloeosporioides*), pink disease (*Corticium salmonicolor*), and cacao pod borer (CPB, *Conopomorpha cramerella*). To overcome the decline in yield in Indonesia, a government-run programme, the National Programme for Increasing Cocoa Productivity and Quality in Indonesia (Gernas Kakao), initiated a rehabilitation project involving side grafting onto the trunks of old trees, as described by Yow and Lim (1994). Field observations in the Konawe, South Konawe and Kolaka regencies of South-east Sulawesi, suggest that about 60% of farmers obtained successful results from side grafting. Results also showed an impressive increase in yield three years after grafting. The technique promoted a five-fold increase in revenue compared to the old cacao plants (IIBCRI 2014). A higher success rate for side grafting was reported by Suharto et al. (2012), quoting Prawoto, for cacao rejuvenation work developed in the Jember area of East Java, in Indonesia.

Similarly, in a rejuvenation programme established in Malaysia in early 1990's, hybrid cacao areas were replaced with clones by side grafting onto unproductive trees (Kelvin Lamin, personal communication, 2013). The programme for rehabilitation of mature cacao was conducted by the Department of Agriculture of Malaysia). The benefit of such 'crop salvation' is to take advantage of the well-established root systems of mature stock trees, in order to achieve rapid growth of scions and early bearing. Side grafting onto the trunk or branches was carried out using new high yielding and disease-resistant clones. Two or more side grafts were made to minimize the possibility of rotting in the stock plant trunk, and to obtain a more balanced canopy. A similar approach has also been used in Bahia (see Figure 6, Annex 4) and in Costa Rica (see Figure 7, Annex 4). Adomako and Adu-Ampomah (2005) analysed the low yields attained by smallholder farmers in Ghana, and suggested that the technique of side grafting onto mature cacao trees could be applied in all West African cocoa-producing countries to upgrade cacao yields, after farmers identify the unproductive trees in their farms. It should, however, be noted that an adequate supply of proven high-yielding clones would be needed to make this an effective approach (Lockwood, personal communication, 2013).

Crown grafting, which is regarded as another 'salvation' technique, is appealing to farmers owing to the fact that the flowering and fruit-bearing processes are continuous. However, these processes may possibly be reduced during the replacement of the old canopy of the cacao trees, which overlaps with the development of new leaves from the grafted clone (Sena Gomes and Castro 1999). Furthermore, the reserves stored in the root and trunk, and in parts of the higher branches, are not eliminated by pruning, and can be used to support the development of the scion and the crop. In addition, parts of the old crown (branches and foliage) are only eliminated six months or so after grafting, and by this time the leaf area of the new crown will already be well developed. Normally, it takes approximately ten months to restore the new leaf area after grafting. Another interesting use of crown grafting is the approach being used by some farmers in Bahia. Due to risks of infection by *Ceratocystis cacaofunesta*, crown grafting has been successfully applied to 12- to 18-month-old seedling rootstocks. The grafts were made at the tips of three fan branches by the split-grafting method described above.

Many successful cacao rehabilitation projects in Bahia utilize a combination of field-grafted chupons (split grafting) to replace unproductive trees (see Figure 3 in Annex 4), and nursery-grown grafted seedlings to increase plant density. This practice was used in the Renascer project, which was implemented in the late 1990s at Lagoa Pequena, a farm in the region of Castelo Novo in Ilhéus, Bahia. The project was implemented on 300 ha of old cacao, planted in the 'cabruca' system of cultivation, whereby trees are planted in native forests (see Figure 3 in Annex 4). At the beginning of the project, the farmer's own selections were used, in addition to some self-incompatible clones recommended by CEPLAC. In 2003, the basal chupons of all 10,150 trees in an experimental block were grafted using one of the following nine clones PH15, PH16, LP06, SL70, SL71, CCN10, CCN51, FB206 and CEPEC2002 (VB1151). All except FB206 are self-compatible clones. The clones were split or top grafted onto basal chupons, in a monoclonal system, and they were managed according to CEPLAC's recommendations, but with special emphasis on sanitation. Fungicidal sprays were not used, and overhead shade was controlled. Between 2005 and 2008, this practice was carried out in about half of the remaining areas of cacao plantings, except for 90 ha of the old Comum variety (Claudio S. Silva, personal communication, 2012).

The data in the Table 3.3 below show results of the Renascer project, which are particularly interesting given the fact that approximately 70% of cacao produced in Bahia originates from the cabruca system of planting.

Projected costs for the establishment and maintenance of one hectare of cacao under Bahian conditions are provided in Table A4.3, Annex 4. The data presented in the table can also be applied to any other rehabilitation projects involving different propagation technologies, such as budding or rooted cuttings.

Table 3.3 Results of the Renascer project, Castelo Novo, Ilhéus, Bahia.

Farm experimental block (11 ha)			Farm commercial production (300 ha)		
Harvesting year	**Dry beans (kg)**	**Dry beans (kg/ha)**	**Dry beans (kg)**	**Dry beans (per ha)**	**Remarks**
2008	8,130	739	55,320	184	Pod losses: 8% affected by witches' broom (WB)
2009	10,125	920	75,510	252	Pod losses: 6% - WB
2010	12,240	1,113	147,870	493	Pod losses: 5% - WB
2011	11,985	1,090	135,330	451	Light drought; less than 3% of pods affected by WB
2012	11,701	1,063	170,160	567	Light weather variation
2013	12,500	1,130	100,000	630	20-30% current mid-crop harvests forecasted

Source: Claudio S. Silva – Chaves Agricola, Itabuna, Bahia. Personal communication, 2012.

3.2.5 Budding

Since the earliest reports of successful patch budding in Jamaica in the 1900s (Harris 1903, Mooleedhar 2000), the use of the technique, especially with green, greenish-brown or brown budwood, has spread to many cocoa-producing countries, such as Brazil, Côte d'Ivoire, Costa Rica, Ecuador, Ghana, Haiti, Indonesia, Jamaica, Malaysia, Nigeria, Peru, São Tomé, the Philippines and others. In general, results vary widely according to plant, environment and management practices; as such, results from only some of the studies are presented in this review. Images of budding in Costa Rica, Indonesia, Malaysia, and the Philippines, are provided in Annex 4, in Figures 1 to 4.

A large-scale nursery operation in Malaysia for the production of conventional budded seedlings of cacao clones, first described by Shepherd et al. (1981), utilized standard rootstocks produced from crossing UIT1 × NA33. It was noted that most cacao smallholder farmers and commercial cacao nursery operators normally grow rootstocks from large seeds obtained from commercial cacao fields, especially following reports that large bean size enhances vigorous seedling establishment for efficient patch budding and top grafting (Shepherd et al. 1981). According to Lee (2000), conventional budding has been successfully performed on one- to four-month-old seedlings in Malaysia. However, Yow and Lim (1994) reported that conventional budding applied to three-month-old seedling rootstocks takes at least six months to produce field plantable budded seedlings. They also emphasized the high costs of preparing and maintaining the budded seedlings in large plastic bags in a nursery shelter for such a long time.

Due to these constraints, many attempts have been made to overcome the high costs of conventional budding, by applying the green-budding technique, in various ways, in order to reduce the time that budded seedlings remain in the nursery. The green-budding technique was pioneered by Jacob (1969) and Rosenquist (1952), who successfully budded cacao seedlings at the cotyledon stage. Studies with similar objectives were carried out by many researchers in various cocoa-producing countries. For example, Giesenberger and Coester (1976) described the green-budding technique, modifying Forkert's method and applying it to hypocotyls of two- to six-week-old seedling rootstocks, and found budding take varied from 90% to 100%, under experimental glasshouse conditions in Amsterdam. They compared the 'T' and inverted 'U' (modified by Forkert) budding methods and found that the latter gave the most satisfactory results. Giesenberger and Coester (1976) also discussed the practice of green budding onto three- to four-month-old rootstocks as developed in Indonesia. Budding techniques are well known in Malaysia, especially the Forkert method (patch budding), due to their use in rubber (Dijkman 1951); as a result, the budding of cacao was quickly adopted in the country. Yow and Lim (1994) conducted extensive studies on the subject and reported successful results for the green budding of two-week-old rootstocks in nursery conditions in Sabah, Malaysia. Similarly, the patch budding of scion clones onto very young seedlings (no older than two weeks old) is being successfully conducted in large farms in Java, Indonesia (see Figure 3, Annex 4) (Smilja Lambert, personal communication, 2013). In contrast, Are (1969), in Nigeria, compared C10 and C57 clones budded onto F3 Amazon seedlings (8, 10, 11, 12.5, and 18-month-old rootstocks), and found that the tested scion clones performed better on the oldest rootstocks tested.

Cacao budding is widely used to multiply selected parents for breeding programmes and to establish seed gardens. Conventional brown budding was the technique utilized by CEPLAC in Bahia for this purpose until the mid-1990s. However, this budding technique has not been commercially utilized as a clonal propagation method in Bahia, or in any of the other cocoa-producing states in Brazil. Among the constraints deterring producers from using this technique include the low rate of take, especially due to excessive handling of potted plastic bags, long period of exposure of plant tissues; and lack of appropriate sanitation to prevent contamination under high humidity in the nursery. In addition, it is a slow-starting technique, requiring a lengthy period of time before the budded plant can be field planted, and involving high costs of production. In contrast, the conventional budding of one- to four-month-old seedling rootstocks has been widely used by Malaysian growers. According to Kelvin Lamin (personal communication, 2013), hybrids were replaced with clones during the 2000s in Malaysia because they have better resistance to prevailing diseases (VSD and black pod diseases), whilst seedling rootstocks (from open-pollinated seeds) were utilized for the production of cacao plants for new plantings through patch budding (see Figure 1, Annex 4). Green-patch budding in the nursery on the hypocotyl stems of three-week-old rootstocks has been used in Malaysia for the commercial multiplication of clonal cultivars (Lee 2000).

Approaches to accelerate bud break and initial growth after budding have also been sought; though in many cases the rootstock is simply decapitated once the bud has taken (Rob Lockwood, personal communication 2013). Kadje and Ndjama (1982) in Ebolowa, Cameroon, found that bending encourages leaf burst and scion development, and improves the success rate of grafts. In their studies, bending was carried out immediately after budding, by inserting a shield-shaped bud into an inverted 'T' cut into the stems of six- to ten-month-old rootstocks, below the cotyledon. Kadje and Ndjama (1982) hypothesized the importance of growth factors on bud burst and leaf development in the bending approach, possibly by action of the naturally occurring growth promoter auxin indol-3-acetic acid (IAA).

In addition, Ramadasan and Ahmad (1986) screened several methods of propagation in Malaysia, and found that chip budding was the most attractive budding technique, owing to the fact that it is cheap and simple, it does not require the use of specialized propagation facilities, and it can be readily adopted for commercial exploitation. Recent results presented by Kelvin Lamin (personal communication, 2013) show that success rates of the chip-budding method when applied to two- to four-week-old rootstocks were very low compared with other budding techniques (see Table A4.4, Annex 4); however, the method was used routinely for many years at Bah Lias Research Station in Sumatra, until the parent company abandoned cocoa production (Rob Lockwood, personal communication, 2014). Nevertheless, researchers formerly based at the station provided detailed guidelines to ensure optimal success levels for the production of young cloned plants through chip budding, as follows:

- Five- to sixteen-week-old rootstocks produce better results than younger rootstocks (within the age range investigated).

- Bud chips, ranging in length from 1.5 to 2.5cm are ideal. If the chips are too small, poor results will be obtained. The depth of the cut into the stock plants should be about 50% of the stem diameter, if the cut is too deep or too shallow the level of success will be lower.
- A critical requirement is that watering should be in the form of a fine mist in order to prevent any disturbance to the grafted plantlets.
- Shade should be no less than 15% to 20% full sunlight, with optimal shade at about 30%.
- The terminal shoot should be excised above the cotyledons at least one week before budding. The hypocotyls become thicker and harder, so that budding is facilitated at about three weeks. Excision should be performed at the latest at the time of budding. Any further delay may lead to failure of the graft union, or of scion shoot emergence.

Traditionally, propagation via budding was extensively used in São Tomé, and was practiced for many years in West Africa, especially Ghana (Wood and Lass 1985). In Nigeria, studies have shown a significant improvement in the rate of take of the brown-budding technique when the ringing method is applied to twigs two days before the budwood is collected, and good sanitation practices are employed (Aikpokpodion, Badaru and Eskes 2003). Indonesia also routinely utilized budding to multiply cacao clones (Ascenso 1968). Gardini et al. (2004) reported the use of an old, traditional brown-budding technique ('un parche') in Peru, which requires highly trained grafters; wastage of seedling rootstocks is minimal as the procedure can be repeated over and over on the same seedling if the first budding fails. Besides this, budding makes very efficient use of scion wood, since it only requires a single clonal bud to graft onto a seedling rootstock (see Figures 2 and 4, Annex 4 for illustrations of the patch-budding technique applied in Malaysia and Costa Rica respectively), in contrast to other traditional methods in which the scion has several buds. This is an important factor when scion wood of a given clone is limited; for example, during the introduction of new accessions into quarantine facilities.

3.2.6 Marcotting

According to Hartmann et al. (2002), rooting by marcotting, or air layering, is highly reliable for hard-to-root, priceless clones; and is commercially used in the production of many plant species, although the costs and labour requirements are high. Therefore, it appears that the propagation of cacao by this method is appropriate for small-scale (family) projects, in which only a small quantity of plants are needed.

Attempts to propagate cacao using this method have been made in the past in some cocoa-producing countries, such as Ecuador, India, Malaysia and Nigeria but they were not very successful. Ramadasan and Ahmad (1986) pointed out that the procedure possesses little potential for commercial exploitation under Malaysian conditions, owing to unsatisfactory production efficiency and various practical considerations, as reported in the results of trials conducted at the Malaysian Agricultural Research and Development Institute (MARDI). In addition, the Board of the National Institute of Industrial Research presented results of trials of Forastero cacao in India, showing that the most successful rooting (58%) was obtained from air layering with the growth regulator indole butryric acid (IBA) at 3,000 parts per million (ppm); rooting in the control group was 40% (NIIR Board 2004).

3.3. Costs of conventional vegetative propagation

In general, the costs of producing conventional cacao rootstocks (seedlings) vary widely among countries, according to several factors such as scale of production; production protocol (sizes, labour, period in the nursery, size of plastic bags, soil mixture, irrigation system, management procedures); facilities required; and administration. As such, the cost of producing a seedling rootstock may vary from US$ 0.07 to US$ 0.17 in Ecuador, to as much as US$ 0.80 to US$ 3.00 in Costa Rica, with seedling age influencing variations in price within each country (see Table A4.5, Annex 4).

It is well known that budding can be a very expensive method in comparison with other propagation technologies. According to Hartmann et al. (2002), the technique is generally considered to be three times more costly than cuttings for many species, and 14 times more expensive than seedling propagation. The high cost of production is normally associated with labour requirements. This may also be the case in cocoa production; however, as with other propagation technologies, production costs vary considerably between countries and depend on the scale of the operation. Therefore, in this review we will only present the projected costs for the conventional brown budding and grafting of four- to six-month-old seedling rootstocks, as well as rooted cutting plants, both under nursery conditions, and based on experiences in Bahia (see Table A4.6, Annex 4).

Projections made by the authors for the production of cuttings from an established clonal garden with 3,333 plants/ha (tree spacing of 3.0 x 1.0m) and intensively managed with available technologies (such as irrigation, fertilization), indicate rates of productivity ranging from 300,000 to 500,000 semi-woody cuttings/ha/year in the third year of management. This estimated productivity is based on well-managed cacao stands that allow multiple harvests per year. Projected costs for the establishment of a clonal garden, with fan cloned or rooted plants, are provided in Table A4.7, Annex 4; orthotropic cloned plants will have similar costs.

Side grafting is reported to have high rates of take (Ivan Garzón, personal communication, 2012); applying this technique with clones recommended by INIAP costs US$ 0.75/successful grafted seedling.

Marcotting is not commonly used for the commercial cultivation of cacao in the main cocoa-producing countries, and not enough information has been published on productivity, costs and potential problems associated with the use of this technique. Nevertheless, a list of materials, supplies and basic procedures for the production of clones using this method is provided in Annex 4 (A4.9).

Cross-cutting issues and projected costs across methods are provided in Annex 4 as follows:

- Comparative productivity, percentage rate of take, cost and general considerations for propagation methods utilized by the Brazilian cacao rehabilitation programme (Table A4.1)
- Projected labour and materials required for the establishment and maintenance of one hectare of clonal garden for three years, in Bahia, Brazil (Table A4.2)
- Projected budget for the establishment and maintenance of one hectare of clonal cacao in Bahia, Brazil, for seven years (values in US$) (Table A4.3)
- Variation in costs (US$) of seedling rootstocks in some cocoa-producing countries (Table A4.5)
- Projected costs (US$) for the production of 100,000 grafted seedlings or rooted cuttings, based on Brazilian experiences (Table A4.6)
- Projected budget for the establishment and maintenance of one hectare of clonal garden in Bahia, Brazil for three years (values in US$) (Table A4.7).

3.4 Advantages and constraints of conventional vegetative propagation

3.4.1 Seedling-derived rootstocks

Advantages:

- Dormancy-free seeds can be used
- Rootstock materials are cheap and easy to obtain
- Technique can be easily adopted by farmers
- Some disease control provided (e.g. against *Ceratocystis* spp., damping-off agents)
- Increased yield of some clones (according to several reports)
- Alteration in scion vigour in some scion/rootstock combinations.

Constraints:

- Seed viability is of short duration
- Sexually propagated cacao seedling rootstocks may show genetic variability in many important agronomic traits, such as growth rate, vigour, crown shape, and disease resistance. If the rootstocks are heterozygous or produced by open pollination, field performance may be reduced in some specific scion-rootstock combinations (according to several reports).

3.4.2 Rooted cuttings

Advantages:

- Easy technique, with a high multiplication rate
- Free from graft incompatibility and rootstock suckering
- Low production costs, with the possibility of using either simple and cheap facilities, or large-scale facilities
- Rooted plants grow on their own roots (fibrous root system), and can propagate both plagiotropic or orthotropic scions (also true for other methods)
- This technique presents medium to high rates of success for many clones.

Constraints:

- Large-scale projects require high investments in facilities, equipment, clonal gardens and supplies
- Intense labour requirements
- Highly trained labourers required
- Strict sanitation needed in the clonal garden before harvesting the scion sticks, during and after rooting process
- Plants, particularly those from fan cuttings, develop shallow root systems in some heavy (high clay content) soil types
- Lack of taproots (fan cuttings) may arrest plant growth and yield during the dry season especially in shallow soils
- Risks of disease infection by soil-borne pathogens in susceptible clones
- Indicated for specific clones and sites (well-drained, preferably deep, flat, but not hilly soil).

3.4.3 Grafting (top or split, side or lateral, and crown or high grafting)

Advantages:

- Quick-starting, easy and simple techniques
- High rates of take and high yields (nursery and field grafting)
- Low to medium production costs

- Clones may be quickly propagated due to fast growth rate
- Techniques can be performed using many different types of rootstocks (seedlings, stems, old tree trunks, high branches).

Constraints:

- Scion-wood sticks required, with four to five buds per graft
- Plastic bags or biodegradable grafting tape required, in addition to plastic cord in some grafting methods (e.g. side grafting in mature trees)
- Medium to high production costs involved
- Highly trained labourers required
- Strict sanitation and post-grafting care needed.

3.4.4 Budding

(Includes other similar vegetative propagation techniques, such as grafting)

Advantages

- Technique can be easily adopted by farmers
- Single bud (patch) utilized to graft a seedling rootstock
- Wastage of seedling rootstocks is minimal (procedure can be repeated if first budding fails)
- Important propagating technique where the quantity of clonal material is limited (overseas transport and quarantine services)
- Clones can be propagated using older rootstocks (old trunks and high branches)
- Clones that cannot be propagated by other techniques (grafting, air layering, and root cuttings) can sometimes be propagated by budding
- Technique can be used as an auxiliary technique to detect the presence of virus-infected plant material (virus indexing).

Constraints:

- Highly trained grafters required
- High production costs
- Largely dependent on strict sanitation measures
- Variable rates of take (low to medium success rates)
- Technique is slow-starting and requires lengthy post-budding care
- Medium productivity.

3.4.5 Marcotting

Advantages:

- Easy and simple method
- Appropriate for family-sized cacao-planting projects
- Reliable for propagating hard-to-root priceless trees
- Technique can be used to multiply onto genetically mature shoots.

Constraints:

- High production costs
- Slow rate of multiplication
- Low rates of take
- Inappropriate for large-scale projects
- Highly trained labourers required.

3.5 Risks and mitigation

Regarding rootstock-scion interactions, since most of the reported results on this issue refer to short-term studies, mainly performed under greenhouse conditions, there is a lack of long-term studies showing possible positive or negative interactions between shoots and roots that could alter cocoa productivity. Many physiological aspects and mechanisms involved in cacao plants are not well known. It is important to investigate and determine parameters that can be used for rootstock selections, particularly including dwarfing. Dwarf rootstocks can partially control the plant shoot size and shape, as well as enhance yield and improve production factors. In Chapter 2 of this review, Lockwood mentioned that dwarfing effects have attracted most attention in cacao. Dwarfing is a valuable trait for two reasons, with apple almost invariably given as the example: a) when associated with improved yield efficiency and maintained/increased assimilate production, it can be used to increase commercial yields; and b) it can make the crop 'pedestrian friendly', with all parts of the tree and especially the fruit within easy sight and reach, greatly simplifying pest and disease management.

Although mechanisms by which rootstocks influence scion vigour and fruit quality, at least in apple, have not yet been fully understood, changes in hydraulic resistance and stem sap flow across the union between stock and scion have been associated with differential rootstock effects on scion vigour, as suggested by Simons (1986). There is considerable evidence, with perennial fruit trees, that the root systems used in composite plants have quantitative differences in anatomical xylem structure and this can be linked to the potential of the root system to dwarf the shoot, perhaps by reducing stem water flow as also emphasized by Beakbane and Thompson (1947). Root systems that reduce shoot-height have a low xylem to phloem ratio, while the opposite is true for root systems that promote shoot growth, and which have xylem tissue with more and larger vessel elements (Beakbane and Thompson 1947). The consistency of this ratio has enabled it to be successfully used, at the seedling stage, in selection protocols for apple 'rootstock' (clonally produced root system) breeding programmes (Beakbane and Thompson 1947, Rogers and Beakbane 1957, Miller et al. 1961). A comprehensive review on the subject was published by Atkinson and Else (2003) for pome species. The authors focused on whole aspects and mechanisms that alter plant growth and agronomic performance, and conclude that rootstocks increase harvest index, and dwarfing increases yield efficiency even more. Cocoa studies can benefit from apple studies on rootstocks, since scion dwarfing in apple rootstocks has been developed over the course of hundreds of years. In spite of the reports in other crops indicating the effect of rootstocks on scion performance, particularly in pome species, not enough information is currently available for cacao. In general, results of research carried out so far on cacao indicate that rootstocks can influence some agronomic traits of scions. However, more research is necessary to expand and consistently examine these effects, especially with respect to new clones recommended worldwide for new planting programmes.

Owing to the wide variation in growth, vigour and disease resistance, using seedling rootstocks derived from open-pollinated seeds from untested mother trees may not be the best approach to minimize general risks.. Seeds should be obtained from reliable sources, such as official or community seed gardens (bi-parental or not), which produce proven hybrid seed combinations or family selections, especially for the control of important diseases in the region. The planting of untested rootstocks in Bahia, Brazil, during the 1990s resulted in considerable losses in cacao when a strain of *Ceratocystis* wilt infected and killed seedlings of ICS1 x Sca6 or Sca12 hybrids. Many areas of Bahia that have been planted with rootstocks originating from clones CCN51, PH15 and PS1319, and from crosses involving ICS1, are at similar risk.

In contrast, selections of Forastero Comum have proved resistant to *Ceratocystis* wilt. Another example of the utilization of proven selected rootstocks can be seen in the planting of CCN51 in Ecuador, where it ensures resistance to various strains of *Ceratocystis* wilt and *Rosellinia/Lasiodiplodia* complex. In addition, growers in Colombia use PA46, PA121, PA150, IMC67, and hybrid seeds of PA46 and EET62 x IMC67, to control root rot caused by *Phythopthora* spp. and *Rosellinia*.

Although production outcomes are markedly influenced by plant, environmental, and management factors, vegetative propagation technology of any kind will not increase the productivity of low-yielding clones, owing to the true-to-type nature of the reproduced plant, which is genetically identical to the mother plant. Therefore, undesirable genotypic and agronomic traits (low productivity, disease susceptibility, high vigour, unbalanced crown, low seed quality) will also be spread through new generations of clonally reproduced plants.

There is a tendency, among smallholder farmers in particular, to propagate a single or a few clones, generally the most productive, resulting in limited genetic diversity and consequently high risks of infection by newly introduced diseases.

The propagation of material which has a latent infection, such as with CSSV in West African cocoa-producing countries, also poses a great risk of spreading the disease into cloned plants reproduced through any of the vegetative propagation methods (budding, grafting, rooted cutting, marcotting). Other diseases, such as *Ceratocystis* wilt and, in some cases in Latin America and Caribbean countries, witches' broom, can also be proliferated in similar ways. Very often, the lack of taproots in rooted cuttings can arrest plant growth and yield during dry seasons, especially in shallow soils, resulting in crop losses and/or tree mortality.

Risks can be minimized using a number of procedures, such as:

- Strict sanitation procedures for the management of clonal gardens, to monitor, eliminate and/or control potentially diseased grafting material, must be included as a routine protocol for any project of clonal propagation.
- Strict use of clones recommended for each specific region in clonal propagation projects.
- Maintenance of high genetic diversity for the most important agronomic traits (production, disease resistance, precociousness, crown, and seed quality) in clonal propagation projects. Diversity can be provided either by planting a mixture of recommended clones on the farm or in a monoclonal fashion with different self-compatible clones in alternate rows.
- Development of projects with rooted cuttings well planned to avoid crop losses due to inappropriate environmental conditions, especially soil type, topography, and rainfall.

3.6 Future perspectives and research

The development of hybrid cacao varieties in the late 1950s led to the wide use of sexual propagation to supply materials for commercial-scale planting. This perhaps explains the choice of seedlings in cacao planting programmes implemented in Malaysia (Lee 2000) and in Brazil (Sena Gomes et al. 2000) until the late 1980s, and, to some extent, the choice made today in most cocoa-producing countries of Central and South America, the Caribbean, West Africa, and Asia (see Table A5.1, in Annex 5). This choice conflicts with the idea that planting high-yielding clones is an important way to increase cocoa productivity (Wood and Lass 1985). The lack of proven clones may have restricted uptake of vegetative propagation for the supply of materials to farmers in some regions. However, the tree-to-tree variation in these hybrid progenies, and genetic variation from other sources, provides opportunities for the selection of individuals with particularly high potential when grown in these regions, which could be captured through vegetative propagation and thus form the basis for future cacao planting and/or rehabilitation programmes.

By comparison, the situation was quite different in Trinidad and Tobago where a cacao breeding programme, which was established in the early 1950s, has continued uninterrupted to the present day. Trinidad selected hybrids (TSHs) from this highly successful programme were traditionally multiplied as rooted cuttings for distribution to farmers. The selections were propagated clonally to ensure high yield potential and tolerance to witches' broom disease, and to maintain good flavour characteristics. The establishment of rooted cuttings was initially difficult, owing to their shallow rooting system, coupled with the significant length of the dry season in Trinidad and Tobago. Because of these constraints, many farmers prefer to plant seedlings, which are more cost effective. Furthermore, the extent of variation in seedlings from present day TSH-crosses is small as a result of this long-established breeding programme (David Butler, personal communication, 2012). Therefore, it appears that clonal plantings are the major trend for some cocoa-producing countries, although hybrid plantings will continue to be utilized.

The rehabilitation of cacao yields in the project Renascer (Ilhéus, Bahia, Brazil) is a clear example that the traditional method of planting cacao in the Cabruca system can be self-sustainable, with the utilization of appropriate technologies and crop management. It is important to note that: a) approximately 70% of Bahian cacao is harvested from this system of planting; and b) the shade requirements of cacao are provided by a mix of forest trees, which is exceptionally important for the conservation of the high biological diversity of the region. It is also important to mention the impressive preliminary results shown by the project Lembrance in Eunapolis, Bahia (see Figure 11 in Annex 4), in which rooted cutting clones were planted as a rotation crop with old papaya, in appropriate soil, with fertigation, and full sunlight. The project Lembrance would be an ideal pilot project that could be replicated in areas in southern Bahia with similar environmental conditions, as well as in many other regions in other cocoa-producing countries in Latin America, Africa and Asia.

The production of orthotropic clonal cacao plants through variations in the conventional propagation procedures of budding, grafting or rooting clonal orthotropic scions from basal chupons is now being tested at CEPLAC, almost 80 years after the first attempts were made to establish this technology by Cheesman in Trinidad. Research on this subject was also recently reported by Miller (2009). Nevertheless, conventional propagation technologies using orthotropic material need to be tested with large numbers of recommended clones that are available in different countries, although results in Trinidad and Malaysia have already shown similar yield potential for clones that were grafted onto seedling rootstocks using plagiotropic or orthotropic budwood.

Research topics that deserve more attention regarding conventional vegetative propagation are the following:

- Production of improved varieties, since successful clonal plantings are entirely dependent upon the availability of reliable varieties.
- Studies aimed at reducing the costs of production of budded or grafted seedlings through shortening the time of maintenance of seedlings in the nursery.
- Production of cuttings from stocks plants grown in containers under greenhouse conditions, a technology successfully utilized for the production of eucalyptus cuttings.
- Testing of yields and costs of rooted cuttings of cacao clones produced by non-mist, low-technology techniques, utilizing the re-designed closed bin system, applied to family-sized projects.
- Investigation of the effects of rootstocks (grafted or budded) of new recommended clones on pod yield.
- Improvement of the storage duration of seeds for the production of seedling-derived rootstocks.

- Long-term studies investigating the rootstock-scion interactions that impact on cocoa productivity; for example, changes in hydraulic resistance and stem sap flow across the union between stock and scion, the potential of the root system to dwarf the shoot, and the influence of the rootstock on some agronomic traits of the scion. Many physiological aspects and mechanisms of cacao plants are not well known.
- Investigation and identification of parameters that can be used for rootstock selections, including dwarfing in particular.
- More research to examine the effects of rootstocks on agronomic traits of scions, especially involving new clones currently recommended for new planting programmes worldwide.

3.7 Acknowledgements

The authors are indebted to Drs Uilson Lopes, José L. Pires and Wilson R. Monteiro for providing valuable insights into CEPLAC's cacao breeding programme. The appreciation is extended to Rob Lockwood and Mark Guiltinan for all the important suggestions they made over the course of this study. The help of the following people in assisting and providing important information on cacao propagation technologies is sincerely appreciated: Basilio Leite (CEPLAC), Cesar (Biofabrica), Claudio S. Silva (Grupo Chaves, Lagoa Pequena Farm, Ilhéus, Bahia), David Butler (Cocoa Consulting), Ivan Garzón (INIAP), Jackson de O., Sebastian A. F., Lopez (Cocoa Consulting) and Wanilson Serra (Bom Retiro Farm, Camacan, Bahia).

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Chapter 4 – Tissue culture

Siela Maximova[4] and Mark J. Guiltinan[5]

4.1 Description of the method

Zygotic embryos (ZE) result from the fusion of female and male gametes formed by meiosis during the sexual phase of plant development within flowers. Gametic fusion (fertilization) is followed by cell division and the differentiation of the dividing cells into tissues and organs, comprising zygotic embryos. These embryos develop within the protective and nourishing environment of the ovule, and at maturity are found within the confines of a fully developed seed (the cacao bean), with 40 or so of such seeds developing within each cacao fruit (pod).

Somatic embryogenesis (SE) is a process that does not involve meiosis or fertilization. The newly propagated plants or embryos are derived from a single parental somatic cell (any cell in the plant other than a gamete or germ cell), or from a group of somatic cells. This process occurs in nature in certain plants that have evolved the ability to propagate asexually. One example is the propagation mechanism of Kalanchoe (commonly known as 'Mother of Thousands'). In these plants, new individuals develop vegetatively as plantlets along the leaves of the mother plant, and the young plants eventually drop off and develop their own roots (Garces et al. 2007).

Plant tissue culture offers a suite of new approaches to speed up the development and deployment of genetically-improved genotypes because of its potentially very high multiplication rate and scalability. The main advantages of cacao tissue-culture methods include the rapid generation of large numbers of genetically uniform plants, for the production of orthotropic plants with normal dimorphic architecture (jorquette and taproot formation); for the production and testing of disease-free materials; and as a tool for germplasm conservation via cryopreservation. To date, these potentials are only beginning to be applied in cocoa-producing countries.

4.1.1 The cacao somatic embryogenesis system

Cacao somatic embryogenesis has been the topic of research of many scientists and laboratories around the world for the last 30 years, and perhaps is one of the most well documented methods for the vegetative propagation of cacao. Efficient methods for multiplying cacao by somatic embryogenesis have been developed as a result of research conducted at DNA Plant Technology Corporation, Cinnaminson, New Jersey, USA (Sondahl et al. 1993); Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), in Montpellier, France (Alemanno et al. 1996a, Alemanno et al. 1996b, Alemanno et al. 1997); Nestlé Research and Development Centre in Tours, France (Masseret et al. 2005, Masseret et al. 2008, Masseret 2009, Florin 1997, Florin et al. 2000, Florin et al. 2001); and at Penn State University (Guiltinan et al. 1997, Guiltinan et al. 2000, Lambert et al. 2000a, Lambert et al. 2000b, Li et al. 1998, Maximova and Guiltinan, 2000, Maximova et al. 2002, Maximova et al. 2005, Maximova et al. 2008, Traoré et al. 2003). Protocols for somatic embryogenesis have been published by Maximova and colleagues (Maximova et al. 2005).

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The somatic embryogenesis process begins with immature flowers, which are collected from trees, surface sterilized, dissected to excise the petals and staminodes, and introduced into tissue culture. The cultures are grown in sterile laboratory conditions using either solid or liquid nutrient media, which contain various mixtures of plant hormones. Hormonal treatments trigger a reprogramming of the somatic cells to de-differentiate, then initiate the embryogenesis developmental programme. During somatic embryogenesis, a single somatic cell or group of somatic cells begins to divide and form pro-embryonic structures, then embryos. Plants derived from somatic embryogenesis differ from those that have developed from zygotic embryos, mainly in the lack of development of the cotyledons and the absence of maternal seed and fruit tissues.

The process of somatic embryogenesis involves four main steps (as illustrated in Figure 4.1):

- *Primary somatic embryogenesis* (PSE), where flower explants are cultured to produce embryogenic calli, which further develop into primary somatic embryos.
- *Secondary somatic embryogenesis* (SSE), in which cotyledons from primary embryos are re-cultured to produce secondary embryos.
- *Conversion*, where mature embryos are converted into plantlets.
- *Acclimatization*, where plantlets are acclimated to greenhouse or field conditions (conversion and acclimation).

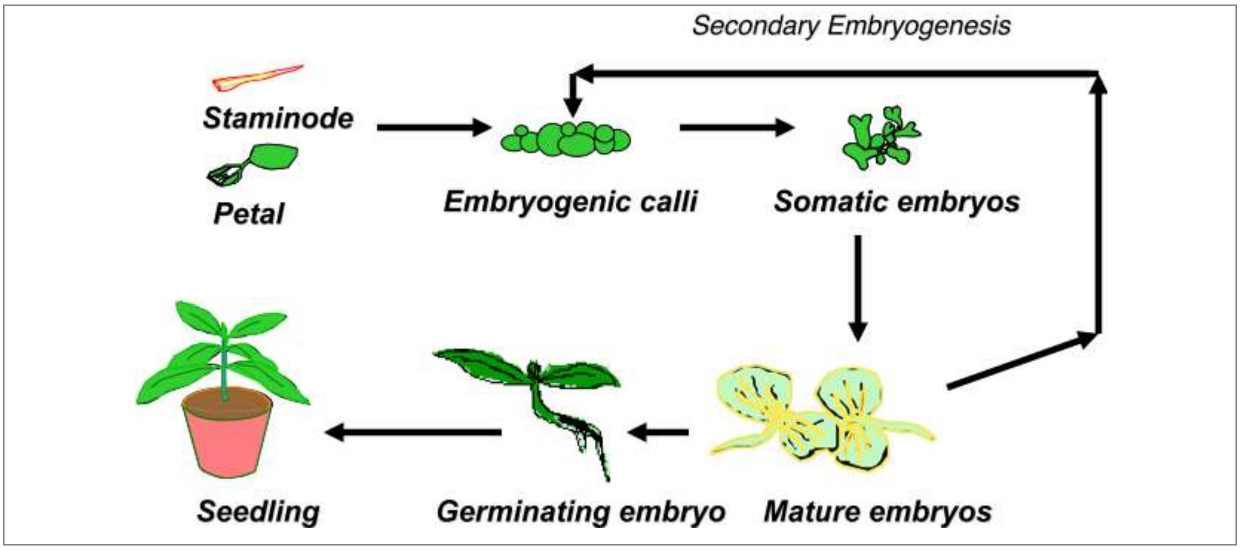

Figure 4.1 Graphic representation of the cacao somatic embryogenesis process. Source: Maximova and Guiltinan 2014

4.1.2 Use of somatic embryo plants for propagation by orthotropic rooted cuttings

In addition to using somatic embryogenesis to produce a large number of clones quickly for distribution to farmers, this technology can also be used for the rapid establishment of seed and clonal gardens. It has been shown that somatic embryo-derived trees (SE trees) managed as a bentwood clonal garden can produce orthotropic shoots from which orthotropic trees can be derived (Maximova et al. 2005, Miller, 2009, Miller and Guiltinan 2003). Thus, orthotropic rooted cuttings with single or multiple nodes can be produced using SE plants as a source of stem cuttings (Guiltinan et al. 2000, Maximova et al. 2005, Miller 2009). Plantlets produced with this method grow in a similar way to seedlings, with an orthotropic growth phase followed by jorquetting, and then the transition to an adult growth phase characterized by the development of plagiotropic branches followed by flower and fruit development.

To test the field performance of rooted orthotropic cuttings collected from SE plants in bentwood gardens, a large-scale clonal garden was established on Nestlé's experimental farm (El Chollo) near Quevado, Ecuador (see Figure 4.2). Ivan Garzón and colleagues at the Pichilingue experimental station of the Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP) have implemented this technology, and are now conducting field tests for the production of rooted cuttings under high-density planting. Results of the first year of the INIAP trial indicated that orthotropic trees can be produced at a competitive cost, and that their agronomic performance is comparable to that of trees produced by somatic embryogenesis or traditional methods. An account of the use of these methods in projects for the mass propagation of cacao was recently reviewed by Maximova and Guiltinan (2012).

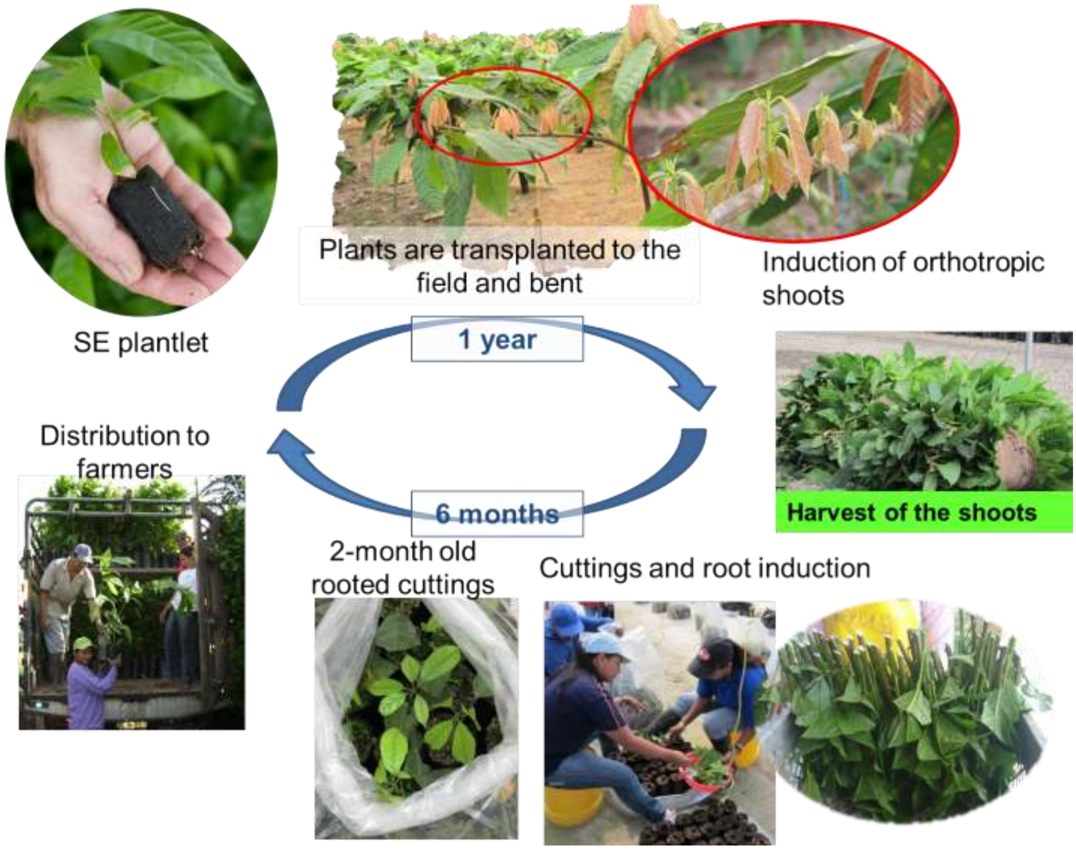

Figure 4.2. Production of orthotropic rooted cuttings from SE trees using a bent-wood clonal garden.
Source: Pierre Broun,Tours R&D, Nestlé

These results are similar to those reported in a publication by Miller (2009), describing the use of bentwood stock plants as a source for orthotropic branches, from which single-node cuttings can be harvested for producing plants with the characteristic jorquette branching pattern. Miller (2009) further noted that occasionally the plants propagated by this method produced jorquettes at heights lower than normal. This limitation can be easily overcome with a single heading cut applied to the first jorquette, which will induce a subtending orthotropic shoot to grow to a normal jorquette height. Complete field evaluation (including yield) of plants produced by this method will require several more years of monitoring. This technique can be used with somatic embryogenesis in the large-scale production of orthotropic cacao trees.

4.1.3 Integrated system for vegetative propagation

A concept for the integration of multiple propagation methods to attain efficient large-scale mass propagation of orthotropic cacao plants was published in 2005 (Maximova et al.). An extension of this idea is presented in Figure 4.3. The protocol combines low- and high-tech approaches, including somatic embryo-derived and orthotropic rooted cuttings in non-sterile greenhouse or field conditions using SE plants as stock plants (Fang and Wetten 2011, Tan and Furtek 2003). For this method, SE plants are acclimated, established as stock plants in field conditions, and further amplified using single-node stem segments to produce rooted cuttings. Plantlets produced with this method grow in a similar way to seedlings, with an orthotropic growth phase followed by jorquetting and the development of plagiotropic branches. This step can be performed at a low cost, greatly reducing the overall expense of a rapid, integrated, orthotropic, clonal multiplication system starting from somatic embryos. Plants produced through these methods could be used for planting in the fields, or as self-rooted stock for budwood and/or cuttings, as required (Fang and Wetten 2011).

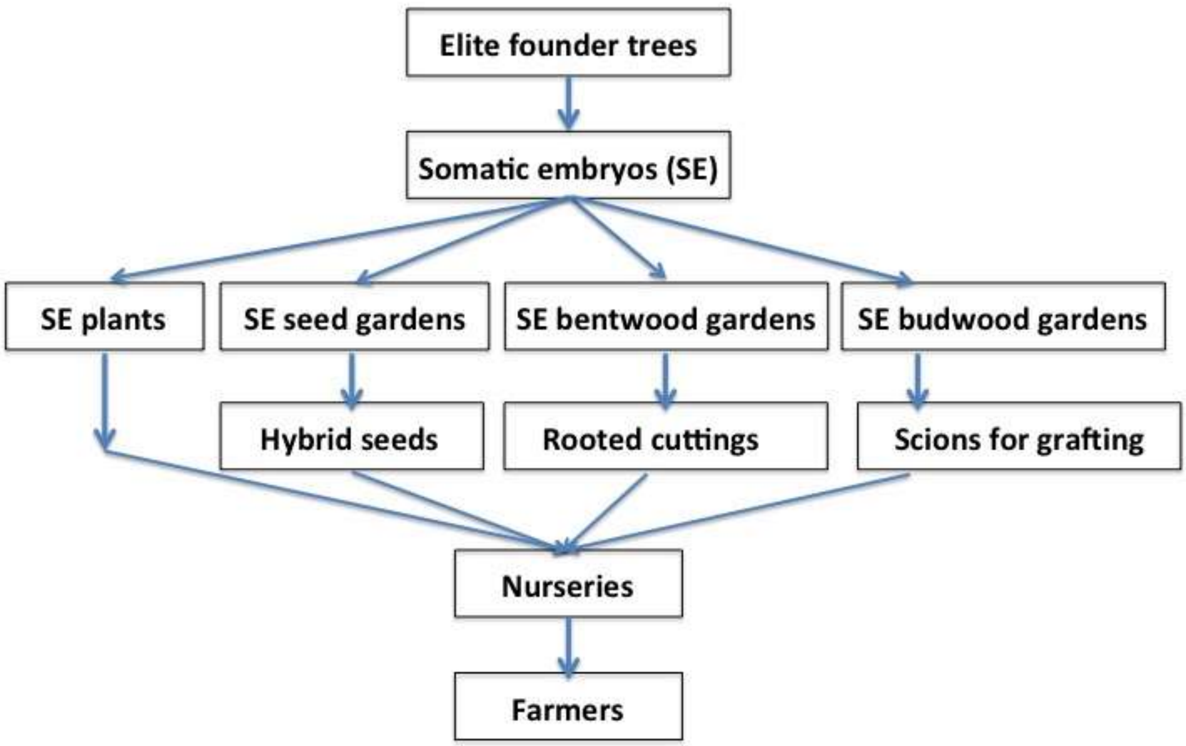

Figure 4.3 Integrated system for the vegetative propagation of orthotropic SE cacao plants. Source: Maximova and Guiltina 2014

Depending on the responsiveness of the individual genotypes to the SE protocols, and the availability of flowers to be used as tissue culture explants, the production of SE plants via primary and secondary embryogenesis requires 12 to 18 months. When the plants are established in the nursery and converted to stock plants for cuttings, the process for producing rooted plants ready for planting in the field requires an additional eight to ten months.

4.1.4 Description of facilities required for somatic embryogenesis

A small, modestly equipped tissue culture laboratory (approximately 100 m^2) is required to propagate between half a million and one million plants per year. The National Programme for Increasing Cocoa Productivity and Quality in Indonesia (Gernas Kakao), was launched by the Indonesian Coffee and Cocoa Research Institute (ICCRI) in 2008. The programme utilized 4,500 m^2 of laboratory space to produce 74.5 million plants between 2009 and 2011, with productivity reaching up to 50 million plants per year. The basic equipment required in SE facilities includes: sterile tissue culture hoods, water purifiers, coolers and freezers, pH meters, weight balances, dissecting microscopes, water baths, autoclaves, and other small-sized laboratory equipment and supplies. An office area with a computer is also necessary. The laboratory should be equipped with climate control, and in most cases in cocoa-producing areas a back-up power supply is necessary.

An acclimation and propagation facility consisting of a shaded nursery is sufficient in tropical areas for acclimating plants from the laboratory, and for maintaining stock plants from which cuttings can be collected for rooting. Depending on the propagation goals of the project, such a facility could be quite large. It should be located in close proximity to the laboratory, and should be staffed by one or two knowledgeable workers, with additional workers being required at various times in the propagation cycle. It should also have the necessary equipment for mixing and moving soil.

Scientists and workers with experience in plant biology and biotechnology may be required to manage plant production in SE facilities. The number of propagation technicians required depends on the number of sterile tissue-culture hoods in the facility, and on the number of established working hours. Field technicians and workers are required to operate production in the nursery. Based on the experiences of ICCRI, a team of approximately 80 people is required to complete all steps in the production of 1,000,000 plants per year. At the peak of production of 50 million plants per year, ICCRI employed about 260 people in the laboratory, and more than 2,000 in the nearby acclimation facility.

4.2 Current status of application of tissue culture

Over the past decade, scientific groups in cocoa-producing countries have begun to integrate the cacao somatic embryogenesis technology into their research and commercial programmes. Training has been provided in to many countries through intensive workshops and visiting scientist and graduate student programmes. Today, more than 150 cacao genotypes worldwide have been introduced into sterile culture for the production of somatic embryos, and since 1999 more than 20 individual genotypes propagated in tissue culture have been planted in long-term field trials in Saint Lucia, Puerto Rico, Ecuador, Brazil and Indonesia. During the past 15 years, the growth of SE plants in the field has been compared to the growth of plants propagated by seedlings, rooted cuttings and grafted plants, by measuring parameters including stem diameter, stem height, longest jorquette branch, number of jorquette branches, occurrence of multiple stems, flowering and fruiting, and others. The results of the studies conducted at all locations demonstrated that there were no major differences in growth parameters among the different genotypes and the propagation methods evaluated. Based on these results it was concluded that SE plants demonstrate normal phenotypes in field conditions and have growth parameters similar to plants propagated by traditional methods. Details of the field evaluation and the application are described below.

4.2.1 Field evaluation of somatic embryogenesis plants

Field evaluation in Puerto Rico - Penn State and USDA

In a collaborative project between the USDA in Puerto Rico, and Penn State, genotypes from USDA cacao field trials were propagated via somatic embryogenesis at the Penn State laboratory between 2001 and 2002. At Penn State, some of the first primary SE plants were used as stock plants to produce orthotropic rooted cuttings. Twenty different genotypes were propagated and approximately 350 plants (orthotropic rooted cuttings) were transported as bare-rooted plantlets back to Puerto Rico. The field site is located directly adjacent to the two sites from which the evaluated accessions were selected by Irizarry, Rivera and Goenaga (Irizarry and Rivera 1999, Irizarry and Goenaga 2000). In Puerto Rico, plants of the same genotypes were generated by plagiotropic rooted cuttings and by grafting plagiotropic and orthotropic cuttings onto EET400 rootstock. The test plots contain approximately ten genotypes propagated by four propagation methods, planted in a split-plot design. The test includes approximately 900 trees. Physiological and yield data were continuously collected from 2001 to 2012, and have indicated that all the plants are growing normally and, independent from the propagation method, are producing on average high yields of 2,007 kg of dry beans/ha per year, with the top yielder averaging 2,538 kg of dry beans/ha per year (Ricardo Goenaga, personal communication, 2013). The results from this study have suggested that somatic embryogenesis followed by orthotropic rooted cuttings propagation is a viable and reliable propagation method for cacao.

Field work at Union Vale Estate, Saint Lucia – Penn State

To compare the growth of the SE plants to plants propagated by traditional methods, the Penn State group conducted a five-year field test at Union Vale Estate, Saint Lucia (2001 to 2006) (Maximova et al. 2008). The results of the study demonstrated that during the first few years of growth there were no significant differences in growth parameters among the different genotypes and propagation methods evaluated, with the exception of the orthotropic rooted cuttings. Trees propagated via orthotropic rooted cuttings appeared to be smaller in size, with smaller average stem diameters, shorter stem heights to the jorquette and shorter jorquette branches. It was concluded that SE plants demonstrated normal phenotypes in field conditions and have growth parameters similar to plants propagated by traditional methods.

Field evaluation in Ecuador (I) - Nestlé

Field evaluation was also carried out in Ecuador, in 1998, on the first 110 SE plants generated in the laboratory at Nestlé's Research and Development Centre in Tours, France. The trees demonstrated a normal development and bean production similar to the control trees in the test (Fontanel 2002). An extensive evaluation of SE plants was initiated in 2000 in two locations in Ecuador, with the objective of evaluating the agronomic performance and conformity of SE plants in comparison with other methods of cacao tree propagation. The test included SE plants; plants from orthotropic and plagiotropic grafts of EET95 on CCN51; rooted cuttings; and also seedlings of genotype EET95 grown in Ecuador (on Nestlé's El Chollo farm). In Ecuador, various data have been collected since 2001, including measurements per tree of: trunk diameter; jorquette height (except for plagiotropic grafts and cuttings that don't develop jorquettes); the length of time required to produce the first pod (in months); the total number of pods produced; and the average weight of 100 dry beans (Masseret et al. 2005). The study reported that no differences could be detected in the average trunk diameters and jorquette formation between SE trees and trees propagated by seeds (Figure 4.4).

It was observed that cacao trees from orthotropic cuttings developed the jorquette at a lower height, similar to the report from the Penn State test in Saint Lucia (Masseret et al. 2005; Maximova et al. 2008). Interestingly, SE plants on average produced the first pods four months earlier than those produced through the other types of propagation. In addition to earlier fruit production, the SE plants displayed good physiological vigour compared with the other trees, which is of particular importance during the early establishment in the field. In the third year after planting, SE trees produced significantly more pods compared with all other propagation methods, and have reached a consistent production rate.

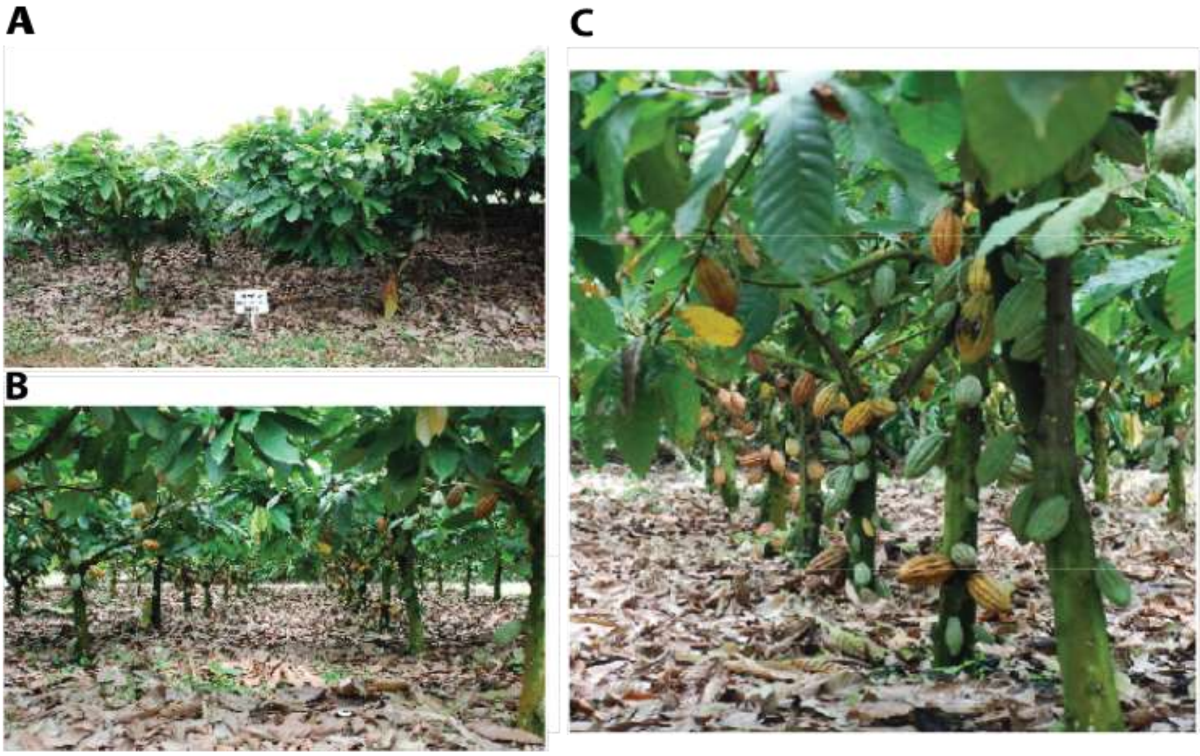

Figure 4.4 Plants propagated by SE at the Nestlé Ecuador five years after planting. Source: Buchwalder et al. 2012

In 2008, yields of 1.7 tonnes per hectare (t/ha) were recorded for grafted orthotropic trees and rooted cuttings; 1.2 t/ha for plants propagated by seeds; and 2.5 t/ha for SE plants. Later trials, some of which were conducted at commercial scale, confirmed the initial findings in terms of early fruiting and morphological characteristics. However, while the productivity of SE trees was also better in the first few years after planting, no significant differences were observed during the later years in comparison with trees propagated using other methods (Figure 4.5), (Masseret et al. 2008).

The main conclusions of these studies were:

1. SE trees had the same architecture as seed propagated trees.
2. Compared with the other vegetative propagation methods, like grafting or rooted cuttings, the SE trees needed approximately two times less pruning during the first three years.
3. The SE trees were more vigorous (larger trunk diameters) and more homogeneous; they developed pods earlier and their first crops produced higher yields.

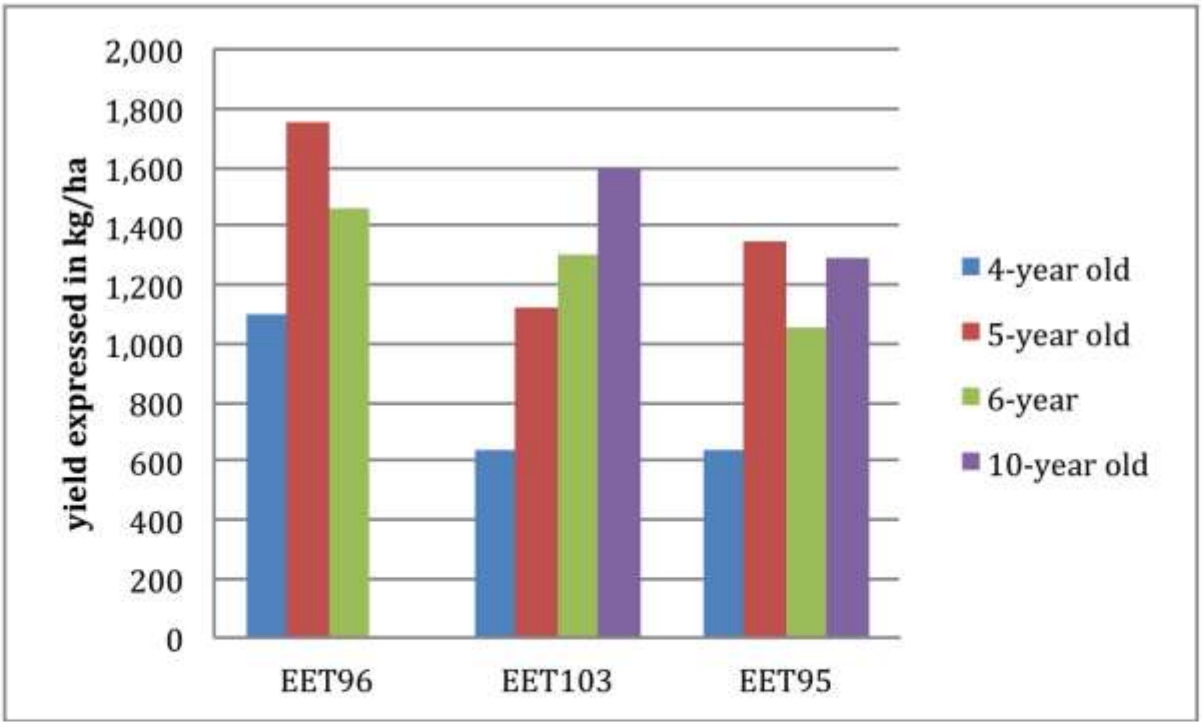

Figure 4.5 Productivity of SE trees planted at the Nestlé farm in Ecuador. Data from years 4-6 is from large-scale trials (at least 1,000 trees); data from year 10 is from the 2000 small-scale trial. Source: Buchwalder et al. 2012

Field tests were conducted at the Nestlé farm in Ecuador between 2000 and 2009 to compare the field performance of SE plants from genotypes EET95, EET96, and EET103 generated in two different locations - the Nestlé research laboratory in France and the research laboratory at Penn State in the USA - using published protocols (Fontanel 2002 and Li et al. 1998). The SE plants were transported to Ecuador in 1999 and data were collected from all trees for a minimum of five years. Similar to the previous reports, all trees displayed normal morphology and growth with respect to the other propagations methods. Primary SE plants from all genotypes generated at Penn State produced pods after two years of planting, which is six months earlier than the rest of the trees in the test. Trees derived from secondary somatic embryos were more productive than those derived from primary embryos. This difference could be expected, considering previous reports that secondary embryogenesis results in higher quality (faster growing, more robust) and more uniform plants (Maximova et al. 2002).

In 2006, Nestlé began planting a collection of cacao genotypes in Ecuador, consisting of SE trees. To date, 70 different accessions have been planted and an evaluation of the agronomic performance of the accessions has been initiated. A replication of this collection is being established in Côte d'Ivoire. This work is ongoing, with the integration of new accessions. It offers a unique opportunity to evaluate the performance of clones presenting all the characteristics of the original selected tree. So far, all the quantitative parameters recorded show a clear homogeneity between trees and all indications are that the trees are developing normally. In order to ensure the long-term survival of the collection, somatic embryogenesis multiplication of each clone is coupled with the cryopreservation of the embryogenic callus. The observations from this collection suggest that somatic embryogenesis preserves 'true-to-type', regardless of genotype.

Field evaluation in Ecuador (II) - CIRAD-INIAP

A collaborative project between CIRAD in France, and the Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP) in Ecuador, initiated in 2002, focused on the adoption and evaluation of the somatic embryogenesis technology. The project involved INIAP's national cocoa programme (Programa Nacional de Cacao del INIAP) and it's tissue culture and biotechnology laboratory in Pichilingue. The project goals were to introduce the SE technology into the country and optimize it for important local Nacional varieties. The project objectives included an evaluation of the embryogenic capacity of multiple cacao genotypes, and the establishment of a field trial to compare the performance of plants propagated by the different methods, including somatic embryogenesis, rooted cuttings, grafting, and seeds (Garzón et al. 2011b). Using protocols developed by Penn State, the embryogenic capacity of 44 different Nacional cacao genotypes was evaluated. The first 30 months of the project included building the infrastructure for tissue culture in Pichilingue, training the personnel, and conducting experiments aimed at optimizing the protocols for local conditions. Large numbers of embryos were successfully converted to plants. Based on the results obtained from the tissue culture experiments, twelve promising genotypes were selected for field testing, and for comparing SE plants to conventional methods of propagation (i.e. grafting, rooted cuttings and seeds). In addition to monitoring the SE field trials, INIAP is currently working on optimizing SE protocols for Nacional clones, and on adapting protocols for the low-tech propagation of orthotropic rooted cuttings. During the first four years of the field trial (2005-2009), data on agronomic, morphological and physiological performances were recorded. Yield data collection is still in progress. The analysis of data recorded during the first three years demonstrated that the SE plants have a high degree of morphological conformity compared with plants propagated by seeds. It was observed that the SE plants had better growth rates and faster jorquette formation, when compared with the other types of plants (Figure 4.6). In general, the physiological parameters and pathological status of the SE plants were the same as those of plants propagated by other methods. The SE plants produced pods earlier than the rest of the plants (20 months after planting in the field), and in all cases SE plants had higher production than the plants obtained by other forms of propagation. The cumulative yield per hectare of plants during the first three years of production reached 3.0 t/ha (fresh cacao), and was 2.4 t/ha (fresh cacao) for the fourth year alone. SE plants yielded 43% more than rooted cuttings, 44- 56% more than grafted plants, and 49% more than seedlings (Garzón et al. 2011). At the INIAP station, it was observed that some SE plants produce more chupons at the base of the tree compared with plants produced from seeds, grafts and rooted cuttings. This could potentially increase the labour requirements for SE plants to periodically remove chupons. Potentially, the new chupons could be used as orthotropic

Field evaluation in Ecuador (III) – INIAP

In 2006, INIAP initiated a project to adopt and evaluate the orthotropic rooted cuttings method for Nacional varieties. SE plants from six Nacional genotypes were established in an experimental plot as bentwood stock plants for rooted cuttings, as described by the Penn State group (see Maximova et al. 2005, Miller 2009). The bentwood stock plants produced numerous axillary shoots and, although there were differences between genotypes, on average a single stock plant could produce between four and eleven shoots within a period of three months. The average length of the shoots was 42-65cm and each produced five to eight quality cuttings (Garzón et al. 2011b). Thus an annual multiplication rate of over 350 rooted cuttings per stock plant was achieved.

Figure 4.6. Somatic embryo plants in INIAP, 2012.
Source: Maximova and Guiltinan 2014

In 2007, an ambitious project was initiated by INIAP, together with the Secretaría Nacional de Planificación y Desarrollo (SENPLADES), and the Secretaría Nacional de Ciencia y Tecnología (SENACYT), aimed at developing a commercial system for the production of planting material from cacao plants of the Nacional cacao. The main goal was to scale up the multiplication process for the production of SE plantlets and orthotropic rooted cuttings obtained from SE stock plants. Somatic embryogenesis cultures were initiated with explants from seven of the ten cacao genotypes of Nacional cacao currently recommended by INIAP for the main cocoa-producing areas of Ecuador, and two promising genotypes recommended for the Amazon region.

Cultures have been continuously initiated since 2008, with more than 20,000 flowers dissected. The resulting SE plants have been planted in the first bentwood clonal gardens established at INIAP's tropical experimental station in Pichilingue, and at central experimental stations in Santo Domingo and Amazonia. Data are being collected on the number of shoots produced per SE stock plant, shoot lengths and the total number of cuttings generated per shoot. Experiments were performed to optimize the light levels, type of shade, and fertilization regimes. The highest yields (31 shoots/plant; average shoot length of 69 cm) were obtained at a planting density of 2,963 plants/ha, under shade (60% plastic mesh or 1,111 plantain plants/ha), with 120 mm irrigation/month during the dry season and 1,000kg of NPK micronutrients/ha per year. Shoots are harvested throughout the year at 132-day cycles. The average number of cuttings per shoot recorded so far was 5.8, yielding approximately 180 cuttings per plant per year, and a potential total yield of approximately 534,060 cuttings/ha per year, at a density of 2,967 stock plants/ha; or potentially 1,000,000 cuttings/ha per year, at a density of 6,667 stock plants/ha. Additional experiments were conducted to optimize the protocols for rooting the orthotropic cuttings and for plant establishment, and to test different environmental conditions, substrates and hormone types and concentrations. The best results obtained were 80% success in producing rooted plants in a period of 120 days from rooting initiation.

Plants propagated by this technology are currently under evaluation in four one-hectare demonstration plots in different regions of the country. The main objective of these tests is to confirm the field performance of plants in different environmental conditions. Based on preliminary analysis of data collected 18 months after planting, it was concluded that the orthotropic plants demonstrated normal growth, but in general the plants were smaller and developed shorter jorquettes in comparison with SE plants, which is consistent with previous reports (Maximova et al. 2008, Miller 2009).

4.2.2 Transfer of somatic embryogenesis technology to West Africa

As part of a long-term goal, and with initial funding from the Sustainable Tree Crops Programme, Penn State and Mars, the team from Penn State has been providing training and education in somatic embryogenesis technology in Africa since 2000. Technology transfer and training have been carried out at CRIG in Ghana, National Centre for Agricultural Research (CNRA) in Côte d'Ivoire; CRIN in Nigeria; and at the Institute of Agricultural Research for Development of Cameroon (IRAD).

The genotypes introduced into tissue culture in Ghana and Côte d'Ivoire between 2000 and 2001 were cacao clones selected in the national breeding programmes. In Ghana, these included clones resistant to CSSV, which were previously selected through breeding. Cacao floral explants from 17 clones in Ghana and from 31 clones in Côte d'Ivoire were cultured; and somatic embryos from 14 clones in Ghana and 24 clones in Côte d'Ivoire were produced. In Ghana, four out of seven CSSV-tolerant clones produced somatic embryos. In Côte d'Ivoire, over 8,000 somatic embryos were produced and maintained in tissue culture, and more than 200 SE plantlets were successfully acclimated. The CNRA group has continued their efforts to optimize the system for local varieties and has described the results of their work in two publications (Issali et al. 2008, Issali et al. 2010).

In 2009, CNRA in collaboration with Nestlé reactivated somatic embryogenesis activities at the new research and development centre in Abidjan, Côte d'Ivoire. The goal of this project is to produce about one million new, disease-resistant cacao trees per year to help with the rehabilitation of the aging cocoa farms in Côte d'Ivoire (Masseret 2009).

Research and adaptation of the cacao somatic embryogenesis methods has been conducted in Cameroon since 1997, mainly involving a group of scientists from the University of Yaoundé. This group has performed research in collaboration with the University of Bamenda in Cameroon, CNRA and the Training and Research Unit (UFR) Biosciences in Côte d'Ivoire; and with laboratories at the University of Hamburg, Germany; CIRAD, France; the University of Perpignan, France; as well as Penn State, USA. The results of these efforts were reported in a number of publications (Ndoumou et al. 1997, Verdeil et al. 2007, Niemenak et al. 2012, Niemenak et al. 2008, Alemanno et al. 2008, Minyaka et al. 2008).

4.2.3 Transfer of somatic embryogenesis technology to Asia

Centre for Cocoa Biotechnology Research, Malaysian Cocoa Board, Sabah, Malaysia

In a study to explore the possibility of using somatic embryogenesis technology for producing planting materials in Malaysia, several commercial cacao clones were evaluated to assess their potential for somatic embryogenesis. Similar to published results, the best regeneration success was achieved using flower staminode tissues (Tan and Furtek 2003). The success of embryo production was influenced by the genotype of cacao and also the time of year in which cultures were initiated (Tan Chia Lock, unpublished data). The protocols used for this research are detailed in Maximova et al. (2008).

As part of this study, molecular fingerprinting and morphological characterization were performed to test for mutations and somaclonal variation resulting from somatic embryogenesis. Two- to three year-old SE plants and their mother plants were fingerprinted using 39 different microsatellite markers (Tan Chia Lock, unpublished data), and no differences were observed between the SE plants and the flower source plants. Cytological analysis of the root tips of the SE plants and the source plants revealed an equal chromosome count of 2n = 20 (Tan Chia Lock, unpublished data). Preliminary morphological examination of leaves and flowers also showed no differences from the mother plants.

Indonesian Coffee and Cocoa Research Institute (ICCRI)

To rehabilitate and intensify the cacao-growing sector, in 2009 the Indonesian government initiated a national programme aimed at improving Indonesian cocoa production and quality (Gernas Kakao). The programme seeks to rehabilitate a total area of 450,000 hectares by side grafting (245,000 ha); intensifying or applying better horticultural practices for existing trees (135,000 ha); and replanting with new planting materials (70,000 ha). All of these activities were implemented in nine provinces in 2009, 15 provinces in 2010, and 25 provinces in 2011.Under the direction of scientists from Nestlé R&D Tours, France, the technology was transferred to ICCRI over the course of 2006 and 2007. About 260 people were working at the laboratory up until 2012, with more than 2,000 working in the nearby acclimation facility.

The first phase of the programme (2009 to 2011) included the provision of training and assistance for farmers in pest and disease control, and cocoa quality improvement, in accordance with the Indonesian National Standard (SNI) (Manurung 2010). As part of Gernas Kakao, ICCRI in Jember established a tissue culture laboratory to scale up somatic embryogenesis to produce up to 50 million plants per year (http://kapurpertanian.com).

The somatic embryogenesis process was initiated for five clones selected for high yield and resistance or tolerance to major diseases of cacao, in particular vascular streak dieback disease (VSD): ICCRI03, ICCRI04, Sca6, Sulawesi1 and Sulawesi2 (Agung Susilo, personal communication, 2012; see also http://www.iccri.net). ICCRI generated and delivered more than 74.5 million SE clones during the period 2009-2011. ICCRI reported that SE cacao clones were found to be normal (true-to-type) and similar to plants propagated by other methods of clonal propagation. ICCRI is currently producing acclimated plantlets (transferred from sterile tissue culture vessels and adapted to nursery conditions) for shipping bare rooted to the cacao-growing regions for distribution by selected nurseries contracted by the Indonesian government. To maintain quality standards, the plants distributed by the nurseries are required to be certified and workers at distribution nurseries in the growing regions are required to complete training in the handling and management of the SE plants, and to obtain certification from ICCRI (Agung Susilo, personal communication, 2012). Today, ICCRI is the most successful institution in scaling up SE technology for cacao for commercial purposes.

In 2012, three independent evaluation reports on the Gernas Kakao programme were made available in Indonesia, including an assessment of the performance of SE plants and surveys conducted by the Indonesian Government, ICCRI and Hasanuddin University. This assessment comprised a summary of the main findings of the surveys, compiled from information available in November 2012, which included documents written in Indonesian, PowerPoint presentations and personal interviews conducted with the different participants.

The reports from the Indonesian Government and from ICCRI both concluded that the SE plants are performing well in the field. The ICCRI survey evaluated the performance of SE trees in five Provinces (Central Sulawesi, West Sulawesi, South Sulawesi, South-east Sulawesi and Bali). The survey indicated that the proportion of SE plants lost during establishment is normal for cacao plants propagated by any method (5-10%) and overall the SE plants perform better than seedlings during the first two years. SE plants were distributed to the five provinces between 2009 and 2011. A total of 5,900 SE trees that had replaced old or unproductive trees on 295 cacao farms were sampled and evaluated with stratified random sampling. Six teams of four people each were deployed to the selected areas to interview farmers and personally collect tree performance data. Characterization of the individual trees was carried out based on plant vigour, growth parameters, number of flowering trees, number of fruits per tree, incidents of VSD, and angle of the main trunk in relation to the ground.

The main conclusions of the survey were:

1. Overall, 73% of the surveyed farms were in the 'good' and 'fair' categories, while 27% were categorized as 'bad'. It was observed that the conditions of SE farms in Central Sulawesi were better than the other locations surveyed. More specifically, the percentages of 'bad' SE farms planted between 2009 and 2010 were on average the lowest recorded: 5% and 11%, respectively.
2. The majority of the SE trees (80%) with good vigour were observed on 'good' and 'fair' farms. 19.6% of all 5,900 SE trees surveyed had low vigour and were mostly found on 'bad' farms. The farms surveyed in West Sulawesi had the highest portion of 'good', vigorously growing SE plants. The analysis of stem girth and tree height data revealed that the SE trees were more vigorous than trees developed by traditional propagation methods. A strong effect of soil and crop management practice on SE plant performance was noted. Few cases of trees with low vigour were found on farms with unsuitable land (low fertility soil with bad drainage) and full sun conditions.
3. Like seed-grown control plants, the cloned SE trees displayed normal dimorphic structure of seed-grown plants with the formation of jorquette branches and taproots (Figure 4.7). The mean jorquette height of the SE trees was 106.5cm with few SE trees (0.04%) forming jorquettes higher than two metres. The majority of the SE trees (95%) were in the upright position, and few had medium (3%) to severe (1%) inclines. Observations of the root systems of some of the inclined trees revealed twisted roots or very short taproots. Further enquires revealed that these symptoms resulted from improper management of the SE plants in the distribution nurseries where the bare-rooted plants received from ICCRI were transplanted into plastic bags. In some cases, where the plants were too large, the taproots were bent, twisted or cut to fit in the bags during transplantation. In some cases, plants were also grown with bad soil drainage likely resulting in water logging and/or poor root development.
4. In 2012, the SE trees exhibited good flower precocity. An average of 56% of the SE trees planted in 2009 (two-and-a-half-years old) were producing flowers. The average number of pods recorded at the time of the evaluation (July-August 2012) for the 2009 plants was 3.97 pods/tree (Figure 4.8). This indicated the flowering precocity of SE trees compared with traditional cacao trees, which normally reach this level of productivity at four years after planting. The number of pods was also greatly affected by the application of agricultural practices by the farmers. Because of this it is impossible to determine how much of the precocity reported is due to somatic embryogenesis technology and how much is the result of better agronomic practices.

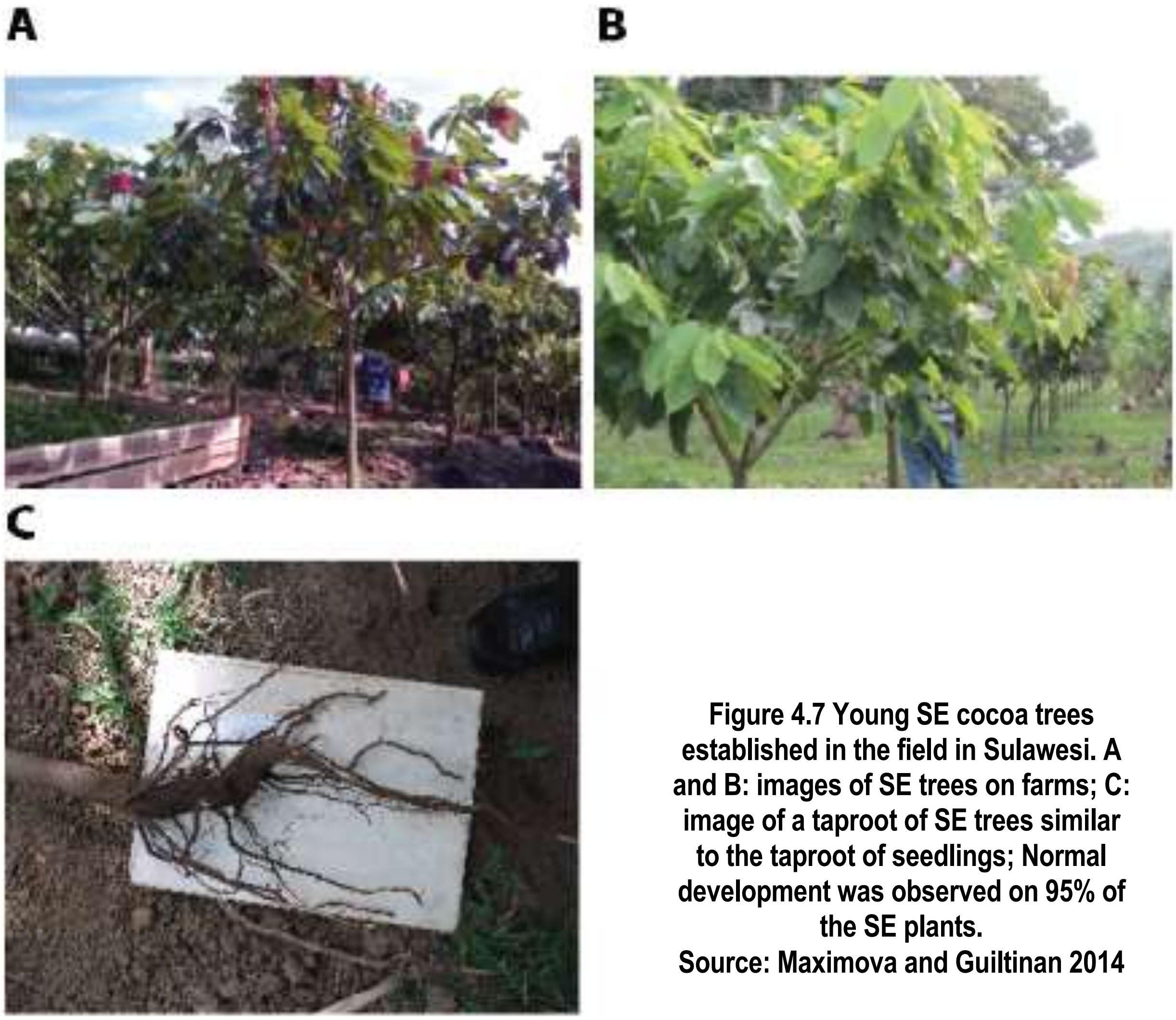

Figure 4.7 Young SE cocoa trees established in the field in Sulawesi. A and B: images of SE trees on farms; C: image of a taproot of SE trees similar to the taproot of seedlings; Normal development was observed on 95% of the SE plants.
Source: Maximova and Guiltinan 2014

However, a study conducted by the Research and Development Centre of Natural Resources at Hasanuddin University (Pardomuan and Taylor 2012) reported that a large percentage of the SE trees planted in South and West Sulawesi between 2009 and 2011 "many trees have fallen down and when you pull them up, it's obvious they don't have taproots". The report refers to a survey performed in March 2012 by a team from Hasanuddin University that included 60 farms in the South and West Sulawesi areas. The conclusions of the survey were based on the analysis of data obtained from a questionnaire designed for the survey. Descriptions of the specific method used in the survey, including the type of questions asked and the way the data was analysed, were not included in the report. The Hasanuddin study reported that the majority of the SE plants surveyed in the specified areas were unhealthy, with poor root structure and suffering from pests and diseases. In part because of this report, and the need to ensure there were no systematic problems with somatic embryogenesis produced plantlets growing in the fields, a site visit of the Gernas Kakao somatic embryogenesis project was carried out by the authors of this chapter in November 2012. Commercial propagation facilities, acclimation and distribution nurseries and demonstration plots were visited. Several extension agents and farmers were interviewed, and a number of local farms where SE plantlets had been established as part of the Gernas Kakao project were surveyed, including: ICCRI field station in Jember, East Java; ICCRI somatic embryogenesis laboratory in Jember, East Java; Dinas Perkpbunan estate - crops and horticulture headquarters in Kendari, South-east Sulawesi; plant distribution nursery, district Konawe, sub-district Amonggedo, Mendikonu village, South-east Sulawesi; extension office, Raterate, South-east Sulawesi; cacao research sub-station, South-east Sulawesi, built for the Gernas Kakao project; extension office in Andolo, South-east Sulawesi; and a new sub-district, South Iwoi Mendoro Bassala in South-east Sulawesi.

Figure 4.8 SE cocoa trees producing fruit in the field in Sulawesi, 2.5 years after planting. Source: Maximova and Guiltinan 2014

Presented here is a summary of the conclusions made as a result of the site visit in November 2012:

1. The ICCRI somatic embryogenesis laboratory utilized basic SE protocols that closely follow the procedures developed at Nestlé and Penn State, with some steps modified for particular genotypes. The laboratory and field sites were well managed and organized. The acclimated SE plants were packaged in boxes and shipped bare rooted from Jember by plane to the various distribution nurseries at the cacao-growing regions of Sulawesi and other islands. The plantlets remained at the destination nurseries for an additional two months after which they were transported and distributed free to project-approved farmers.

2. In the field, a large number of SE plants were observed at multiple locations in Java and South-east Sulawesi at all growth stages, from plantlet to three years old (the oldest plants in the project at the time). The SE plants appeared normal and adapted to local conditions. They seemed to flower earlier than other types of plants by six to ten months, excepting side-grafted plants, which benefit from having large, well-established root systems and thus also flower very early.
3. Experiences with horticultural specialists, extension agents and farmers were discussed. In general, the opinions were that the SE plants are the same or better than those plants propagated by seeds or through grafting. It was observed that some plants in the field were short, weak, etc.; however, no more than about five percent. Some fields were worse than others. Farmers who were interviewed all said that poor performance in such fields was noted for all kinds of plants and not just the SE plants.
4. It was observed that some of the plants that were grafted using locally adapted varieties selected by field staff seemed to perform better. This appears to be one limitation of this project in that the varieties used were not specific for each region.
5. Some logistical problems were recorded regarding the distribution of the plants. Although workers at the distribution nurseries are required to be trained by ICCRI and to obtain certification for managing and handling SE plants, it seemed that some of the nurseries were not well prepared logistically for such a large influx of plantlets. It was noted that plants that were larger than the standard size (20 cm) were transplanted in standard bags by pruning or bending the roots to make them fit. Such practices have a detrimental effect on the cacao plants and they usually do not recover. Additionally, some farmers did not plant the trees for a few extra months, for various reasons, which further adds to the problems.

4.3 Costs of tissue culture propagation

Compared with traditional propagation methods, somatic embryogenesis is more expensive; this is mainly because of the cost of building and operating a tissue culture facility, as mentioned above. The experience of INIAP in Ecuador shows that in an intensive, but still relatively low output experimental research laboratory, the cost of developing one plant through somatic embryogenesis using a solid medium is US$2.70 (Freddy Amores, INIAP experimental station, Pichilingue, personal communication, 2012). It is possible that with scale up and the utilization of a liquid medium, production costs could be lowered to US$0.30-0.50 per SE plant, as has been achieved for other plant species, like coffee. After the SE plants are established as stock plants, the cost of one SE orthotropic rooted cutting is US$0.20 (calculation based on the establishment of 10,000 SE plants).

In Indonesia, SE plants from ICCRI retail at approximately US$0.50 per plant. The price of the plants following establishment in distribution nurseries is approximately US$0.75 per plant. ICCRI also distributes open-pollinated seedlings for approximately US$0.40, hand-pollinated seedlings for US$0.50, and grafted plants for US$0.70, per unit. Although the precise production cost per unit of the ICCRI somatic embryogenesis process has not been publicly released, ICCRI staff members have confirmed that they are operating at a reasonable profit margin.

Facilities required

- A small modestly equipped tissue culture lab (approximately 100 m2) is required to propagate between 0.5 and 1 million plants per year. The equipment required includes:
  - sterile tissue culture hoods
  - water purifiers
  - coolers and freezers
  - pH meters

  - weight balances
  - dissecting microscopes
  - water baths
  - autoclaves
  - climate control equipment
  - back-up power supply
  - other small lab equipment and supplies
  - office and computer.
- An acclimation and propagation facility consisting of a shaded nursery is sufficient in tropical areas for acclimating plants from the lab, for maintaining stock plants for rooted cuttings and propagation by rooted cuttings.

4.4 Advantages and constraints of tissue culture

The main advantages of using the somatic embryogenesis technology for propagating cacao are:

- SE can produce genetically uniform plants, and capture high-value genetic traits.
- The SE technology is applicable to many different genotypes; although variation in efficiency has been observed between different genotypes, most genotypes can be propagated by this method.
- The SE process can be conducted year round; the work takes place in a laboratory setting and it is not strongly dependent on the time of year. However, plant propagation activities should be timed to ensure the plants will be ready for the planting seasons of individual countries.
- SE plants have morphological characteristics similar to plants grown from seeds. Cacao seedlings have characteristic dimorphic growth that is preferred by farmers because the young plants establish better owing to a single main taproot and jorquette formation that requires less formative pruning. SE plantlets have a similar morphology to seed plants.
- SE plants develop their own roots, unlike grafted plantlets that are commonly grown on rootstock obtained from seedlings. It is possible that genetic segregation for root traits may cause a variation in the yield potential of the resulting grafted populations.
- SE can be used to scale up from one or a few mother plant(s) more rapidly than other clonal propagation methods due to the multiplication rate of the SE process and the large number of floral explants possible per mother plant (rates of up to 50 embryos per flower are common). Since a healthy cacao tree can produce thousands of flowers per year, a potential multiplication rate of 50,000 fold is therefore possible. The use of embryogenic suspension cultures can further increase the multiplication potential.
- Cacao SE can be used for the production and testing of disease-free materials (See Quainoo, Wetten and Allainguillaume 2008).

The main disadvantages of using the SE tissue culture method for cacao propagation are:

- Some genotypes exhibit very low SE potential. For these, optimization of media and hormone concentrations may be necessary.
- Risk of somaclonal variation; this has been observed with other plant species and is a result of mutations occurring during the tissue culture process. While field testing has shown that this is not a major problem for SE cacao plants, there is a potential for problems if the protocol is modified, if cultures are carried out for too long or, possibly, for certain genotypes. Careful monitoring of plantlet production and performance in the field can mitigate this potential problem.

- Cacao SE is more expensive than traditional propagation methods. In fact, the cost of building and operating a tissue culture facility is much higher than that of a traditional propagation facility (see previous section on costs of applying the tissue culture technology).
- Embryogenesis has to be conducted in sterile laboratories that are expensive to build and operate, and are often located far from the cacao farms. Whilst SE cannot be carried out by the farmers themselves, clonal propagation by cuttings and grafting/micro-cutting can be conducted on farm by the farmers.

4.5 Future perspectives

Tissue culture has been commercialized for more than four decades. It is estimated that the current market of tissue-cultured products is 500 million to 1 billion plants annually. Perennial species produced commercially through somatic embryogenesis include teak; shade trees like maple, birch and hawthorn; roses; fruit trees; shrubs; lilacs; sugar cane; banana; coconut; bamboo; date palms; and forest trees (http://www.monfori.com; http://www.agriforestbiotech.com; http://www.indvitrolab.com; http://www.davaomusat.com; http://www.lapandybio.com; http://www.vitrobiovalen.com; http://www.marionnet.com; http://www.dbtmicropropagation.nic.in). Additionally, plant tissue culture has been used for the commercial propagation of a large number of coffee trees (http://www.ecomtrading.com/) and plantain (http://www.galiltec.com). Initially, abnormalities related to clonal oil palm were reported in 1986 (Corley and Tinker, 2003). However, over the course of the two decades that followed, SE protocols were optimized and by 2010 twelve tissue culture laboratories in Malaysia were producing clonal oil palm ramets (Kushairi et al. 2010). In 2011, oil palm clone producers in Malaysia produced 3.5 million ramets (http://www.myjurnal.my/public/article-view.php?id=48496).

The success of these ventures encourages us to consider the use of tissue culture for cacao and has driven the investments in research and field testing, as reviewed above. The effectiveness of this technology has been proved in many field tests, which have positively demonstrated the successful and reproducible growth of test-tube plantlets into fully producing mature trees. The recent experience in Indonesia suggests that somatic embryogenesis could be a viable commercial technology for cacao.

The demand for clonally propagated cacao plantlets is expected to increase dramatically over the coming years, due to the intersection of several synergistic drivers: increased cocoa demand; the release of new, high value, disease-resistant varieties; increased demand for special high-flavour cocoa varieties; the need to replace a large number of aging or diseased cacao trees currently in production; demand for the supply of specialty cocoa varieties; and demand for planting new land with cacao. We conservatively estimate that there are about nine billion cacao trees in production worldwide and if these are replanted at a rate of once every 25 years, about 450 million new cacao plantlets will be required each year just to keep up with current replanting demands. As new varieties are developed, demand for new planting materials will increase dramatically because more farmers will want to grow the new varieties.

Based on the promising results presented above, we conclude that cacao tissue culture holds a very strong potential to help address the future demand for cacao planting material. Furthermore, with the rapid pace of scientific advancement in the fields of cacao genetics and breeding, it will become even more important to implement fast clonal propagation systems for cacao to provide more efficient and rapid testing and deployment of new varieties.

Perhaps SE technology would be most important in the initial scale up of new varieties by saving two or three years when only one or a few mother trees exist. Possibly cacao SE technology should be utilized not as a stand-alone technology, but in conjunction with other methods of propagation such as rooted cuttings and grafting.

4.6 Acknowledgements

The authors would like to express their appreciation to all the organizations and individuals that contributed to the information presented in this chapter, including: Pierre Broun, Anne Buchwalder, Bernard Masseret and Vincent Petiard from Nestlé, France; Ivan Garzón and Freddy Amores from INIAP, Ecuador; the ICCRI team, and especially Agung Susilo; Ricardo Goenaga from USDA, Puerto Rico; Andrew Wetten from the University of Reading, UK; and Jean-Philippe Marelli from Mars Centre for Cocoa Science, Brazil.

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Chapter 5 - Cross-cutting issues and conclusions

High performance planting materials form an essential component of a package of measures to improve the productivity of cocoa farms. Breeding programmes in many countries require strengthening to ensure a steady stream of new, recommended materials that have been validated for local use. They also require integration with the means of multiplying them rapidly in order to ensure that they can be delivered to the farmers in sufficient quantities to satisfy demand in a cost-effective and timely way. Currently, even though certain improved planting materials have been recommended for use, together with the necessary technologies to propagate them, in most cocoa-producing countries many farmers do not have access to these materials. Rapid improvement of this unsatisfactory state of affairs is one of the higher priorities for all stakeholders in the global cocoa economy.

The decision to adopt a specific propagation method, or combination of methods, for the production of improved planting materials will depend on a number of factors, including the planting materials, resources and timeframe available; as well as the farmer's needs and ability to invest in new materials. It will also depend on whether the farmer aims to plant a new area, or rehabilitate existing plantings where productivity is low owing to factors such as poor planting materials, low tree density, pest/disease problems and tree age. Since these factors will vary tremendously both within and between regions, it is not possible to provide a simple recommendation here on the most appropriate techniques. However, this review does provide information on the main cacao propagation methods (by seed, by conventional vegetative propagation, and by tissue culture) and the status of their application. In this chapter, we discuss some of the factors that need to be considered when developing a specific propagation strategy, such as the type of variety and the amount of foundation material recommended for propagation; the facilities, labour and skills required; the phytosanitary conditions necessary; the costs involved; and the social and economic context. In Annex 5, we provide summary tables of the main advantages, constraints and possible improvements for each method, by drawing together information from the individual review chapters.

5.1 Importance of making improved planting material available for propagation

As discussed in detail in Chapter 1.3, the availability of appropriate source materials proven for their suitability in a particular region is of overarching importance when considering the most appropriate propagation method to use to supply farmers with new planting materials. It is important that these materials have been evaluated using the same propagation method that will eventually be used to mass propagate them so that they will perform as expected in the farmer's field. For example, the uniformity and yields from trials established using bi-parental seed from carefully controlled manual pollination could be quite different to the results obtained by farmers supplied by seed gardens relying on the self-incompatibility reaction to favour, but not guarantee, the production of bi-parental seed. For clonally propagated varieties, the method used may have substantial effects on vigour and tree architecture, such that a clone selected following trials established using budded material, for example, may not achieve the same performance when propagated by somatic embryogenesis, particularly if no adjustments are made to planting density and management practices. The process of developing and registering new varieties, and establishing selection criteria and requirements for trial data, will vary between countries, but it is essential that the materials that are recommended and propagated for distribution have been thoroughly evaluated to reduce the risk to the farmers who will depend on the performance of this material for their livelihoods.

In areas where true-breeding traditional varieties are still grown, farmers can obtain seed from their own trees, or from those of neighbouring farmers, to produce new planting materials that satisfy their needs at minimal cost. However, in many cacao-growing areas these traditional varieties have been replaced, at least to some extent, with hybrid/improved types. Since these types are generally not true breeding when sexually propagated, they will not necessarily reproduce the characteristics of the mother tree and the resulting populations may be highly variable.

Breeding efforts in a number of research institutes have resulted in recommendations for seed-parent crosses that have been tested for their suitability for local growing conditions and, usually, for industry and consumer. The extent of the implementation of these recommendations, in both large-scale production, and community and village seed gardens, is reviewed in Chapter 2. It is apparent that even though high performance seedling varieties were identified in West Africa and Indonesia over fifty years ago, there is still limited capacity to produce these varieties in bulk in most areas. Although seed gardens may take some seven years to come into full bearing, with good management they would be expected to generate seed pods for at least thirty years. During this period of productivity, new clones could be introduced into the seed garden for use as male parents to pollinate the existing mature pod-bearing trees. This means that the output of seed gardens can be improved during their lifespan as breeding work identifies new recommendations for the pollen parents. There are clear advantages therefore for closely linking the breeding and seed production operations to ensure that seed gardens have been planted with sufficient parental materials to incorporate newly recommended materials as soon as they have been confirmed. In effect, this will involve the vegetative propagation of the candidate parental materials during the period when their progeny are undergoing the later stages of evaluation and in on-farm trials.

Vegetative propagation, whether by conventional techniques or tissue culture (somatic embryogenesis), will produce planting materials that are genetically identical to the source material, though there may be variation in vigour, and therefore in the optimum planting density, depending on the propagation method used and on agronomic and environmental factors. There are a number of clones available in the Americas and South-east Asia, which have been selected for their suitability to local growing conditions, agronomic practices and quality requirements. Examples of programmes using conventional vegetative propagation techniques and the clones used are reviewed in Chapter 3, and examples of the use of tissue culture are given in Chapter 4. However, few clones have been evaluated for their suitability in West Africa, and although selection work is underway it may be many years before a range of proven clones is available. Again, close linkages between breeding programmes and the agencies carrying out conventional vegetative propagation are advocated so that clonal gardens of candidate clones are planted with stock plants in the areas where the planting materials will be required, and supplies of budwood, graftwood and/or cuttings are available as soon as the recommendations are confirmed. For somatic embryogenesis, unopened flower buds are required as the initial starting material, and tissue cultures of these can be established remotely from the sites where the planting materials are needed. Normally, however the resulting plantlets are weaned in nurseries closer to the areas where they will be required eventually.

In large-scale planting and rehabilitation initiatives, it is particularly important that consideration is given to the genetic background of the materials distributed to ensure appropriate levels of diversity are present. This reduces the risks that monoculture might pose with respect to changing local climatic conditions and the evolution of more aggressive strains of endemic pests/diseases or the introduction of new ones. In several countries, farmers are recommended to alternate monoclonal strips with strips planted with a diversity of self-compatible clones, and possibly with other agroforestry species, to minimize risks associated with monoculture, whilst maintaining sufficient uniformity and bean quality to satisfy farmer and market requirements respectively.

Attention should be paid to the legal conditions attached to materials' distribution. For example, the two international collections at CATIE and Cocoa Research Center (CRC) use the Standard Material Transfer Agreement (SMTA) of the International Treaty on Plant Genetic Resources for Food and Agriculture. Care should be taken to ensure that there are no infringements to benefit sharing and plant breeders' rights by any organisation involved in propagating materials for distribution to farmers.

An analysis of the breeding work that is underway, and the scale of the work required to deliver future improved seed and clonal varieties is outside the scope of this review. However, the importance of well-managed, large-scale and long-term breeding programmes, which are fully integrated with the mechanisms needed to supply their outputs to farmers, is clear. In determining the breeding objectives, careful consideration should be given to the suitability of the material to the proposed propagation method. In addition to characteristics directly related to the technique (e.g. ease of rooting where propagation by cuttings is envisaged), it is also important to consider whether the materials have appropriate tree architecture characteristics (e.g. branching angle, jorquette height, vigour) for the desired propagation method and planting density. For example, a type that produces a good tree shape with minimal pruning requirements when propagated by plagiotropic budding, may not necessarily produce an easily manageable tree when propagated by orthotropic cuttings. For conventional vegetative propagation by budding and grafting techniques, there is also scope to investigate the rootstocks-scion interactions that may impact on cocoa productivity with a view to selecting rootstocks that optimize the performance of the scions whilst still offering other advantages such as disease resistance.

5.2 Technologies and facilities for propagation

The establishment of a clonal garden is an early requirement for most propagation techniques, whether the garden will be used to supply budwood/graftwood directly to the farmers, provide materials for propagation in an associated nursery, or produce materials for the establishment of a seed garden. Careful management is needed to ensure that the source material is correctly identified. This could be accomplished by providing training and materials so that off-types could be quickly recognised based on their morphological characteristics or through the use of genetic fingerprinting. Moreover, clean stock schemes are required to ensure that materials are maintained free from graft-transmissible disease, particularly from virus infection in areas where CSSV or similar viruses are present. Optimization of irrigation/fertigation, shade and pruning regimes may be helpful in ensuring that the material is maintained under conditions that stimulate vigorous vegetative growth. Systems based on somatic embryogenesis combined with conventional propagation techniques may be useful in rapidly increasing quantities of stock plants where the source material for seed garden development is initially available only in limited quantities. Combined systems can also be used to generate plants which are the same in terms of growth form and propagation method, as those used by the breeders when they selected the clone, thus increasing the likelihood that the plants will perform in the field in the way that the breeders would expect.

The timing of the availability of seeds is important both for farmers intending to conduct plantings when local weather conditions are favourable and to ensure a steady supply of rootstocks for vegetative propagation operations such as budding and grafting. It is difficult to store seed for more than two weeks after harvesting (Mumford and Brett 1982) and thus seed may not be available when the farmers or propagators need it. The timing of pod production in seed gardens can be manipulated to some degree by extending the normal flowering period through the frequent manual stripping of young pods (cherelles). Irrigation may be required for maintaining the conditions needed for flowering and crop development, especially in places where this would occur during the dry season. Another approach would be to develop technologies that could allow seed to be stored for a period of several months after harvest, perhaps building on the preliminary research that has shown the potential of polymer gels in this respect.

Although some farmers plant seed at stake, i.e. directly into the ground, in many cases the seedlings are raised in nurseries for several weeks or even months before they are ready for planting or budding. Often seeds are planted in plastic bags containing soil, and the size and weight of these container-grown plants can be a constraint in getting the plants from the nursery to the final planting position. This can be a major problem, especially where the use of vehicles is limited/impossible and plants have to be head-loaded by the farmers and their family members for at least part of the distance. The development of low-cost, locally sourced, light-weight potting media and planting containers, suitable for use in farmer- and community-level nurseries, would greatly facilitate the transport of such plants and could contribute to the wider adoption of container-grown planting materials in areas where planting at stake is the norm.

Well-managed nurseries are a key part of large-scale propagation operations, whether the objective is to supply farmers with seedlings, rooted cuttings, grafted/budded plants or weaned plantlets. They offer the possibility of supplying planting materials to farmers at the most convenient time and when weather conditions are favourable for planting. However, such nursery operations can be expensive, thus increasing the costs of supplying the materials to farmers. This is especially the case in areas where labour costs are high, electric power is either unavailable or unreliable and automatic irrigation/fertigation is not available. As identified in the earlier chapters, there is considerable scope to improve operations. This could be along the lines adopted in forestry practice such as: optimizing the time that materials remain in the nursery, utilizing light-weight potting materials to reduce the use of top soil whilst improving water and nutrient-use efficiency, therefore improving the root system resulting in plants that are easier to handle and transport.

The production of somatic embryos requires well-equipped laboratories, but the acclimation (weaning) and further growth of plantlets to a stage at which they can be distributed to farmers can normally be accommodated within a conventional nursery. Future developments could include the up-scaling of laboratory facilities and adoption of a liquid culture system to increase throughput and reduce cost per plantlet.

5.3 Labour, skill requirements and farmer training

Although smallholder farmers may be able to manage their own small-scale nurseries and on-farm propagation activities, most propagation operations have considerable requirements for labour: manual labour for activities such as preparing land/potting media and watering; skilled labour for activities such as budding/grafting, manual pollination, and laboratory operations; and management staff with the necessary skills for operating large facilities to rigorous standards. Clearly they must also be operated in a cost effective manner and to ensure that survival and vigour of the plants is maximized. The availability of local labour and training needs will thus be important factors when considering the establishment of new propagation facilities, and the development of technologies to reduce labour requirements, such as irrigation, may become increasingly important in areas where there are likely to be inadequate rainfall, and/or where labour is in short supply / expensive.Farmers may require training in order to be able to manage their new planting materials appropriately. They will need to have an understanding of the soil, climate, planting density, shading and fertilizer regimes required to reach the reported yields for those materials. Even if the materials underperform due to poor agronomy, rather than the quality of the materials themselves, farmer dissatisfaction may lead to rejection of future opportunities to obtain new planting materials. Training will be of even greater importance in situations where the new planting materials are propagated clonally from plagiotropic material, whereas previous experience has been with seed raised materials to ensure that the plants are well pruned and rootstock outgrowths removed appropriately.

5.4 Phytosanitary considerations

Particular care must be taken at all stages of propagation and dissemination of planting materials to minimize the risk of spreading pests and diseases. Many cocoa-producing countries have phytosanitary legislation and procedures in place to minimise the risk of the introduction and/or spread of pests and pathogens within their territories, often adhering to the International Plant Protection Convention (IPPC, https://www.ippc.int). The use of intermediate quarantine, for example via the International Cocoa Quarantine Centre at the University of Reading (http://www.icgd.reading.ac.uk/icqc/), and post-entry quarantine is strongly recommended when moving material between regions which do not share the same pest and diseases. CacaoNet, the Global Network for Cacao Genetic Resources, has recently updated the 'Technical Guidelines for the Safe Movement of Cacao Germplasm', which provides information on the principal pests and pathogens of cacao, and recommendations for minimizing the risk of accidentally introducing these organisms when cacao planting material is distributed. Although the guidelines were developed to provide the best possible phytosanitary information to institutions involved in small-scale plant exchange for research purposes, the information will be also be relevant to those involved in producing and distributing planting materials on a larger scale (End, Daymond and Hadley 2014).

Viruses such as CSSV, which is endemic in many cacao-growing areas of West Africa, and the virus that affects cacao in Java, pose a particular risk when cacao is propagated vegetatively by conventional techniques, since they can be spread through graft transmission and cannot be eliminated by pesticide treatments. Depending on the strain of the virus and the cacao variety, symptoms may appear within weeks of infection, but in some cases the plants may remain symptomless for several years; for example, plants of the Amelonado variety may not show symptoms of infection with a mild strain CSSV for over a year even though they usually show symptoms of severe strains within three months of infection. Therefore, where vegetative material is to be propagated and distributed it is important that a clean stock scheme is in place to ensure that the stock plants have been confirmed to be free of virus, through frequent inspection by trained disease spotters, and ideally using molecular probes or virus indexing.

Although molecular studies have demonstrated that seed/seedlings from CSSV-infected mother trees do give positive reactions to some molecular probes developed for CSSV, there is no evidence to suggest that the virus will be transmitted to the seed in a viable form (Ameyaw, Wetten and Allainguillaume 2009). Therefore, seed is often considered to be the safest way to distribute planting materials in the areas of West Africa affected by CSSV, in terms of avoiding disease transmission.

Particular care must be taken to avoid the spread of fungal spores and insect pests associated with pod husks, poorly cleaned seeds, young plants and packaging materials, especially when moving materials between regions that do not share the same species/strains of pests and diseases. In such cases, the movement of whole pods is not recommended and some countries have placed their own restrictions on the movement of seeds/whole pods within their country or region. In Indonesia and Malaysia, for example, there is a perceived risk that larvae of boring insects such as the cacao pod borer (*Conopomorpha cramerella*) could be carried with poorly cleaned and treated seeds; while in Brazil and Ghana, different species and/or strains of diseases are endemic to particular regions. Particular care must be taken when moving plants in containers, especially when soil has been used in the potting medium, owing to the risks of spreading soil-borne pests and diseases, which can include *Rosellinia* spp., *Phytophthora* spp., nematodes and soil-borne mealy bugs.

Contamination from microorganisms and pests is generally eliminated at an early stage in tissue culture, and somatic embryos and plantlets in aseptic media are considered low risk from a phytosanitary perspective. Research results suggest that the somatic embryogenesis process considerably reduces the risk of CSSV transmission; however, since the possibility cannot be ruled out absolutely, it would be prudent to confirm that the source materials used are virus-free.

5.5 Predicting demand for planting materials

Estimating demand for planting materials for new plantings and rehabilitation has to be undertaken at a country/regional level, taking into account factors such as planting density, area to be planted, the efficiency of the utilization/establishment of planting materials, farmer demand and national policies, if any, towards diversification of perennial cash crops. Efforts to estimate demand are underway in many countries as part of the national cocoa plans being developed under the Global Cocoa Agenda, as proposed frequently by ICCO and re-stated during the first World Cocoa Conference, held in Abidjan in October 2012. The challenges in modelling the seed garden capacity required to match actual demand in West Africa are discussed in Chapter 2 and further research will be needed to gain a better understanding of some of the factors involved and to improve the efficiency of the utilization of the materials produced.

Experience gained from initiatives such as Project Lembrance in Brazil, where rooted cuttings were supplied to farmers for intercropping with papaya (see Chapter 3), and initiatives involving the side grafting of young tree trunks or high branches with improved materials, may well be useful in developing propagation strategies for other areas where there is a need for the rehabilitation of existing cacao farms to rapidly increase their productivity.

Somatic embryogenesis, possibly in conjunction with conventional vegetative propagation techniques, may also offer unique opportunities, particularly in the initial scale up of new varieties, by saving up to two or three years in cases where only one or just a few mother trees exist.

5.6 Considerations on estimating costs

Cost considerations for large-scale plant propagation will usually include selecting one or more propagation methods specifically suitable for a given region or country, and describe the resources needed, such as infrastructure (propagation laboratories, greenhouses, nurseries, irrigation, shade, and electricity); plant materials; equipment and supplies; labour; technical assistance; and administration.

In this review, we set out to compare the costs on a like-for-like basis, such as the set-up costs and costs of production per plant/per million for easy comparison. However, this has been particularly challenging since very few data on costs of cacao propagation technologies have been published, and so estimates are only possible based on specific examples (case studies) in a specific location/region/country, as described in Chapters 2.3, 3.3 and 4.3. In general terms, improved seed that is destined to be planted at stake or raised in a farmer's nursery is by far the cheapest option, with the cost of producing seed in an established seed garden in West Africa, using manual pollination, estimated at US$0.002 per seed, or US$0.088 per seed if the costs of establishing and maintaining the seed garden are taken into account.

The estimated cost of raising a seedling in a nursery, whether for supplying to farmers or for budding/grafting in subsequent operations, is around US$0.50 per plant, depending on the country (due mainly to labour costs) and on the length of time that the seedling is maintained in the nursery (see Chapter 3). In West Africa, the average cost per plant is US$0.45, plus the cost of the seed, when considering the lowest possible labour costs (see Chapter 2); in Ecuador, the cost of raising seedlings is estimated to be US$0.07 per plant, while in Costa Rica, the cost is US$3.00 per plant.

Estimates for the costs of budding and grafting should take into account the costs of establishing/maintaining the clonal garden and producing rootstocks, as well as nursery costs and the labour needed to carry out the propagation technique. The cost of establishing/maintaining a clonal garden is generally comparable to seed garden estimates, which in Brazil, according to Table A4.7 in Annex 4, can be estimated at US$23,428 per hectare over a three-year period). In West Africa, the estimated costs of establishing a seed garden range between US$ 5,876 per hectare at the lowest labour rate that was modelled, to US$ 28,606 at the highest rate, over a three-year period (Rob Lockwood, personal communication, 2014).

From the Brazilian experience, the cost estimates for nursery-grown budded plants and grafted plants are approximately US$0.60 and US$0.75 respectively (see Table A4.1, Annex 4). Cuttings, although they do not require seedling rootstocks, do require propagation facilities and nursery care. In Brazil, the average cost per plant produced from rooted cuttings is US$0.58, though this will vary according to the scale of the operation; in West Africa, the average cost per plant produced from rooted cuttings is US$ 1.61 at the lowest labour rate.

Compared with traditional propagation methods, cacao somatic embryogenesis is more expensive. This is mainly due to the cost of building and operating a tissue culture facility in addition to the nursery costs. In Ecuador, the cost of somatic embryogenesis production in a small-scale research laboratory is US$2.70 per plant. When the somatic embryogenesis technique is used to establish stock plants, which are then used to produce orthotropic cuttings, the estimated cost per rooted cutting is US$0.20 (calculation based on the large-scale commercial production of rooted cutting plants in Ecuador). The retail price of a plantlet in Indonesia produced through somatic embryogenesis is estimated at US$0.50 (or US$0.75 after establishment in the distribution nurseries), although the production costs are not publicly available (see Chapter 4).

For propagation in the field (for example, side grafting onto established trees), estimated costs need to include the establishment of a clonal garden, distribution of materials to the site, and labour for the grafting operation. Estimates from Brazil range from US$0.40 to US$0.60 per plant depending on the technique used (see Table A4.1, Annex 4).

It is difficult to compare these cost estimates, or use them to estimate costs in other countries or situations, for the following main reasons:

1. The production objectives may vary. The relative cost of establishing new plantings vs. rehabilitating old, low-performing trees on an existing farm depends on different sets of criteria and assumptions. There will clearly be more possibilities to invest in large infrastructures and expensive equipment if the production objective is on a large scale.
2. A large proportion of the costs of propagation, particularly for the techniques involving nursery care, are dependent on local labour rates, though other factors such as local availability/cost of materials, and distribution costs will also have an impact.
3. Although the cost of nursery management and distribution of a 'ready-to-plant' propagule might be similar for a given region or country, there may be considerable variation in the costs of producing the materials entering the nursery. This will depend on the scale of the operation and the starting point of comparison, i.e. whether or not the costs of establishing

the laboratories, seed gardens, budwood gardens and nursery facilities should be taken into account, and, if so, how the increase in productivity/efficiency over time can be taken into account - for example, as procedures are optimized, source plants mature, and management and labour become more skilled.

5.7 Factors to be considered when choosing propagation methods

Based on the current knowledge and status of the different propagation methods, as described in Chapters 2 to 4, the following important factors need to be taken into consideration when deciding on which propagation method to use: (1) farmer demand for a specific type of material; (2) the main objective of production, and the scale and timeframe required to achieve the objective; (3) the availability of planting materials that have been proven to be superior under farmers' management when propagated by the chosen mass propagation method; (4) technical and institutional issues; and (5) financial considerations. These factors are expanded on below.

5.7.1 Farmers' demand

- Total cost of plants to farmer.
- Financial situation of farmers, their access to credit and willingness and capacity to invest in planting materials.
- Production objective – see 5.7.2
- Preference for type of planting materials.
- Technical skills and knowledge required by the farmer to implement.

5.7.2 Production objective, scale and timeframe

- The current and potential demand from farmers for planting materials (a 25-year planting cycle is suggested), including:
  - Demand for rejuvenation of old trees (area and/or number of trees).
  - Demand for new planting area (area and/or number of trees).
  - Demand for replanting an area affected by a pest and/or disease.
  - Scale of demand – individual farm, village, region, country.
  - Timeframe for reaching the production objective (short-/medium-/long-term investment).
- Scale and size of the operation targeted or projected, i.e. hectarage and number of trees per hectare (ha).

5.7.3 Planting materials

- Availability of planting materials that have been proven/recommended for use in a particular area, using a specific propagation method.
- Availability of planting materials with the quality characteristics required by the intended market.
- For seed propagation, the availability of plants of parental clones that are mature enough to bear pods (maternal parent), and flowers (pollen parents).
- For vegetative propagation, the availability of vegetative material that has been confirmed as healthy and virus free in areas where viruses are endemic.
- For somatic embryogenesis, the availability of parental material with flower buds.
- Multiplication rates and growing conditions in the field.
- Timescale for a tree to yield after planting.
- The existence of an independent seed/plant certification system.
- Legal conditions attached to materials distributed from genebanks and breeding programmes.

5.7.4 Technical issues and institutional support

- Technical skills and knowledge required by the farmer for the implementation of the propagation technique, and/or the management of trees resulting from that form of propagation.
- Availability of high quality production sites (seed gardens/clonal gardens/somatic embryogenesis laboratories) and nurseries in favourable agro-ecological conditions, and/or investment in irrigation where appropriate.
- Availability of land/facilities that could be developed for use as quality production site/nurseries.
- Professional management, and appropriately trained staff and labour.
- The existence of an efficient seed/plant distribution system, using intermediary nurseries where appropriate and with the possibility of seed storage facilities in the future.
- Support/commitment from local, regional or national agricultural research services, including integrated breeding programmes.

5.7.5 Financial considerations

- Availability of funds (government, donor, private) to develop and run propagation and nursery facilities.
- Cost of production of the plants/seeds, noting that this will often be dependent on the scale of production (for example 100,000 plants, one million plants, five million plants and 20 million plants per year).
- Cost of transporting planting materials to the farms.
- Total cost of plants to farmer.
- Financial situation of farmers, their access to credit and willingness and capacity to invest in planting materials.
- Projected financial returns (through increased yield) vs. the loss of income and risk associated with (re)planting.
- Availability of financial support from donors and/or government subsidies at the farm, local, regional or national levels.

When considering the implementation of one or more propagation method, each specific situation and case must be studied very carefully. No single solution can be recommended without evaluating the factors listed above. Once these are further defined, this review can assist in considering a specific approach based on the current experience of practices described in Chapters 2 to 4.

5.8 Conclusions

Current Status: the need for action

It is estimated that globally only 25% of all cacao farms currently consist of improved varieties. Often, farmers obtain planting materials by propagating from their own trees, or those of their neighbours, especially where improved materials are difficult to obtain or thought to be too expensive. Thus, not only are they unable to take advantage of the materials that have been selected for higher yield and good quality, but also often face problems of non-uniformity and high losses to local pests and diseases. There is also the risk that these plants could be highly susceptible to diseases that have not yet become established in the area, but which may have caused heavy losses in other cocoa-producing regions. Moreover, these non-uniform local materials may offer little resilience to environmental stresses, which may be increasing in some areas due to local climate change. The governments of cocoa-producing countries, and the wider cocoa industry, recognize that the supply of planting materials to farmers is a challenge that must be urgently addressed to improve the sustainability of the world cocoa economy.

This review provides a status report and information that will be of value in developing strategies for delivering improved productivity by supplying improved planting material to farmers. Each of the propagation techniques available for cacao has its own advantages and constraints, particularly in terms of availability of source materials, scalability, timescale and cost. Moreover, there are prospects for developing the technologies and/or integrating them to provide cost effective, high throughput systems.

Availability of improved planting materials: performance, agronomic suitability, uniformity v. vulnerability

The availability of improved types, proven for their suitability in a particular region, is of overarching importance when considering the most appropriate propagation method to use to supply farmers with new planting materials. Furthermore, although farmers might be looking for uniformity of production within their individual fields, widespread use of very closely related or clonal material across an extensive area increases the vulnerability of the crop to climate change, and evolution to to pests and disease. It is important to ensure that farmers are provided with an appropriate mixture of clones, or seed varieties, which have been confirmed as suitable for planting together, taking into account agronomic and genetic factors such as tree architecture/vigour, timing of flowering, ability to self- and/or cross-fertilize (i.e. sexually compatible pollen), together with quality and flavour aspects.

Building on existing capabilities, expanding capacity and development needs

Most countries already have some capacity to produce improved seed at very low cost, though in many cases considerable further investment would be needed to increase this capacity and meet demand. The development of seed storage technology, or mechanisms to alter the timing of production from seed gardens, would also help ensure sufficient seed is available to farmers wherever they are located and whenever they need it. Some countries, particularly those in Latin America and the Caribbean and South-east Asia, have successfully used traditional vegetative propagation techniques to supply clonal material to farmers either as ready-to-plant propagules or for *in situ* grafting to rehabilitate existing plantings. There is scope to expand the clonal gardens and optimize large-scale propagating facilities, or develop low-cost systems for community level use, to make these materials more widely available. Once suitable clones have been selected for use in West Africa, then this technology can be introduced for large-scale use in that region. Somatic embryogenesis has the advantage that, once suitable clones have been identified, it can provide a rapid means to produce clonal plantlets with the seedling growth habit familiar to most farmers. Although the technique requires substantial investment to set up and run the laboratories/nursery facilities, and consequently results in higher costs per propagule, it can be used as a means to provide materials directly to farmers. There are also possibilities for its use in combination with orthotropic cuttings to increase the output of clonal materials with a seedling habit. Whichever technology is used, it is important that the nursery and distribution networks work effectively and efficiently in order to supply farmers with healthy and robust materials ready for planting.

It is clear that the availability of recommended source materials, that have been thoroughly evaluated using the same propagation technique as that which will be used to mass produce the materials for the farmers, will be a key factor when considering the most appropriate technology or technologies to include in a propagation strategy. In this review, we identify some of the materials currently being propagated for distribution, but it would be useful to gather further information on the recommended varieties (true breeding, bi-parental crosses or clones) in each country/region, and the breeding strategies, evaluation criteria and depth of evaluation that have been used to select them. If the objective is to supply planting materials to farmers as soon as possible, then the choice will have to be based on the current knowledge and performance of the locally available varieties for each method. If the production objective is on a longer timescale, further investment in breeding programmes will enable the development and thorough evaluation of candidate materials locally, or regionally, to ensure that only the most appropriate materials are distributed to farmers. This thorough evaluation will minimize the risk that farmers take when replanting/rehabilitating, and also the risks to the country's export earnings and cocoa industry if large areas were to be planted with materials that did not meet expectations, either in terms of yield, disease resistance or quality.

The need for coordinated efforts to improve the sustainability of cocoa production

The production of improved types is a very long and expensive process and the breeding programmes must be fully integrated with propagation systems so that materials are tested for their ease of propagation, and stocks of candidate materials should be built up during the later stages of evaluation so that adequate quantities of planting materials are available for distribution as soon as new recommendations are made.

The improvement of production calls for an overall coordinated effort such as the Global Cocoa Agenda lead by the ICCO, and particularly the development of national cocoa plans, looking at sustainable production beyond 2020. The success factors of any effort to supply new planting materials to farmers will depend not only on the characteristics of the material to be propagated but also on the effectiveness of quality control during the propagation procedure and throughout the distribution system. The ability of farmers to ensure the materials they receive flourish may also be influenced by the levels of farmer training and access to inputs such as fertilizers. Ultimately, the benefits of the new materials will have to be seen by the farmer to outweigh the costs and risks involved in replanting if they are to be widely adopted.

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Current status:

- The low rate of adoption of improved plant materials is a major constraint to cocoa productivity and thus the sustainability of the world cocoa economy.
- Estimated ~ 25% cacao planted globally currently consist of improved varieties.
- Farmers often propagate their own/ local materials, or those of neighbours which can lead to problems with lack of uniformity, little resilience to biotic and abiotic stresses and low productivity.
- Better strategies are needed to supply farmers with new and affordable planting materials.
- Supplying farmers with new planting materials is highly context-specific.
- Need an appropriate/ agronomically compatible mixture of clones or seed varieties to meet farmer needs for uniformity whilst minimising the risks associated with monoculture.

Propagation by seed:

- Well-managed seed gardens produce large quantities of improved seed cost effectively. However, further investment needed to increase capacity, and develop seed storage or other mechanisms, to ensure sufficient seed is available to farmers when they need it.

Propagation by conventional vegetative methods:

- Some countries (especially in Latin America and the Caribbean and South-east Asia) have used traditional vegetative propagation techniques to cost effectively supply clonal material to farmers (as ready-to-plant propagules or for *in situ* grafting).
- Scope to expand clonal gardens and optimize large-scale propagating facilities, or develop low-cost systems for community-level use, to allow wider availability.
- Once proven clones are available in West Africa, technology can be introduced for their large-scale use.

Propagation by somatic embryogenesis (SE):

- Once suitable clones are identified, SE can provide a rapid means to produce clonal plantlets which have the seedling growth habit familiar to most farmers, i.e. the adoption of SE material foregoes the major agronomic opportunity offered by well chosen clones grown as plagiotrops.
- SE requires substantial investment to set up and run the laboratories/nursery facilities.
- Can be used to provide materials directly to farmers or used to provide stock/parental materials for budwood gardens/seed gardens.
- Possibilities for SE use in combination with orthotropic cuttings to increase output of clonal materials with seedling habit.

Nursery Operations and Distribution networks

- Effective phytosanitation, well managed nursery operations, and efficient distribution networks are essential in order to provide farmers with healthy, robust materials in a timely way.

Which technologies to use?

- Each propagation technique has specific merits and constraints (particularly for availability of source materials, scalability, timescale and cost).
- When considering the most appropriate technologies to include in a propagation strategy, a key factor is the availability of recommended source materials that have been thoroughly evaluated using the same propagation technique as that to be used to mass produce the materials for farmers.
- If aiming to supply planting materials as soon as possible, the choice should be based on current knowledge and performance of locally available varieties for each method.

Next steps:

- This review identifies some materials currently being propagated for distribution.
- For future planning, it would be useful to gather further information on:
- Recommended varieties (true breeding, bi-parental crosses or clones) in each country/region.
- Breeding strategies, evaluation criteria and depth of evaluation that have been used to select them.
- On a longer timescale, further investment in breeding programmes will enable development and evaluation of candidate materials locally or regionally to ensure that only most appropriate materials be distributed to farmers.
- Such evaluation will minimize farmer risk when replanting/ rehabilitating and minimize risks to country's export earnings and cocoa industry.
- Production of improved types is lengthy and expensive. Breeding programmes must thus be fully integrated with propagation systems so that materials are tested for ease of propagation, and stocks of candidate materials should be built up during later evaluation stages so adequate quantities of planting materials are available for distribution as soon as new recommendations are made.

Successful adoption of propagation methods will depend on:

- Characteristics of material to be propagated.
- Effectiveness of quality control during the propagation procedure and distribution.
- Care taken after delivery of plants, (may be influenced by levels of farmer training and access to inputs such as fertilizers).
- Benefits of new materials will have to be seen by farmers to outweigh costs and risks involved in replanting.

For production and productivity improvements an overall coordinated effort is needed such as that proposed in the Global Cocoa Agenda, and development of national cocoa plans, looking at sustainable production beyond 2020.

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Annexes

ANNEX 1 – List of participants to the review workshop

Review workshop 'Supplying new cacao planting material to farmers: a review of propagation methodologies'
13-15 May 2013, London, UK

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ANNEX 2 – List of acronyms and abbreviations

ACIAR	Australian Centre for International Agricultural Research
CATIE	Centro Agronómico Tropical de Investigación y Enseñanza (Tropical Agricultural Research and Higher Education Center), Costa Rica
CDC	Commonwealth Development Corporation
CEPEC	Centro de Pesquisas do Cacau (Cacao Research Centre), Brazil
CEPLAC	Comissão Executiva do Plano da Lavoura Cacaueira (Executive Commission for Cacao Farm Planning), Brazil
CFC	Common Fund for Commodities, the Netherlands
CIRAD	Centre de coopération internationale en recherche agronomique pour le développement (Agricultural Research Centre for International Development), France
CNRA	Centre national de recherche agronomique (National Centre for Agricultural Research), Côte d'Ivoire
Cocobod	Ghana Cocoa Board
CPB	Cacao pod borer
CRA	Cocoa Research Association Ltd : A UK-based non-profit scientific research association that is supported by Mars Chocolate, Mondelez International and the London Cocoa Trade ICE Futures Europe
CRC	Cocoa Research Center, University of the West Indies, Trinidad and Tobago
CRIG	Cocoa Research Institute of Ghana
CRIN	Cocoa Research Institute of Nigeria
CSSV	Cocoa swollen shoot virus
FAO	Food and Agriculture Organization of the United Nations
Gernas Kakao	Gerakan Nasional Peningkatan Produksi dan Mutu Kakao (National Programme for Increasing Cocoa Productivity and Quality), Indonesia
IBA	Indole butyric acid
IBGE	Instituto Brasileiro de Geografia e Estatística (Brazilian Institute of Geography and Statistics)
IAA	indol-3-acetic
ICCO	International Cocoa Organization, London, UK
ICCRI	Indonesian Coffee and Cocoa Research Institute
ICGD	International Cocoa Germplasm Database
ICO	Instituto de Crédito Oficial (Official Credit Institute), Ecuador

ICS	Imperial College Selections
IIBCRI	Indonesian Industrial and Beverages Crops Research Institute , Indonesia
IICA	Inter-American Institute for Cooperation on Agriculture
INGENIC	International Group for the Genetic Improvement of Cocoa
INIAP	Instituto Nacional Autónomo de Investigaciones Agropecuarias (National Institute for Agricultural Research), Ecuador
IPPC	International Plant Protection Convention
IRAD	Institute of Agricultural Research for Development of Cameroon
IRCC	Institut de Recherches du Café et Cacao (Coffee and Cacao Research Institute), Togo
MARDI	Malaysian Agricultural Research and Development Institute
MCB	Malaysian Cocoa Board
MMSP	Mabang Megakarya Selection Programme
NIIR	National Institute of Industrial Research, India
NPK	Nitrogen, Phosphorus, Potassium
OAS	Organization of American States
Penn State	Pennsylvania State University, USA
PLANAFORO	Rondônia Natural Resource Management Project
PSCC	Programme Semencier Cacao et Café (Cacao and Coffee Bean Programme), Cameroon
PSE	Primary somatic embryogenesis
PVS	Plant vitrification solution
RRS	Reciprocal recurrent selections
SE	Somatic embryogenesis
SENACYT	Secretaría Nacional de Ciencia y Tecnología del Ecuador (National Secretariat of Science and Technology,) Ecuador
SENPLADES	Secretaría Nacional de Planificación y Desarrollo (National Secretariat of Planning and Development,) Ecuador
SIDRA	Sistema IBGE de Recuperação Automática (IBGE System of Automatic Recovery)
SMTA	Standard Material Transfer Agreement
SNI	Indonesian National Standard
SODECAO	Société de Développement du Cacao (Cocoa Development Corporation,) Cameroon
SSE	Secondary somatic embryogenesis

STCP	Sustainable Tree Crops Programme, based at the International Institute of Tropical Agriculture (IITA-Ghana)
TSH	Trinidad selected hybrids
UFR	Unité de formation et de recherche (Training and Research Unit)
USDA	United States Department of Agriculture, USA
V4C	Vision for Change project, funded by Mars
VSD	Vascular streak dieback disease
WACRI	West African Cocoa Research Institute
WBD	Witches' broom disease
WCC	World Cocoa Conference
ZE	Zygotic embryos

ANNEX 3 – Report on cacao planting materials from various cocoa producing countries

Rob Lockwood

Seed production capacity is summarized for the principal cocoa-producing countries, with an account of each country provided, including the genetic background to cacao cultivation and, where available, the estimated uptake of improved planting material.

A3.1 Brazil

Production: 200,000 tonnes (t). Area: 653,921 hectares (ha)

Cacao varieties in Brazil

The early history of cacao cultivation in Bahia (and other extra-Amazon states of Brazil) was reviewed by Bartley (2005), who surmised that a single variety, 'Comum', of unknown provenance, was involved during the first century of establishment of the crop. A second introduction, known as 'Pará', of uncertain Amazonian origin, divides into two varieties, Maranhão with two forms, and Pará. The narrow genetic base of these cultivars has long been recognized, and undoubtedly contributed to the impact of the epidemic of witches' broom that commenced in 1989.

Distribution of planting material

BAHIA

Bi-parental seed gardens were established in Bahia in the 1960s to produce 18 bi-parental crosses (see below Table A3.1) by natural cross-pollination, with reliance on the incompatibility mechanism to ensure out-crossing of self-incompatible parents.

Table A3.1 Bi-parental crosses produced in Bahia by natural cross-pollination in the 1960s.

UF613 x SIAL325	UF613 x SIC813	IMC67 x SIC813
IMC67 x SIAL70	UF613 x SIAL70	IMC67 x SIAL325
Pound7 x IMC67	Pa30 x IMC67	Pa121 x IMC67
Pa150 x IMC67	TSH-565 x IMC67	TSH656 x IMC67
TSA644 x IMC67	Sca6 x UF667	Sca12 x UF613
Sca6 x ICS39	Pa150 x SIC864	Pa169 x SIC864

Source: Vello et al. 1969.

About 177 ha of seed gardens were planted at several experimental stations in Bahia beginning in the early 1960s and continuing up until the late 1980s. The clones used in these seed gardens were: Catongo, CEPEC15, ICS1, ICS6, ICS8, IMC67, Ma15, Pa30, Pa121, Pa150, Pound7, Sca6, Sca12, SIAL7, SIAL70, SIAL88, SIAL169, SIAL325, SIAL505, SIC5, SIC17, SIC23 and SIC328. The clones were planted in separate blocks for manual pollination, such as Sca-6 and Sca-12; or in alternate double rows for natural pollination, for example SIC23 x ICS1, and SIAL505 x SIAL169, and so on (Roberto Sena Gomes, personal communication, 2013.

Initial target production was 40m seeds per year from a planned 60-70 ha of seed gardens, but additional seed gardens were planted as above and in 1979 Alvim (1981) reported a distribution of 100m seeds per year. It is understood that the seed gardens fell into disuse following farmers' disappointment with the field performance of the seeds that they received. A recent impact on farms has been an expression of resistance to witches' broom in seeds originating from Scavina parents, allowing for the implementation of a large-scale clone selection programme on farm.

RONDÔNIA (WEST OF THE BRAZILIAN AMAZON)

In 1973, a seed garden was established at the experimental station of the Executive Commission for Cacao Farm Planning (CEPLAC), in Ouro Preto do Oeste, Rondônia, with several additions made during the 1980s, and the last in 1998.

- Production of hybrids: IMC67 x Ma11, IMC67 x Be10, Sca12 x Be10, Sca12 x Ma11, Sca6 x Ma11
- Followed by: IMC67 x Be8, Be9, SIC813, SIAL169
- Followed by: Pound7 x Be10, Ma12, Ma15, SIC864
- Followed by: Pa150 x SIC328, SIAL325, CA5, Ma11
- Followed by: Pound12 x SIC329, SIC831, SIAL505, Ma14
- Followed by: Sca6 x ICS1, ICS6, SIC801, Be9

After 1990, crosses of the following were made:

- Sca6 x ICS1, ICS6, Mocorongo1, CAS2, CAS3
- Pa150 x Ma11, Ma15, Be7, Be10, SIC328, SIAL325
- IMC67 x Be8, Be10
- Pa121 x Ma13
- Be10 x Ma11, Ma15
- Pa195 x SIAL325
- Ma13 x Mocorongo1

Between 1976 and 1998 approximately 151.2m seeds were produced. Seventy-one (71) million cacao seeds were distributed to smallholder farmers, of which 58.1% of had been produced from manual pollination (Almeida and Neto 1999).

CLONAL MATERIAL

Information on clones used in Brazil is given by Roberto Sena Gomes in paragraph 3.2.1 of this Review.

Acknowledgements: The author is most grateful to R. Sena Gomes for providing information on the distribution of cacao planting material in Brazil; and to U. Vanderlei Lopes, M. Morais, and Dario Ahnert.

A3.2 Cameroon

Production: 229,000 t. Area: 670,000 ha

Cacao varieties in Cameroon

The cocoa industry in Cameroon has a different genetic base to the rest of West Africa, reflecting the country's different colonial history. The predominance of Trinitario material in the early introductions explains why Cameroon has a reputation for supplying red cocoa powder. It also differs from the other West African countries in that clonal material has been supplied to farmers from time to time.

Bartley (2005) provided a comprehensive review of the cacao introductions programme in Cameroon, the earliest record being a shipment of 13 Trinitario plants from the Royal Botanic Gardens at Kew in 1876, followed by Preuss's introduction of 'better' varieties from São Tomé to the Botanic Garden at Victoria. This may be the introduction referred to by Ngatchou (1981), although Efombagn et al. (2008) stated that a Lower Amazon Forastero type, presumably Amelonado, was introduced from São Tomé around 1892. Bartley (2005) also refers to the introduction of 332 plants from Trinidad, including 'Forastero'. Wood (1991) refers to an introduction from Fernando Po (now Bioko, in Equatorial Guinea).

Bartley summarizes Preuss' (pp. 165-277, 1901) large introduction of Trinitario and other material from Central and South America and the Caribbean, with a 15 ha collection established at the Botanic Garden at Victoria. There seems little doubt that seed from some of the introduced types were used to develop cacao farms in western Cameroon, but West and Voelcker (1942) reported that yields were low. Cameroon received clonal material from the West African Cocoa Research Institute (WACRI) up until the reunification of the country in 1961. Thereafter, Cameroon enjoyed access to the material selected elsewhere in Francophone West Africa.

Seed production and crosses produced

Liabeuf (1969) listed eleven bi-clonal seed gardens in 1967. The crosses produced in those gardens are presented in Table A3.2

Table A3.2 Crosses produced in 11 biclonal seed gardens in Cameroon in 1967.

Year planted	Area (ha)	Cross produced
1960	1	SNK37 x SNK37
1960	1.5	SNK13 x ICS95
1960	1.4	UPA143 x SNK64
1960	1.7	ICS43 x SNK37
1960	1.4	SNK109 x SNK37
1962	1	UPA143 x ICS40
1962	1	SNK16 x ICS6
1962	1	ICS60 x SNK456
1962	1	ICS46 x SNK460
1964	1.3	UPA134 x SNK12
1964	1.7	UPA143 x ICS95

Source: Liabeuf (1969).

Table A3.3 The list of seed gardens was greatly extended by Ngatchou (1981).

Field	Cross	Notes
CSI	UPA143 x SNK64	See Table A3.2 above
CSII	SNK13 x ICS95	Also manual pollination SNK13 x T79/501
CSIII	UPA143 x SNK64	See Table A3.2
CSIV-XI	T60/887 x ICS46	And reciprocal
CSXII	UPA143 x ICS95	See Table A3.2
CSXIII	IMC67 x SNK10	Also manual pollination SNK10 x UPA138
CSXIV	ICS43 x UPA143	And reciprocal. See Table A3.2
CSXV	UPA143 x ICS40	
CSXVI	ICS40 x UPA134	
CSXVII	SNK109 x IMC67	And reciprocal
CSXVIII	T79/501 x SNK109	And reciprocal, also manual pollination SNK109 x UPA134
CSXIX	SNK10 x IMC67	See above and Table A3.2; also reciprocal
CSXX	T79/501 x SNK13	And reciprocal
CSXXI	T79/467 x SNK13	Also manual pollination T79/467 x SNK16
CSXII	T79/467 x SNK64	Also manual pollination T79/467 x SNK16
CSXIII	SNK48 x IMC67	And reciprocal
CSXXIV	T60/1774 x SNK64	And reciprocal; also manual pollination SNK16 x SCA12

Source: Ngatchou (1981).

The seed gardens occupied 90 ha, including 15 ha planted in 1978-79. Production was 800,000 pods.

Today, Cameroon relies on four seed gardens: one maintained and managed by the Programme Semencier Cacao et Café (PSCC), in the south-west (Kumba); and three maintained and managed by the Société de Développement du Cacao (SODECAO), in the south (Ebolowa) and in the centre (Abong Mbang and Mengang) of the country. The Sustainable Tree Crops Programme (STCP) reported that no more than 100,000 seed pods are produced each year; however, the study only involved three of the sites: Nkoemvone (Ebolowa), Barombi (Kumba) and Mengang (Oliver Sounigo, personal communication, 2007).

According to Lanaud et al. (1987), the crosses produced in the earlier seed gardens included:

IMC67 x SNK109	IMC67 x SNK10	T79/501 x SNK64
$ScaF_1$ x SNK16	T60/1774 x SNK16	

Asare et al. (2010) summarize the clones in the various plantings below in Table A3.4:

Table A3.4 Bi-parental crosses produced in earlier seed gardens in Cameroon.

Location	Year established	Nominal (ha)	Effective (ha)	Clones
Nkoemvone	1960	103.0	9*	ICS40, ICS46, ICS95, IMC67, Sca6, Sca12, SNK13, SNK10, SNK16, SNK64, SNK109, T79/467, T79/501, UPA134, UPA143
Mengang	1981	34.6	10*	ICS40, Sca12, SNK13, SNK16, SNK413, T79/467, T79/501, UPA13, UPA134
Marombi Kang	1981	12.0		SNK13, SNK16, SNK64, SNK413, T79/501, UPA143
Abong Mbang			*5**	

Key: *Estimates of effective areas, as well as updated lists of clones, provided by Olivier Sounigo (personal communication, 2012)

At Mengang, SNK13 x T79/467, UPA13 x ICS40 and SNK16 x Sca12 are listed as biparental crosses. Asare et al. (2010) reported that new seed gardens are being planted at Kumba (10 ha), Mamfe (Obang) (10 ha) and Menchum (Modele) (5 ha). The clones in newly planted seed gardens are: BBK109*, BBK726, BBK1016*, Gu144c, Gu225v, ICS84*, IMC60, IMC67, Pa7, Pa70, Pa107, Pa150, Pound7, Sca6, Sca12, Sca24, SNK15*, SNK64*, SNK413*, SNK608*, SNK620*, SNK630*, T60/887, T79/501, TIKO32*, UPA134, UPA143, and UPA337, where * refers to those clones used only as male parents.

Manual pollination for commercial seed production was introduced in 2005 (Efombagn *et al*., 2009). However, the seed production plots are designed to limit the risk of self-fertilization under natural pollination (Sounigo and Efombagn, 2012). About 185,000 seed pods are produced annually from the 24 ha of effective seed gardens (Sounigo, personal communication, 2012), equivalent to a yield of about 350 kg dry beans/ha.

CLONES

A programme to produce and distribute up to 650,000 rooted cuttings of nine clones selected at Nkoemvone (SNK clones) was described by Braudeau (1958) and by Liabeuf (1960). In 1967, Liabeuf (1969) reported annual production and distribution of 500,000 cuttings of 35 clones. Use of clones was phased out when seed gardens began to produce bi-parental crosses, because the seedling material was thought to be higher yielding, as well as cheaper to produce (Ngatchou, 1981).

FARMERS' PLANTINGS

Efombagn et al. (2006) reported results from a survey of 193 leaf samples taken from smallholder farms in the southern, eastern and central provinces. No pure West African Amelonado or Trinitario types were recognized in the central and southern provinces. However, some trees were thought to be 'near Amelonado' (because they were relatively homozygous, rather than having the molecular profile corresponding to the central type recognized by Aikpokpodion (2005) or near to the early SNK clonal Trinitario selections. Some trees were close to the SNK-600 series of clones, selections from Trinitario x Upper Amazon crosses, and were assumed to have originated in seed gardens, although about half of these arose from self-fertilization, in line with the findings in Lanaud et al. (1987). In another study (Efombagn et al. 2009), 25% of trees were related to the 'Amelonado' type, 21% were bi-parental crosses from seed gardens, 25% were selfings from seed gardens, and 28% were from farm seed.

A3.3 Costa Rica

Production: 700 t. Area: 4,453 ha

Costa Rica has been supplying cacao seeds to its own farmers and to other countries, mostly in the Caribbean and the Americas, but also including Malaysia, at least since the late 1960s. According to Wilbert Phillips-Mora's presentation at the Bioversity cacao review workshop held in May 2013, in London, the crosses have included:

Catongo x	Pound7, Pound12
CC267 x	Catongo
IMC67 x	Sca6, UF613, UF654
Pound12 x	Catongo, Sca12, UF12, UF667
SPa9 x	UF613
UF12 x	IMC67, Pound7
UF29 x	Catongo, IMC67, UF668
UF296 x	CC9, CC18
UF613 x	IMC67, Pound7, Pound12, SPa9, UF29
UF654 x	Pound7
UF667 x	IMC67, UF29
UF668 x	IMC67, Pound7, Pound12
UF676 x	IMC67
UF677 x	IMC67, Pound7, Pound12, Sca6

Source: Wilbert Phillips-Mora, Presentation titled: "Cacao propagation practices and distribution of planting material in Central America", Bioversity workshop, London, May 2013.

About 26 million seeds were distributed to 19 countries between 1976 and 1989. About half of the seeds were used in Costa Rica and about a quarter went to Panama, with Guatemala, Honduras and Nicaragua all receiving over half a million seeds. Over 300,000 seeds were sent to Malaysia.

A3.4 Côte d'Ivoire

Production: 1,511,000 t. Area: 2,150,000 ha

Cacao varieties in Côte d'Ivoire

European settlers first planted cacao in Côte d'Ivoire, in small areas, in 1895. The origin of the planting materials is not known. Ten years later, a Fanti, William Gaume, imported pods from Ghana (Burlé, 1962). Besse (1964) reported that when selection work began in 1958, the bulk of the cacao was of the West African Amelonado type with some Trinitarios in the vicinity of Bingerville (near to Abidjan) and

Upper Amazon derivatives on the border with Ghana. Capot and Besse (1962) described the arrival in Abengourou of lorry loads of mixed Amazon pods from Ghana in 1957.

Formal transfer of Upper Amazon material from Ghana to Côte d'Ivoire occurred in 1954 (Capot and Besse 1962), including seed of at least ten crosses between trees of Upper Amazon origin in Posnette's (1951) introduction to Ghana[6]; Capot and Besse mention seven F_3 hybrids and two series of F_3 progeny - it appears that the latter refer to inter-crosses within T16 = IMC24 open pollinated and T17 = IMC53 open pollinated. More than 3,000 trees of Upper Amazon origin were growing in Divo in 1957. This material became the foundation of cacao breeding in Côte d'Ivoire and subsequently elsewhere in Francophone West Africa. The development of the first bi-parental crosses in Côte d'Ivoire was described by Besse (1977).

Seed production in Côte d'Ivoire and crosses produced

Besse (1964) reported that the first biclonal seed garden was established in 1962, with two females and two males, with a ratio of eight female trees to one male tree (Besse 1969). Distribution of seed pods began in 1975 (N'Goran et al. 2000).

The parents of 12 bi-parental crosses were planted in biclonal seed gardens between 1967 and 1971 according to Bastide and Sounigo (1993); or between 1967 and 1974, according to Besse (1977). Besse reported that the crosses included:

UPA402 x UF676*	UPA405 x C412
UPA409 x C1*	UPA413 x C1
UPA419 x C1**	UPA603 x UF667*
UPA608 x C412	UPA701 x C10
UPA701 x UF676	UPA710 x C5
T79/416 x E1 (correctly E1:J9/2/70)	T85/799 x S84 (correctly S84:E10/4/90) (withdrawn)
UPA409 x IFC1 (withdrawn)	T79/416 x E1:J9/2/70 (tested and rejected in Ghana)*
UPA400 series = T87/1329 x T87/1311	C1 (also known as IFC1), an Ivorian Amelonado selection
UPA600 series = T79/378 x T72/1433	C5 (also known as IFC5), of Cameroon origin
UPA700 series = T87/78 x T87/1309	C10 (also known as IFC10)

* 8:1 and ** 5:1 ratio of Upper Amazon to Trinitario (Lanaud et al. 1987).

Besse (1977) reported that in 1975 there were 80 ha of seed gardens in Divo, with a further 20 ha divided between Abengourou, San Pedro and Zagné. 750,000 pods were harvested and distributed to farmers in 1974. UPA620 and ICS95 were planted in one seed garden in Divo in 1967, and UPA620 and UF667 in another (Snoeck 1977). There are said to be six hectares of 'new' seed gardens divided between Divo (1 ha), Abengourou (1 ha), Soubré (2 ha) and Zagné (2 ha). According to Tahi (2012) there were 91.6 ha of seed gardens in Côte d'Ivoire in 2012 (49.6 ha et al. in Divo; 20 ha in Abengorou; 13 ha in Soubré; and 9 ha in Zro-Troya). All seed pods are produced by manual pollination of freshly opened flowers, sufficient to plant 25,000 ha in 2011-12.

Farmers' plantings

In Côte d'Ivoire, extension service assisted farmers in creating nurseries, and supplied 45 seed pods from seed gardens per planned hectare of cacao, and later five further pods to raise replacement plants (Niamke 1982). It is understood that the seed gardens fell into disuse in the late 1980s. Freud,

6Note from Rob Lockwood: I was unable to find a reference to this but the parentage of the UPA material (Lockwood and Gyamfi 1979) shows that legitimate seeds were supplied from Tafo.

Petithuguenin and Richard (1999) reported on a 1994 survey of farmers' planting material: 25% Amelonado, 62% from 'selected' trees and 13% 'hybrid'. Variety had no effect on yield.

Between 1995 and 2002 use of on-farm seed became more common (Aguilar et al. 2003), with the proportion of farmers accessing improved hybrids falling from 7.5% in 1994 to 4.5% in 2005. Results from a 2001-2003 survey of planting material on 280 farms were reported by Pokou et al. *(*2009). It was estimated that less than 10% were planted with Amelonado, much of the remainder being Upper Amazon derivatives. 71% of farms were established with seed taken from the same or a neighbouring farm; 23% of farmers said that they had received seed from extension services. Many farms were established at 2,000 or more trees/ha, a practice that has implications for seed garden capacity.

A3.5 Dominican Republic

Production: 54,000 t. Area: 183,000 ha.

Van Hall (1932) reported that the most common cacao variety in Santo Domingo was an Amelonado type (referring to the pod shape) originating in Trinidad, with yellow pods more common than red, and that it was being replaced by types of superior quality. Forastero hybrids of finer quality had been imported from Venezuela together with seeds from Ecuador. Bartley (2005) provides greater detail, saying that a 'Créole' from Martinique has become the prominent type in the Dominican cacao population.

Between 1968 and 1974, six triple hybrids were widely distributed in the Dominican Republic (Batista 1982) by controlled manual pollination of a local selection, SB, onto six bi-parental crosses:

IMC67 x TSH565	
IMC67 x TSH644	TSA644 = (Sca6 x IMC67), if 644 is correct
IMC67 x Sca6	
TSA644 x IMC67	
IMC67 x Pa218	
IMC67 x TSA655	

A3.6 Ecuador

Production: 161,000 t. Area: 360,025 ha.

Ecuador has a long history of cacao cultivation and cacao populations that Bartley (2005) considered to be of great interest. The first cultivated population on the Pacific coast was a single variety with a small genetic range, known as 'Nacional'; fermented and dried cacao beans of this variety were exported from the Arriba region, which gave its name to the characteristic cacao type. Posnette (personal communication, 1985) considered that the best available description of this variety was Pound's (1945), until Bartley published his seminal work in 2005. Lerceteau et al. (1997) considered that Nacional is closer to Forastero than to Criollo, but according to Delgado et al. (2003) it is not a Forastero sensu Sca-6. While Loor et al. (2009) were able to define the Nacional type using molecular data, they were unable to trace its ancestry.

In the late nineteenth and early twentieth century, cacao was introduced to the Pacific coastal region from several sources, certainly Venezuela and Colombia and possibly Trinidad and Mexico too (Bartley 2005). Bartley described how this material may have hybridized with the Nacional type, and he detailed the search for 'Refractario' trees that survived the initial epidemic of frosty pod rot (*Moniliophthora roreri*) and witches' broom disease (*Marasmius perniciosa*) in 1916 and 1919 respectively. Pound (1938) gave a full account of this material. Zhang et al. (2008), using molecular methods, showed that the Refractarios are separate from the Upper Amazon Forasteros, Trinitarios, Criollos and French Guiana material, and intermingle with known Nacional hybrids, confirming Bartley's hypothesis. It is thought that the Refractarios were derived from multiple parents that appear to have a close genetic relationship with each other.

Farias (1958) described seed gardens that were planted at Hacienda Clementina. One-hectare blocks were planted with Sca6 x ICS1, Sca6 x ICS67 (?IMC67), Sca12 x ICS6 and Sca12 x S62 (it is unclear what clone this is), with alternate rows of the two parental clones; seeds were to be produced by natural cross-pollination, relying on the self-incompatibility of the Scavina clones. The blocks were separated from each other by at least 100m.

Agama et al. (2006) studied smallholder cacao farming in three areas of Ecuador's Pacific coast. Most of the cacao in the farms comes from seed collected in the farmers' farms in the north, commercial nurseries, and the Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP) in Llanuras and Piedemonte. In the Northern area, a large part of the traditional farms show Criollo-like traits, whereas in the other two areas the Nacional and, to a lesser extent, Trinitario types predominate in the traditional farms. Young farms are still mainly planted with seedlings in the north, whereas clones have become increasingly more important in the other two areas. The most important clone in Ecuador is CCN51, selected by Homer U. Castro out of (IMC67 x ICS95) x O1, where O1 came from Valle de los Canelos in eastern Ecuador (Stern 2011). Farmers often use the best trees ('Super Trees') as clones.

A3.7 Ghana

Production: 1,025,000 t. Area: 1,625,000 ha

Cacao varieties in Ghana

The history of cacao introductions to Ghana is well documented (Lockwood and Gyamfi 1979). Although a diversity of material was introduced at the beginning of the twentieth century, primarily to the agricultural station at Aburi (now the Botanic Gardens), the Lower Amazonian Forastero that is known as 'West African Amelonado' ('Amelonado') became the dominant cultivar. Posnette (personal communication, 1969) estimated that when he arrived in the country in 1937, 95% of the farmers' trees were of the Amelonado type.

Amelonado was an excellent variety for the agricultural system that prevailed in the early twentieth century. High density planting of seeds in newly thinned forest quickly dominated sites; trees yielded well with minimal intervention by farmers (see for example Patterson, 1927; Beckett, 1945) and provided excellent financial returns on patient sweat equity. However, as conditions changed due to increasing loss of the forest cover, exhaustion of soil fertility and the advance of cocoa swollen shoot virus (CSSV), the following shortcomings of Amelonado became apparent:

- Susceptibility to infection with CSSV and sensitivity to infection from severe strains
- Difficulty to establish under sub-optimal conditions, such as a replant
- Relatively slow to come into bearing.

The vigour and precocity of the examples of Pound's (1938) Upper Amazon material in Posnette's 1944 introduction of cacao to Ghana (Posnette 1951) prompted a 1948 commission of enquiry into the civil disturbances arising from CSSV control measures, to recommend the rapid multiplication of the Upper Amazon material pending its use in the development of superior varieties (Anon. 1948). The development and release of 'mixed Amazons' was described by Rogers (1955) and by Hammond (1955, 1958). 6,650,000 pods and 6,050,000 seedlings were distributed up to March 1961.

The development of what became known as the 'CRIG Series II hybrids' was described by Glendinning (1963, 1967) and by Lockwood (1976), while Glendinning and Edwards (1961) provide details on the seed gardens that produced the hybrids in bulk. In 1973, there was an estimated 192 ha of productive seed gardens in Ghana (Lockwood 1974). A review (Anon. 1970) of the performance of the Series II hybrids in trials prompted the replacement of all the locally selected Trinitario parents with Amelonado, as the latter crosses were higher yielding, had a lower incidence of black pod disease, and were easier to manage. This change was possible following the introduction of mass manual pollination for seed production.

During the 1970s, a series of pollen parents were evaluated as substitutes for the Amelonado pollen parents of the four Upper Amazon clones in existing seed gardens, which would maintain the agronomic performance of the crosses and their sensory quality, while conferring resistance to the spread of CSSV (reviewed by Adomako et al. 1999a). The candidate pollen parents were pre-multiplied and planted at three sites. A recommendation to use a suite of pollen parents, which was made in 1985 and updated in 1998 (Adomako and Allen 2001), has not been implemented because the pollen parents remain to be proliferated.

Systematic evaluation of candidate female parents (Adomako et al. 1999b) led to the choice of Pa7, Pa150, Pound7 and T60/887 as the female parents for new seed gardens. Prospective pollen parents for these clones are under evaluation in the Mabang Megakarya Selection Programme (Nsiah et al. 2009).

Seed production in Ghana and crosses produced

Nine clones are used as female parents in Ghana, mostly planted in monoclone blocks. Table A3.5 provides estimates for the composition of seed gardens according to each clone, including immature plantings.

Table A3.5 Composition of seed gardens in Ghana.

Clone	Seed garden code	Area (ha) in 2011
Current recommendation		
Pa7	C85	57
Pa150		5
Pound7		5
T60/887	C42	128
Previous choices		
T63/967	C75	39
T63/971	C74	59
T79/467	C69	9
T79/501	C67	7
T85/799	C77	49
Mixed clones		16
Others		2
Total area		**378**

Source: Ghana Cocoa Board - Seed Production Unit, 2011.

Seed garden expansion is limited by the availability of suitable land with secure title. T85/799 (C77) is used as a universal pollen donor for the other eight clones. When used as a female parent it is crossed with T79/501 (C67). By 2010-11 seed pod production had reached 6.9 million (see Figure A3.1).

Farmers' plantings

Based on a 1994 survey, Freud, Petithuguenin and Richard (1999) reported farmers' planting material as being 14.1% Amelonado, 62.1% from selected trees (12.1% Amazon, 47.9% progenies from Amazon trees, 11.6% progenies from hybrid trees) and 14.4% hybrids. The use of Amelonado possibly had a negative effect on yield. By contrast, Edwin and Masters (2005) reported a 42% gain in yield on farm when farmers used the most recent varieties.

Ghana's CSSV Disease Control Unit (Anon. 2012) identifies the varieties encountered during its routine surveys for CSSV infection. The most recent data (Figure A3.2) suggest that only 14% of the cacao was

Amelonado in the area surveyed, with roughly equal parts of hybrids and Amazons making up 70%. Opoku et al. (2006) took leaf samples from 218 Ghanaian cacao farms in 2004. Analysis with micro-satellite markers showed that 26% of the farms had some Amelonado trees, 48% of the farms had some hybrids, and 69% of the farms had some Amazons (see Figure A3.2). A higher proportion of Amazon material in the Western Region was confirmed by surveys of the farmers, which showed that Western Region farmers were more likely to obtain seed from neighbours' farms, due to lack of nearby seed gardens.

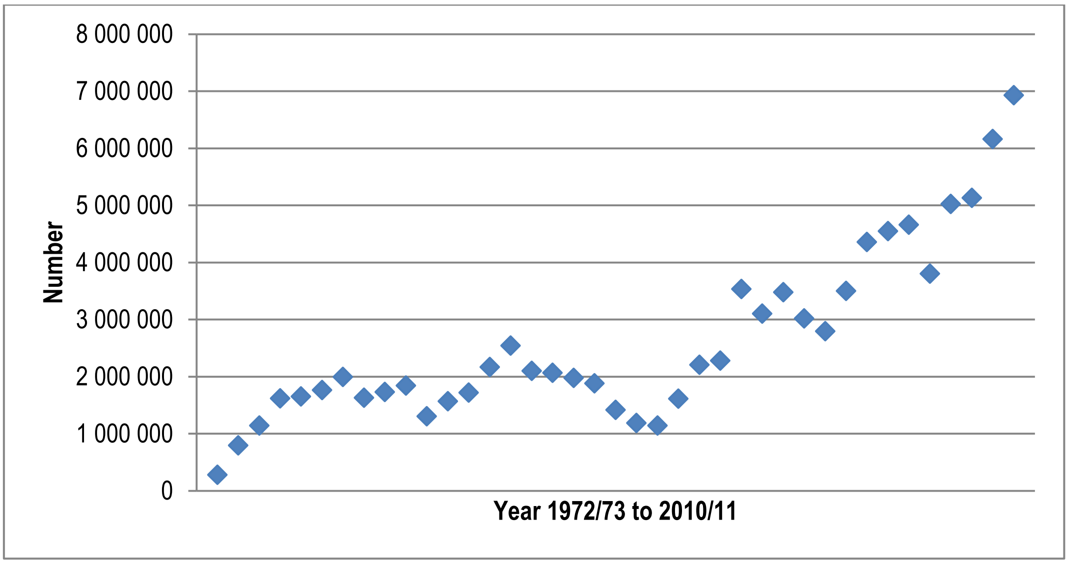

Figure A3.1 Number of seed pods produced in Ghana, 1972-73 to 2010-11.

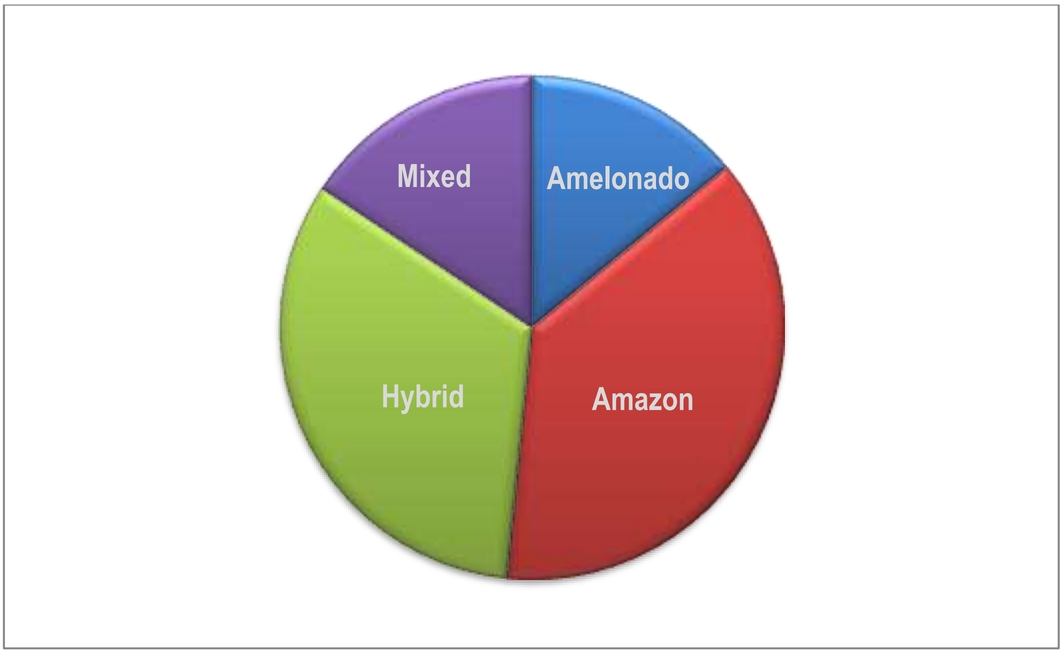

Figure A3.2 Genetic origin of Ghana's stock of cacao trees - Ghana 2006-2011 CSSV survey (1,232,973 ha).

A3.8 India

Production: 12,000 t. Area: 3,900 ha

Seven bi-parental crosses are produced in a seed garden at Kerala State University. The Upper Amazon parents originate either directly from the Intermediate Cocoa Quarantine Facility at Reading University or are derived from them. The pollen parents are either obtained directly from farms or are derivatives of on-farm selections, coded CCRP. A new seed garden is being developed at Tamil Nadu University. Between six and seven million seedlings are distributed each year.

Clones are not used in India.

A3.9 Indonesia

Production: 400,000 t. Area: 1,026,000 ha

Cacao varieties in Indonesia

Toxopeus and Giesberger (1983), Wood (1991) and Bartley (2005) traced early cacao introductions to Indonesia. It is assumed that the first cacao cultivated in Indonesia descended from an early introduction, possibly a single plant of red-podded Criollo type, which was taken from Mexico to the Philippines. It appears that this introduction led to the variety known as 'Java red', 'Java cacao' or 'Java porcelaine', which was characteristically weak and low yielding. According to Van Hall (1930) the finished cacao was not of the same standard as 'Caracas cacao'.

Two plants were introduced from Venezuela in 1888 (Bartley 2005), one of which survived. Van Hall (1930) described how the plant proved to be a Forastero and not the expected high value Caracas type. When the first seeds grew out they proved to be hybrids of 'Java cacao'. The so-called 'Djati Roenggo' hybrid (named after the estate on which it was selected) was more vigorous than the Java cacao, much more variable, and with quality only slightly inferior to the old Criollo. Further selection work in this population and derivatives of it continued for several years on Djati Roenggo, Getas and other estates, leading to clones known by estate names. A high level of genotype diversity and heterozygosity, and moderate allelic richness was confirmed in Java Criollo cacao by Agung et al. (2009).

Seed gardens

A 1987 review of availability of cacao seeds in Indonesia (Lockwood 1987a, unpublished) provides information on the seed gardens at the following five sites: Indonesian Coffee and Cocoa Research Institute (ICCRI) at Jember, PT Hasfarm Products, PT London Sumatra, PTP-II and PTP-VI.

ICCRI

There were two seed gardens at the ICCRI site in Jember, each with four hectares:

- Seed garden 1 comprised single rows of ICS1, ICS6, ICS60, Sca6 and Sca12, with the ICS and Sca clones arranged in alternate rows. ICS6 was misidentified.
- Seed garden 2 comprised double rows of ICS6, ICS60 and GC7 (a selection out of Getas8; Getas is an estate in Java), alternating with Sca6 and Sca12. Again, ICS6 was misidentified.

The seed gardens were established by field budding of seedling rootstocks, some of which had contributed to the development of fruiting composite plants. All seeds were produced by natural pollination with seed pods harvested from all clones, including those that were known to be self-compatible. Annual production was said to be two million seeds.

PT Hasfarm Products

Seed production was conducted on a privately developed 5,500-hectare cacao estate (Pinang Mas) in the Samarinda district of East Kalimantan. The parental material came from Sabah. There were nine biclonal blocks, isolated by 50 m of land planted with leguminous cover crops.

Table A3.6 Crosses produced in 1986 on Pinang Mass Estate in Kalimantan, Indonesia.

Cross produced	Notes
Na-32 x Pa-35	Pa-35 is an unidentified Trinitario
Na-32 x UIT-1	UIT-1 is a Nicaraguan Criollo, possibly ICS-39, ICS-40 or ICS-60
Na-33 x UIT-1	
Sca-6 x UIT-1	
817-B x 354-A	817-B* is Pa-7 x Amelonado, 354-A is UIT-1 x Na-33
Pa-7 x 63-A	63-A is UIT-1 x Na-32
63-A x SCA-12	
369A x UIT1	369A is Pa7 x Na32
246A x 151B	246A is Pa35 x Na32

Legend: * The A and B clones were single tree selections in the aforementioned crosses.

PTP-II

In 1987, four seed gardens had been established, totalling 17 ha (Lockwood, 1987a). Single crosses were produced from alternating double row strips of the two parents. A nine-row link zone of the common parent was planted when one parent changed, with the pods discarded from the centre five rows of the link zone. A nine-row strip was planted at the end of each row, and the blocks were surrounded by 50 m of rubber.

Seed garden (1), 1979

Sca12 x TSH539	Sca12 x Pa150	Sca12 x IMC67		
Sca6 x Pa150		Sca6 x TSH858	Sca6 x TSH654	Sca6 x ICS60
Pa150 x Sca12	Pa150 x TSH908	Pa150 x TSH539	Pa150 x ICS60	Pa150 x Sca6
ICS60 x TSH908	ICS60 x TSH858	ICS60 x Pa150		
IMC67 x Sca12	IMC67 x TSH654			
TSH539 x Sca12	TSH539 x Pa150	TSH539 x ICS60		
TSH858 x ICS60	TSH858 x Sca6			
TSH654 x IMC67				
TSH908 x Sca6	TSH908 x ICS60	TSH908 x Pa150		
Sca12 was misidentified				

Seed garden (2), 1980

ICS60 x TSH539	
IMC67 x TSH858	IMC67 x TSH866
Pa150 x TSH654	Pa150 x TSH858
Sca12 x TSH654	Sca12 x TSH866

[including all seven reciprocal crosses].

Seed garden (3), 1984

Pa150 x Sca12	ICS60 x TSH908	Pa150 x TSH908

[and all three reciprocals].

Seed garden 4, 1984

TSH858 x ICS60	TSH858 x IMC67	TSH858 x Pa150

[and all three reciprocals].

Small seeds were discarded before the larger seeds were peeled, a practice that was thought to maintain germination power. Ten million seeds were despatched in 1986; this number was expected to rise to 20 million in 1987.

PTP-VI

The variety was 'semi-synthetic III', a development of the variety described by Toxopeus (1969a). The variety originated in 100,000 seeds received from the Harrisons and Crosfield seed garden at Bagan Datok, in western Malaysia:

Na32 x ICS60
Na32 x Pa35
Na32 x Amelonado
Na33 x Pa7
Na33 x Amelonado

and 200,000 seeds from Harrisons Malaysian Plantations Bhd Flemington Estate, taken from a mixed population of Nanay, Parinari, Scavina, ICS, GS, DR, Poerboyo (an estate in Java) and Getas clones (this appears to be the introduction mentioned by Iswanto et al. 1999, referring to a paper by Tri-Hutomo and Suhardjo).

The seeds were mixed, and 150,000 seedlings were planted in Adolina and Pabatu Estates in 1973. Two years later, 4,642 trees were selected for precocity and habit, which was reduced to 793 on the basis of yield. A re-selection among the 793 trees gave 131 trees. In 1977, a further 2,257 trees were selected in the original planting and eventually reduced to 70 trees. These and the 131 from the earlier selection were reduced to 105 on the basis of butter fat content, and planted in Piasu Hulu Estate in 1980 as 'Synthetic III' (Lockwood 1987b, Soepadiyo 1985).

Adolina Estate had 49 ha of budded plants of the Synthetic III parents, with each represented by a row of 5-10 buddings scattered through the area at random. The clones were highly variable, predominately of Nanay, Pa-7 and Amelonado parentage, with unexpectedly little influence of the Nicaraguan Criollo type considering that bean weight was a principal selection criterion. There were a few red-podded types derived from DR-1 (Lockwood 1987b).

Three 100-hectare seed gardens for producing bi-parental crosses were either planted or planned:

September 1985:	UF667 x IMC67; IMC85 x Pa81; TSH858 x IMC30; IMC30 x Pa30
November 1986:	UIT1 x BLC4; UIT1 x IMC10; UIT1 x Na33; UIT1 x Sca12; UIT1 x TSH858 and their reciprocals
1988:	UF667 and UIT1 x IMC30, Pa35 (a selfcompatible Trinitario) and Pa300 and their reciprocals

Source: Lockwood (1987b).

PTPP London Sumatra

Four one-hectare seed gardens were planted in 1984 to produce speculative crosses. Each garden included one Parinari clone (among Pa121, Pa300, Pa303 and Pa310); five Trinitarios (GC29 and K21 from Keravat, Papua New Guinea; KA2101 also from Keravat, mostly a green pod off-type; UF667 and UF713); and two local selections of unknown parentage (BL621 and BL629). The Parinari clones were planted in alternate rows throughout the seed gardens, separated by one of the other clones, with all seven of the latter used. Table A3.7 is an up-to-date compilation of seed gardens in Indonesia (Agung, personal communication, 2011).

Table A3.7 Cacao seed gardens in Indonesia (1990-2011).

Producer	Clone composition	Location
Pusat Penelitian Kopi dan Kakao Indonesia	ICS60, GC7, TSH858, Sca6, Sca12	Jember, East Java
Pusat Penelitian Kelapa Sawit	ICS60, TSH858, IMC67, Pa150, Sca12	North Sumatera
PT Perkebunan Nusantara II	TSH539, TSH654, TSH858, TSH908, TSH866, ISC60, Sca6, Sca12	North Sumatera
PT Perkebunan Nusantara IV	ICS60, TSH858, Pa35, UF667, Na32, Na33, IMC67	North Sumatera
PT Hasfarm Agro Niaga	Na32, Na33, Na34, Pa35, UIT1, UIT2, Sca6, Sca12, 63A, 354A, IMC67	Southeast Sulawesi
PT London Sumatera	Pa300, Pa121, Pa303, Pa310, GC29, UF667, UF713, BLC3, BLC4, BL621, BL693	North Sumatera
PT Perkebunan Nusantara VII	ICS60, IMC67, Pa150, TSH858, TSH908	Lampung
PT Perkebunan Nusantara IX	Amelonado	Central Java
PT PP Jember Indonesia	ICS60, ICS12, ICS13, DR1, Sca6, Sca12	Jember, East Java
PT Inang Sari	GC7, ICS60, Sca6, Sca12	West Sumatera
PT Perkebunan Nusantara XII	ICS60, ICS13, GC7, Sca6, Sca12	East Java
PT Glenmore	ICS13, ICS60, GC7, DR1, Sca6, Sca12	Banyuwangi, East Java

ICCRI is producing a new hybrid: the cross between TSH858 and Sul1.

Total seed garden production was estimated at 30m seed per year (Agung Susilo personal communication to Nick Cryer, June 2012).

Indonesia: on farm

Iswanto et al. (1999) described how the Trinitario clones derived from the 1888 introduction are grown only on the estates in Java. Smallholder farmers and private enterprises grew Upper Amazon hybrids, Trinitario hybrids, Amelonado progenies and Trinitario clones, especially BR25 selected in Sabah by Anselmi (unpublished) and PBC123 selected by Ang Boon Beng (Ang and Shepherd 1980).

Lambert et al. (2006) described how smallholder farmers returning to Sulawesi from Sabah, Malaysia, had brought with them pods of high yielding seedling material and budwood of superior clones, especially BR25, KKM22 (Sapiyah et al. 1987) and PBC123, the last of which proved to be particularly popular.

Currently, large numbers of plantlets obtained by somatic embryogenesis are being distributed from a tissue culture laboratory at Jember, reportedly several millions per year. The clones are Sul1, Sul2, ICCRI3, ICCRI4 and Sca6. There are government sponsored schemes to upgrade plantings by side-grafting with Sul1 and Sul2, with farmers preferring one of them, and other clones.

Clone recommendations for fine cocoa

No.	Clone name	Note
1.	DR1	Trinitario
2.	DR2	Trinitario
3.	DR38	Trinitario
4.	DRC16	Trinitario
5.	ICCRI01	Trinitario
6.	ICCRI02	Trinitario
7.	ICCRI05	Trinitario

Clone recommendations for mainstream cocoa

No.	Clone name	Note
1.	ICS60	Forastero
2.	TSH858	Trinitario
3.	UIT1	Trinitario
4.	GC7	Trinitario
5.	ICS13	Trinitario
6.	RCC70	Forastero
7.	RCC71	Forastero
8.	RCC72	Forastero
9.	RCC73	Forastero
10.	RCC74	Forastero
11.	RCC75	Forastero
12.	ICCRI03	Trinitario
13.	ICCRI04	Trinitario
14.	ICCRI07	Forastero
15.	Sca6	
16.	Sulawesi01	
17.	Sulawesi02	Forastero
18.	Sulawesi03	Forastero

Acknowledgements: the author thanks Nick Cryer for the clone recommendations.

A3.10 Malaysia

In Peninsular and East Malaysia (Borneo), both the public sector - in the form of the Federal Land Development Authority and the Sabah Department of Agriculture - and the private sector - including Harrisons' Malaysian Plantations Bhd (later Golden Hope Plantations Bhd), United Plantations Bhd (both in Peninsular Malaysia) and BAL Plantations Sdn Bhd (in Sabah) - produced and sold cacao seeds. The focus below is on Sabah as it was a particularly important area for cacao development, and more information was available to the author.

In Sabah, breeding trials were conducted by both Sabah Department of Agriculture and BAL Plantations Sdn Bhd (e.g. Chee 1976, Lim 1976). Sabah Department of Agriculture identified the following six crosses (Series I hybrids) for production in their first seed gardens at Quoin Hill Cocoa Research Station (QHCRS):

- UIT1 x Na33
- Pa7 x Na32
- Aml x Pa7
- Aml x Pa35
- UIT1 x Na32
- UIT1 x Na34

These were followed by representatives of a Second Series, also described by Chee, among which the crosses of Sca6 and Sca12 with UIT1 and UIT2 (UIT1 and UIT2 were unidentified, introduced Trinitarios, morphologically close to the Nicaraguan criollo types ICS39, ICS40 and ICS60) were produced in bulk in polyclonal seed gardens (Phua 1981). The Third Series (Phua 1982) was crosses involving IMC67, Pa76, Pa137, Pa138, Pa156, Pa173, Pound4, Sca6, Sca9 and 'Sca20', and again some were produced in bulk at QHCRS.

Edwards (1969) described a 30acre (12.1 ha) polyclonal seed garden at QHCRS comprising Na31, Na32, Pa7, 'Pa35' and UIT1 ('IMC60'). The aim was to produce Pa7 x Na32, Na32 x IMC60, IMC60 x Na32, Na32 x Pa35, Na32 x Pa35 and reciprocal and 'Pa35' x Na31 by natural pollination, these being some of the crosses that were approved in Ghana for distribution as second and third generation openpollinated seeds (F_2 and F_3 Amazons, assuming that the clones were correctly identified).

The early cacao trials at BAL Plantations Sdn Bhd were described by Lim (1976) and Anon (1982). Many of these trials were conducted in collaboration with Sabah Department of Agriculture. These trials led to the establishment of seed gardens in 1969, 1971/72, 1974/75 and 1986, as detailed in Tables A3.8 and A3.9.

Table A3.8 Cacao seed gardens at BAL Plantations Sdn Bhd: planting year, area and planting points.

Field	Planting material	Year planted/ budded	Area (ha)	Area planted (ha)	Spacing (m)	Density (per ha)	Total points	Guard trees
CA24	F_0 clonal hybrids	1969	0.44	0.36	3.05 triangle	1,236	380	16
CA29 plot 1	F_0 and F_1 selected clonal hybrids	1971/72	1.18	1.13	3.96x 1.83	1,380	1,564	1,334
CA29 plot 2	F_0 and F_1 selected clonal hybrids	1971/72	1.28	1.21	3.96x 1.83	1,380	1,663	
CA29 plot 3	F_0 and F_1 selected clonal hybrids	1971/72	2.9	2.76	3.96x 1.83	1,380	3,811	
CA36 plot 1	F_0 and F_1 selected clonal hybrids	1975/76	2.55	2.40	3.66x 1.83	1,493	3,593	1,068
CA36 plot 2	F_0 and F_1 selected clonal hybrids	1975/76	2.7	2.58	3.66x 1.83	1,493	3,859	
CA42 plot 1	F_0 and F_1 selected clonal	1980/81	3.5	3.33	3.66x 1.83	1,493	4,991	5,756

Field	Planting material	Year planted/ budded	Area (ha)	Area planted (ha)	Spacing (m)	Density (per ha)	Total points	Guard trees
	hybrids							
CA42 plot 2	F_0 and F_1 selected clonal hybrids	1980/81	3.51	3.31	3.66x 1.83	1,493	4,936	
CA42 plot 3	F_0 and F_1 selected clonal hybrids	1980/83	4.16	3.91	3.66x 1.83	1,493	5,838	
CA62 IMC67		1986	1.08			3,333	3,590	
Na32		1986	0.59			3,333	1,980	
Na33		1986	1.32			3,333	4,408	
Pa76		1986	0.88			3,333	2,944	
Pa138		1986	0.89			3,333	2,976	
Pa156		1986	1.32			3,333	4,408	
Pound4		1986	1.49			3,333	4,958	
UIT2		1986	0.54			3,333	1,800	
Amelonado		1986	0.15			3,333	486	
ICS95		1986	0.09			3,333	195	
Sca9		1986	0.08			3,333	0	
Sca12		1986	0.19			3,333	250	
UIT1		1986	0.27			3,333	365	
Total			22.22	20.99			58,995	

Source: Rob Lockwood, unpublished data.

Table A3.9 Cacao seed gardens at BAL Plantations Sdn Bhd: clones and numbers of trees.

Clone	Parentage	Field					Total
		CA24	*CA29*	*CA36*	*CA42*	*CA62*	
ICS95						195	195
IMC67					1008	3,590	4,598
Na32		121	776	1,247	600	1,980	4,724
Na33		25	80	156	300	4,408	4,969
Na34			68	156	300		524
Pa7		121	507	638	1,200		2,466
Pa76					784	2,944	3,728
Pa138					832	2,976	3,808
Pa156					672	4,408	5,080
‘Pa’35			412	208			620
Pound4					672	4,958	5,630
Sca6			715	652	337		1,704
Sca9					179		179
Sca12			813	1,112	3,424	250	5,599

Clone	Parentage	Field					Total
		CA24	*CA29*	*CA36*	*CA42*	*CA62*	
'Sca'20					112		112
UIT1		90	1,635	1,044	4,526	365	7,660
UIT2			52		204	1,800	2,056
Amelonado		5				486	491
63A	UIT1 x Na32		299	970	200		1,469
151B	Aml x UIT2		416				416
246A	'Pa'35 x Na32		423	120	256		799
354A	UIT1 x Na33		375	827			1,202
369A	Pa7 x Na32		467	208			675
817B	Pa7 x Aml		114				114
Total		362	7,152	7,338	15,606	28,360	58,818

Source: Rob Lockwood, unpublished data.

BAL's seed sales from 1981-1986 are summarized in Table A3.10. There were very few sales subsequently (Source: Rob Lockwood unpublished information).

Table A3.10 BAL Plantations Sdn Bhd cacao seed sales, 1981-1986.

Year	Number of seeds sold
1981	27,150,980
1982	24,007,518
1983	25,448,831
1984	20,567,850
1985	18,809,066
1986	8,449,135

Source: Rob Lockwood unpublished information.

The author has been unable to obtain any information on the seed gardens that were developed in Peninsular Malaysia. An indication of the scale of the operations is given by Anon. (1981) (Table A3.11).

Table A3.11 Prang Besar Research Station cacao seed sales, 1978-1980.

Sector	1978	1979	1980	Total
Department of Agriculture/government agency	2,226,000	2,667,000	1,500,000	6,435,000
Smallholder farmers	1,700,000	2,500,000	1,320,000	5,520,000
Estates	2,300,000	4,200,000	7,050,000	13,555,000
Total	6,268,000	9,367,000	9,870,000	25,505,000

Source: Anon. (1981).

A3.11 Nicaragua

Production: 3,400 t. Area: 5,939 ha

According to a presentation given by Wilbert Phillips-Mora at the Bioversity workshop held in London, in May 2013, seed production commenced in Nicaragua in 1982 using the following 28 clones, which were supplied by the Centro Agronómico Tropical de Investigación y Enseñanza, Costa Rica (CATIE):

Catongo	
EET	48, 62, 64, 95, 96, 162, 399, 400
ICS	6, 8
IMC	67
Pound	7, 12
Sca	6, 12
SPa	9
UF	12, 29, 221, 296, 613, 650, 654, 667, 668, 676, 677

Source: Wilbert Phillips-Mora 2013.

There were 40 bi-parental crosses.

A3.12 Nigeria

Production: 240,000 t. Area: 1,344,500 ha

Cacao varieties in Nigeria

The earliest known cultivation of cacao in Nigeria was at Agege in 1880 (Webster 1962), with the seed possibly obtained from the Basel Missionaries at Akropong in Ghana, who sourced material from Cape Palmas in Liberia - although the origin of the Cape Palmas material remains unknown (Anon.1951). However, Toxopeus (1993) quotes Webster as stating that the material had originated in Fernando Po. According to Ayorinde (1966) there were other cacao introductions from Fernando Po.

A red-podded cacao type, thought to be a Trinitario from Trinidad (Aikpokpodion 2005), was introduced to the Lagos Botanic Gardens, which were founded in 1888. Presumed descendants of these trees survived at Agege into the 1960s (Toxopeus 1993).

West (1938 p.371) commented ‘Nigerian cacao appears to be remarkably uniform but outstanding trees exist whose progeny must be tested’. The initial breeding work in Nigeria (for a summary, see West 1945) led to the establishment in 1948 of several monoclone blocks of N-38 (also known as T-38) in western Nigeria, for the production of clonal seed. In 1956, bi-clonal seed gardens were planted to produce N-38 x Amelonado. However, this material was overtaken by bi-parental crosses involving Upper Amazon material, and it is unclear what if anything these early seed gardens contributed to Nigeria’s cocoa industry (Toxopeus 1969b).

The first important variety recommendations in Nigeria were broadly the same as those in Ghana, as both countries were members of the West African Cocoa Research Institute (WACRI): initially mixed Upper Amazons, sometimes referred to in Nigeria as a ‘semi-synthetic variety', (Toxopeus 1969c p.102), and the WACRI Series II hybrids. The yield advantage of mixed Amazons over Amelonado in western Nigeria was estimated at 147% by Abidogun (1982).

The development of seed gardens to produce the WACRI Series II hybrids started later in Nigeria than in Ghana because of the time it took to transfer the parental material and build up stocks. The most recent variety recommendation from Nigeria comprises four Series I and eight Series II crosses (Aikpokpodion et al. 2010).

Series I	Series II	
T65/7 x N38	T65/7 x T22/38	T82/27 x T12/11
T101/15 x N38	T12/11 x N38	T86/2 x T53/8
Pound7 x Pa150	T65/7 x T57/22	T86/2 x T57/22
T53/5 x N38	T53/5 x T12/11	T65/7 x T101/15

Seed production in Nigeria and crosses produced

Seed gardens to produce WACRI Series II hybrids and later crosses generated in the CRIN breeding programme were established at 14 locations (see Table A3.12).

Table A3.12. Seed gardens established in Nigeria.

Location	Year	Nominal (ha)	Effective (ha)	Material planted
Ondo State				
Ile-Oluji	1968-78	12.03	8.03	Not stated
Ibule	1962	33.40	11.00	C69 (T79/467) x C20 (S72:J11/4/49) = WEA2
Ibule (CRIN)	1977	4.00	2.10	Trinitario, CRIN Series I and II
Alade	1956	4.50	0.50	IT CRIN Elite
Idanre	1966	28.00	16.50	Na32 x Pa35 (latter = unidentified Trinitario)
Owena				C75 (T63/967) x C18 (P4/9:J11/4/5)*
				C75 (T63/967) x C25 (E1:C4/3/291) = WBE2
	1976	4.30	2.00	C75 (T63/967) x C14 (W41) = WBE6/WEB3, C77 (T85/799) x C27 (S84:E10/4/90) = WEA3
Owena (CRIN)	1962	20.23	9.20	C75 (T63/967) x C25 (E1:C4/3/291) = WBE2 C74 (T63/971) x C24 (K5:C4/4/353)*
Osun				
EWS Ilesa	1961	5.20		C77 (T85/799) x C27 (S84:E10/4/90) = WEA3
Ilerin Ilesa	1962	6.00		C74 (T63/971) x C18 (P4/9:J11/4/5) = WAE5
Esa-Oke	1979	5.00		C77 (T85/799) x C27 (S84:E10/4/90) = WEA3
Oyo				
CRIN HQ IdiAyunre	1964 (onwards)	255.49 (total)	229.98	F_1 and F_2 Amazon CRIN Elite Planted in CFC Plots: T65 (/7(presumed, not stated)) x T10/15 T86/2 x T22/28 T86/2 x T9/15 Pound7 x Pa150 Pound7 x T60/887 T9/10, T10/15, T22/28, T65/, T86/2 are CRIN selections from Posnette's (1951) 1944 material from Trinidad
South South				
Edo	1967-75	31.50		Trinitario, F_3 Amazon
Cross River				
Ajassor	1975	4.40		F_3 Amazon, CRIN Elite

Location	Year	Nominal (ha)	Effective (ha)	Material planted
South-East				
Abia				
Ibeku	1971-86	28.50		F_3 Amazon (not seed production)

Legend: * These are not WACRI Series II hybrids. Source: Asare et al. (2010).

As of 2008, two hectares of seed gardens have been planted in each of 14 cacao-producing states, to reproduce the following eight newly recommended crosses (Adewale and Aikpokpodion 2012):

T65/7 x N38
T101/15 x N38
Pound7 x Pa150
T65/7 x T57/22
T82/27 x T12/11
Pa150 x T60/887
T82/27 x T16/17
T65/7 x T9/15

Adewale and Aikpokpodion also reported the establishment of four community seed gardens of a quarter of a hectare each. These gardens comprise 50 female and 10 male plants, and produce the following four crosses:

Pound7 x Pa150
C77 (T85/799) x C67 (T79/501)
T65/7 x N38
T101/15 x N38

Target seed pod production in 2012 was 2.6 million.

Farmers' plantings

A small-scale farm survey by Esan et al. (1999) suggested that by the mid 1990s Amelonado had become a minor variety in Nigerian cacao cultivation, providing about 10% of the tree stocks. Based on a farm survey, Aikpokpodion et al. (2006, 2009) found that about 10% of the material was Amelonado, 10% Trinitario, 8% Amelonado x Trinitario hybrids, 48% Upper Amazon x Amelonado hybrids, and the balance Amelonado-Trinitario-Upper Amazon hybrids. Given the shortage of improved seed in Nigeria, this is consistent with the observation made by Aikpokpodion et al. (2005) that when sourcing seed from their own trees or trees of neighbouring farms, the farmers prefer Upper Amazon derivatives.

A3.13 Papua New Guinea

Production: 47,000 t. Area: 128,000 ha

Cacao was introduced to Papua New Guinea from Samoa (by way of Java and Ceylon) before the First World War. There may have been a direct importation from Venezuela in about 1907 (Henderson 1951). The next introductions were from Java in 1932 (Bridgland 1960), with inter-Amazon crosses being imported from Trinidad in 1960. From about 1980, clonal material was introduced from intermediate quarantine stations. The country produces a premium cocoa.

Seed Garden (1), covering an area of 9.6 ha, with 4m square spacing, was planted at Keravat in 1979 with the following six clones (Byrne 1984):

KEE2 (660 trees; KEE2 = Pa15 x Pa127)	KA2101 (2,031 trees)
KEE5 (693 trees; KEE5 = Na406 x Na222)	KA5201 (1,032 trees)
KEE52 (660 trees; KEE52 = IMC67 x IMC46)	K20 (996 trees)

The KEE clones were planted in separate portions of the seed garden, in four- or five-row strips separated by a row of a Trinitario parent. Internal and external guarding was provided by ten rows of Trinitarios. The seed garden relied on self-incompatibility and natural pollination, and was expected to yield enough seed to plant two million seedlings a year.

A 20-ha seed garden of the same design, also expected to produce 20 million seedlings a year, was planned for planting on the nearby Tavilo Plantation, with further seed gardens planned for North Solomons (Bougaineville) and New Ireland Provinces. The six parental clones were planted at Lejo Cocoa Station (near Popondetta in Oro Province), which was acquired by Higaturu Oil Palms Pty Ltd and used for seed production (by manual pollination) in the late 1980s and early 1990s (Rob Lockwood, unpublished information).

Seed Garden (2) was planted in 1985 with the following five KEE clones: KEE5 = Na-406 x Na-222, also in Seed Garden (1); KEE12 = Na-33 x Na-34; KEE22 = Pa-100 x Pa-20; KEE23 = Pa-207 x Pa-138; and KEE 43= Pound-7c x Pound-26a; and three Trinitarios (KA2-101 - as in Seed Garden (1), K-82 and KA2-106). Some of these crosses were produced at Lejo Cocoa Station (Rob Lockwood, unpublished information).

The most recent seed gardens were planted in Rabaul (10 ha) and Madang (8 ha), in 2006 and 2009 respectively. The seedbearing parents are KEE5, KEE12, KEE23, KEE42 (Pound7 x Pound21), KEE43 and KEE47 (Pa128 x Pa149); the pollinators are the Trinitarios K82 and KA2106, in other words a modification of Seed Garden (2). These two gardens are currently producing three million seeds/year by manual pollination (priced at 0.3 Kina each one).

Eight clones have been released for commercial use: four vigorous and four less vigorous; all were selected out of bi-parental crosses involving Upper Amazon parents.

A3.14 The Philippines

A 20-ha seed garden (including discard areas) was established in about 1980 by Cocoa Investors Inc., on Christiansen Estate, to produce bi-parental crosses that had been developed at the former BAL Plantations Sdn Bhd in Sabah. See Table A3.13 below.

Table A3.13 Bi-parental crosses developed at the former BAL Plantations Sdn Bhd, Sabah.

Female		Male
UIT2	X	817B
UIT2	X	Pa7
UIT2	X	‘Pa35’
UIT2	X	Sca6
Sca6	X	151B
Sca6	x	354A
Sca6	x	Na32
Na32	x	‘Pa’35
‘Pa’35	x	Sca12
Sca12	x	369A
Sca12	x	UIT1
UIT1	x	Sca12
63A	x	Sca12
63A	x	Sca6
UIT1	x	Sca6
UIT1	x	369A
UIT1	x	Na32
UIT1	x	Na33
UIT1	x	Na34
UIT1	x	63A
UIT1	x	246A

Source: Lockwood, Sergeant and Lim (1985).

63A = UIT1 x Na32	151B = AmI x UIT2	246A = ‘Pa35’ x Na32
354A = UIT1 x Na32	369A = Pa7 x Na32	817B = Pa7 x AmI

The seed garden was laid out as six row strips. It was designed to produce seed by natural pollination.

A3.15 Peru

Production: 54,000 t. Area: 77,192 ha

Evans et al. (1998) reported that hybrids produced by controlled pollination are available at certain centres. A mixture of about five hybrids is recommended, and sometimes even compulsory, to ensure good cross-fertilization. No detailed information has been obtained.

A3.16 Sierra Leone

Production: 12,000 t. Area: 39,000 ha

A seed garden was planted in 1977 at Pendembu in eastern Sierra Leone. The overall area was said to be 6.47 ha, comprising four equal-sized blocks of bi-parental crosses.

Table A3.14 Composition of seed garden at Pendembu, Sierra Leone.

Clone A	Clone B	Ghanaian codes	Comment
C67 (T79/501)	C77 (T85/799)	WE6, Series IIF	Not an approved hybrid
C68 (T79/416)	C26 (E1:C4/3/270)	Not tested in Ghana	Not an approved hybrid
C74 (T63/971)	C20 (S72:J11/4/49)	Not tested in Ghana	Not an approved hybrid
C75 (T63/967)	C25 (E1:C4/3/291)	WBE2, Series IIB	Approved hybrid

The seed garden was laid out as alternate rows of the two clones, relying on self-compatibility and natural cross pollination for the production of hybrid seeds. The spacing was 2.44m within the rows and 3.05m between the rows. The possibility that perimeter guard rows were planted around each block requires further investigation. In January 2013, less than half the cacao trees remained in the seed garden. A budwood garden that was planted at Kupwabu in 1971 was used for seed production by manual pollination up until the war that started in 1992. Very few of the trees have survived.

Clones are not used in Sierra Leone. (Rob Lockwood, unpublished information).

A3.17 Togo

Production: 142,000 t. Area: 133,500 ha

Cacao varieties in Togo

Agbodjan (1985) stated that in 1984 Togo's cacao was an Amelonado/Trinitario mixture, but mentioned only Amelonado in the discussion of his paper (pp.22-23 of the conference proceedings). This suggests that Amelonado was the predominant variety at that time, as it was in the early days of Ghana's cocoa industry.

Seed production in Togo and crosses produced

Deuss (1981) stated that 16 ha of seed gardens were planted at Zozokondji from 197175. According to Lanaud et al. (1987), the crosses to be produced included UPA402 x UF676, and UPA603 x UF667, with UPA603 x UF667 produced at Tomegé. In the production of UPA crosses in the seed gardens, the ratio of female to male parents was lower in Togo (5:1) than in Côte d'Ivoire (8:1) (Lanaud et al. 1987). Tsatsu and Bekou (2003) listed the following six crosses of Ghanaian origin as coming from the seed gardens at Zozokondji:

T79/416 (C68) x E1:J9/2/70 (C26) (not an approved Series II hybrid)
T79/467 (C69) x S72:J11/4/49 (C20) (Series IIM)
T63/967 (C75) x W41 (C41) (Series IIK and its reciprocal L)
T63/967 (C75) x E1:C4/3/291 (C25) (reciprocal of Series IIB, an approved hybrid)
T63/971(C74) x P4/9:J11/4/5 (C18)
T16/613 (C73) x E1:C4/3/270 (C23) (should be reciprocal, Series IID)

Source: Tsatsu and Bekou (2003)

Seeds from six crosses Pound7 x IMC67 and reciprocal; Pa7 x Pound7; Pa7 x P30 (= Amelonado); Pound7 x T60/887 and reciprocal produced in a 4ha seed garden, have been distributed since 2005 (Wegbe et al. 2012).

A3.18 References

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ANNEX 4 – Conventional vegetative propagation: planning and management

Roberto Sena Gomes and George Andrade Sodré

A4.1 Materials and inputs required to produce seedling rootstocks for planting one hectare of cacao

Conventional nursery facilities:

- To plant one hectare of cacao, using 3.0 x 3.0m spacing, 1,100 seedlings are required. However, 1,400 rootstock seedlings should be prepared, since an estimated 10% will be lost, either through death or selection; 5% will be replanted in the field; and 15% will be used for grafting/budding.
- About 35-40 selected mature cacao pods are required to supply 1,400 seeds.
- 1m^2 of nursery floor can hold 70-80 (tightly packed) or 40-50 (less packed) seedlings, allowing room for circulation (the lower number of 40 seedlings/m^2 of nursery floor is used here).
- A nursery area of 35 m^2 (suggested dimensions 5 x 7m) is suggested for 1,400 rootstock seedlings.
- Nursery facilities include wooden poles, palm trees or coconut leaves (from the farm) for overhead shading, lateral wind protection, and sand for the floor.
- 10 wooden poles (2.2m long) are required for the basic frame.
- Wires stretched between upright poles support overhead covering.
- Floor drainage comprises 1.00-1.50 m^3 of coarse sand or fine gravel distributed in gutters on the nursery floor.
- Coarse sand is required for the nursery floor (3.0 litres/m^2 x 35m^2 = 1.05m^3).
- Shading materials comprise 35m^2 of palm or coconut tree leaves.
- Lateral wind protection materials, measuring 24m in length x 1.8m in height, comprise palm tree or coconut leaves; saran or polypropylene (50% light interception) provide options for more sophisticated nursery facilities.
- The size of plant containers (such as polyethylene bags) should be 28cm x 12cm x 10µm (about 2 litres of substrate); 1,400 polyethylene bags are needed for the 1,400 seedlings.
- Total substrate volume for 1,400 seedling rootstocks is 2.8m^3.
- Substrate components are soil, manure and sand at a ratio of 60:20:20 respectively - ratio varies depending on composition of local soil; plus fertilizers (1-2kg NPK/m^3 + 200g FTE-Br.12/m^3)$^+$, lime (2-5kg/m^3)$^+$.
- 500 g of aluminous wire No.14 (1kg = 36m) is required.
- Tools and equipment required include wheelbarrows, buckets, watering cans, hoes, shovels, hole diggers, rakes, small sprayers, machetes, grafting knives, pruning shears (one unit of each).
- For pest and disease control, fungicides (2-3g or ml/100 seedlings per application)$^+$, insecticides (2-3 ml or g/100 seedlings/application)$^+$, and herbicides 0.3-0.5g or ml/m^2 of nursery floor)$^+$ may be applied.
- Six manual labour days are required to collect and transport wood material and palm leaves; mark and open holes; work on floor drainage; and establish the nursery structure - including shading and lateral wind protection.

- Four to six months of manual labour, of one man, three hours per week, is required to perform weeding, watering, pest and disease control.

(+) The use and quantities of fertilizers, lime and other chemical inputs will vary according to soil analysis, pests, fungal disease and weeds, and following local recommendations. Systemic fungicide/insecticide normally varies from 1-3 ml per litre of water.

A4.2 Materials and inputs required to produce seedling rootstocks for medium- and large-scale projects

Non-conventional nursery facilities:

- Building facilities required include a main office, laboratory, workers' rooms, storage room, planting room, warehouse, water tank, water treatment unit.
- Clonal garden (*).
- Nursery facilities can be built using wooden poles or a metal frame, saran or polypropylene (50 % light interception), wires, and sand or gravel for the nursery floor.
- Wires stretched between upright poles can support overhead (moveable) and lateral shading. The saran netting (50% light interception) should be stretched 2.40m above the nursery floor.
- An overhead, automated irrigation system is an essential part of this type of nursery due to intermittent daily watering required by seedlings grown in plastic tubes and in a commercial pot mixture.
- Floor drainage can be created using drainage pipes, stones or gravel (1.50m^3 for 35m^2 of nursery floor), distributed in gutters on the nursery floor (*).
- Fine gravel or coarse sand is required for the nursery floor (1.00m^3 for 33m^2 of nursery floor).
- Nursery facilities can be constructed using a variety of different materials and technologies, but that which is most recommended for a medium- to large-scale operation comprises galvanized metal for the frames, and polypropylene (150 micron plastic film; 50% light interception) for the roof and the lateral protection; lateral wind protection should be 2m in height. Wires stretched between upright poles provide lateral support to hold the trays.
- 1m^2 of nursery floor can hold about 86 seedlings per six-month period, using plastic tubes of 288cm^3 capacity in 54-cell plastic trays; or over 280 seedlings/m^2 per six-month period, using smaller tubes, e.g. 56cm^3 capacity in 96-cell trays (*).
- Substrate should be composed of commercial compost and coconut powder fibre (50% each), in addition to complete NPK and slow-release micronutrient fertilizers, and lime (*).
- Automated irrigation mist system and pipe-water system may be used (*).
- Equipment, tools and machinery required include substrate-mixer, vibrating table, wheelbarrow, plant-tray shelves on wheels, manual and motorized sprayers, trucks and pick-ups, basic laboratory equipment, buckets, watering cans, hoes, shovels, hole diggers, rakes, machetes, grafting knives, pruning shears, etc. (*).
- Planting materials required include pre-germinated seeds, rooting hormones, herbicides, insecticides, fungicides (*).
- Number of days of manual labour required depends on the size of the project.

(*) Quantities and size vary according to project scale (e.g. in the case of tubes/trays, 1,000 rootstock seedlings require 1,000 plastic tubes, 0.3m^3 of pot mixture, and 19 plastic trays (54 cells per tray for holding 280 cm^3 tubes); see for example www.mecprec.com.br).

A4.3 Preparation of seedling rootstocks

Conventional technique utilized in most cocoa-producing countries:

- Seeds (variety/family recommended by technical assistant agent) are selected and duly cleaned with dried sawdust to remove the mucilaginous seed pulp and let it pre-germinate in a semi-saturated substrate (cured sawdust, compost, jute or heavy cotton tissue) for about three to five days. Pre-germinated seeds are then transplanted to pre-prepared polyethylene bags, filled with soil or a mixture of compost, soil, and sand (see A4.1 above) in the nursery (50% overhead shade).
- The seedlings remain in the nursery for four to six months, under a regime of irrigation, fertilizer application, pest control and weeding, before being transplanted into the field, where they are grafted some months later.
- The procedure used to grow the seedlings will vary according to local environment and must follow the recommended practices and use the products indicated by local technical assistants (general recommendations for basic inputs are listed above in A4.1).
- Alternatively, the young seedling rootstocks can be grafted and/or budded in the nursery, at different ages by green- or brown-budding techniques, according to recommendations. Success rates of take may vary greatly, according to many factors (genetics, prevailing environmental conditions, methods, labour efficiencies, and management practices used by different countries). Estimates of possible outcomes are presented in Tables A5.2 and A5.5, Annex 5.

Non-conventional technique utilized in Bahia, Brazil:

- In a nursery facility, pre-germinated seeds are transplanted to pre-prepared plastic tubes (288cm^3 capacity), filled with a mixture of composted pine bark and powdered coconut fibre (50% each), in addition to a complete slow-release fertilizer.
- Tubes are placed in perforated plastic trays supported by horizontal metal wires, which are stretched between vertical poles specifically to hold the trays (90cm above the ground).
- Young cacao seedlings are grown for four to six months in the greenhouse facility. They are managed under favourable conditions of watering and mineral nutrition (which varies according to environmental conditions, age and seedling requirements) provided by an overhead automated irrigation system, and following strict sanitation procedures, which continue after grafting.
- In the next stage, the rootstocks, 1cm in diameter (about two-and-a-half- to three-months old), can be grafted and/or budded with suitably sized clonal semi-woody scions (see A4.4 for details).
- The cloned seedlings normally remain in the nursery for more than three months under a regime of acclimation for field planting, with reduced water supply and increased light levels in the final four to six weeks.
- Rates of take may vary widely according to factors such as genetics, prevailing environmental conditions, method, labour efficiency, and management practices. Estimates for grafting success using the aforementioned technique in Bahia, Brazil, are provided in Table A5.2, Annex 5.

A4.4 Materials and inputs required to produce 1,400 cloned seedlings for planting one hectare of cacao through brown budding or split grafting

Materials required:

- 1,400 cacao seedling rootstocks, stem diameter 1.00-1.50cm, duly sanitized.
- 120 brown budwood scions for budding (with an average 12 buds each), or 560 brown budwood scions for grafting (with 10-12 buds each); quantities vary according to clone.

- Inputs: fertilizer (3-5 g NPK/seedling per application) [+]; NPK + micronutrient leaf fertilizer (2-3 ml/100 budded seedlings per application)[(+)]; fungicide (2-3mg or ml/100 seedlings per application) [(+)], insecticide (2-3ml or mg/100 seedlings per application) [(+)]; herbicide (0.3-0.5g or litre/m^2) [(+)].
- Supplies: wrapping tape (2-3kg) or biodegradable tape (200g), plastic tags (50 units), transparent plastic bags (15 x 30cm x 8 to 10µm).
- Other supplies: paper, pencils, pens, markers, cotton cloths (3 units-40 x 40cm), knife sharpeners (3 units), alcohol gel (1 litre), commercial chlorine (1 litre), soap (1-2 units), paraffin (1-2kg), small pan (1), jute (4 large bags).
- Tools: a bucket, watering cans (2), grafting knives (2), pruning shears (2), manual saws (2), and a sharpener (1).
- Equipment: workbench (1), stools (2), two mini-sprayers (500cm^3), 20-litre manual sprayer (1), wheelbarrow (1).

(+) Quantity and use of fertilizers, other chemical inputs will vary according to soil analysis, pests, fungal disease and weeds, and following local recommendations. Systemic fungicide/insecticide will normally vary from 1 to 3ml per litre of water.

Methodology: conventional brown-budding technique

In most cacao nurseries, the brown-patch-budding method, very similar to Forkert's method developed for rubber (but without chip wood), has been performed in the past with scion sticks and rootstock stems of comparable diameter sizes (1.0-1.5cm) as follows:

- All budding tools must be sterilized before, during (every 10-15 budding grafts) and after budding work, using 8-10ml/litre of water with 2-2.5% a.i. of chlorine. Budwood scions must be sanitized by spraying a solution of 1-2ml/litre Carbendazim in water (500g/litre).
- All leaves are pruned from the scions immediately after harvest to minimize dehydration.
- For long distance transportation, after the sprayed solution has dried out in the shade, the cut ends of the scions are sealed with liquid paraffin (by quickly dipping them into molten paraffin – slightly above 37°C). The budwood scions are then packed in damp paper (old newspaper), in loose bunches of 20-30.
- A patch of bark containing one bud (5.0 x 1.0 to 1.5cm) is cut from budwood; another cut is made in the rootstock stem of similar size, in the form of an inverted 'U', just below the cotyledon scar.
- The bud patch is lightly trimmed along one or both sides by about 2mm to achieve a better fit in the patch window.
- Then, the bark lid of the patch is lifted up and the bud patch is inserted firmly underneath the bark in the opened window on the rootstock.
- When the bark lid has been closed the patch is ready for wrapping. A plastic tape is carefully used to tighten and cover all the cuts. Since the recent introduction of biodegradable grafting tape (for any grating methodology), there is no longer any need to leave the bud uncovered; the bud will burst through the tape.
- The plastic tape is removed 10 to 15 days after grafting, and the lid cover is cut and removed five days later. The procedure is completed within one more week, pruning successful seedlings by removing the crown at 15cm above the grafting height on the stem.
- A second cut is made on the stem just above the graft, after the development of three pairs of fully expanded leaves, and then the slanted cut surface is protected with grafting paint.
- Rates of take normally vary according to factors such as genetics, prevailing environmental conditions, methods, labour efficiency, and management practices. Estimates of success using this technique in Bahia, Brazil, usually vary from 60 to 70% (see Table A5.2, Annex 5).
- A cloned seedling rootstock will be ready for field planting about 10 to 12 weeks after budding.

A4.5 Conventional split-/top-/splice-grafting technique for cloning seedling rootstocks in nurseries, field-planted seedlings and basal chupons

- All grafting tools must be sterilized before, during (every 10-15 grafted seedlings) and after grafting work, using 8-10ml/litre of water with 2-2.5% a.i. of chlorine. Scions should be sterilized by spraying a solution of 1-2ml/litre of Carbendazim in water* (500g/litre). This same solution should be sprayed over the growing scions, beginning just after the removal of the plastic bags, around two to three weeks after grafting.
- All the leaves should be pruned from the scions immediately after harvesting to minimize dehydration, although sometimes about 3cm of the leaf blade and petiole remain.
- For long distance transportation, after the sprayed solution has dried out in the shade, the cut ends of the scions should be sealed with liquid paraffin (by quickly dipping them in molten paraffin – slightly above 37°C). The scions are then packed in damp paper (old newspaper), in loosely packed bunches of 20-30.
- Semi-hardwood scions (about 15cm in length) are obtained from orthotropic branches of selected varieties, by cutting a long V-shape, gradually tapering into a wedge, at the base of the branch; the scion is then carefully inserted into a vertical cut of a similar size in a stem rootstock seedling (grown in a plastic bag or tube) or a basal chupon in the field.
- Rootstock diameter normally varies from 1cm (plastic tube seedlings; see Figure 5, Annex 4) to 1.5cm (plastic bag seedlings; see Figures 2, 3, 4, 10, Annex 4); in the field, rootstock diameter, especially that of basal chupons, may be greater than 2.5cm and will require more than one scion – using top-/lateral-grafting technology (see Figure 6, Annex 4).
- After the wedge has been inserted, the graft union should then be wrapped with a plastic cord or tape, and the graft should be enclosed in a transparent plastic bag to avoid dehydration, as shown in Figures 5, 7, 8, 10, Annex 4.
- The plastic bag can be removed about two to three weeks later and the scion should then be immediately treated with fungicide and insecticide, as recommended.
- Alternatively, a special biodegradable grafting tape can be used instead of plastic bags. The tape is firmly wrapped around the entire scion. There is no need to remove it since the buds normally grow through the thin film of plastic tape.
- Grafting basal chupons and rootstock seedlings in the nursery or field requires stems that have been cut off at a given height, and split tops for scion insertion. Some farmers carry out grafting at about 20cm below the seedling apex, leaving two to three pairs of fully expanded leaves below the grafting point. The functional leaves left will keep contributing with the production of photoassimilates (see Figures 2, 3, and 8, Annex 4, for top grafting in several countries).
- Old basal chupons (with diameters greater than 2.5cm) may require two scions, which are inserted at the top of the chupon under the bark through lateral trunk incisions (Figure 6, see Annex 4.
- Young basal chupons, free of pests and diseases, grown from the root/stem collar region of mature cacao trees, are recommended for successful grafting. Chupons that grow from other heights on the trunk should not be selected for grafting.
- It is important to manage the chupons after grafting, as rootstocks, by periodic pruning to remove the development of new chupons from their base or lower stem.
- Cloned potted seedlings, either grown in plastic bags or tubes, will be ready for field planting around 90 to 100 days after bud burst; during this period, water should be reduced and light increased in order to harden the seedlings in preparation of field transplanting.

A4.6 Materials and inputs required for managing one hectare of old cloned plants using side-/lateral-grafting methodology

Estimated 1,100 trees; two grafts per trunk and a 50% grafting success rate of take

Materials required:

- Approximately 1,320 clonal scions, each measuring 15cm in length, and comprising 10-12 buds (or 3,300 scions, with four to five buds on each); for young trees, the amount of scions and related supplies may be reduced by 1/2 to 1/3.(*)
- Inputs: fertilizer (150-250g NPK/tree, per application)(+); NPK plus micronutrient leaf fertilizer (about 5ml/100(+) grafts per application), beginning after leaf expansion); fungicide (about 5g/100(+)grafts per application, beginning after bud burst); insecticide (about 5 ml/100(+) grafts per application, beginning after bud burst); herbicide (3-5kg/ha per application)(+).
- Supplies: wrapping tape (8kg/ha); plastic tags (50 units); 2,200 transparent, plastic bags (15 x 30cm x 8 to10µm).
- Other supplies: paper, pencil, pen, marker, cotton cloth (3 units – 40 x 40cm), knife sharpeners (5 units, No.400), alcohol gel (1 litre), commercial chlorine (1 litre), soap (1-2 bars), paraffin (2-3 kg), small pan (1), jute (4 large bags).
- Tools: 20-litre bucket, grafting knives (2), pruning shears (2), manual saws (2), and a sharpener (1).
- Equipment: stools (2), mini-sprayers (2 units, each with a 500cm^3 capacity), 20-litre manual sprayers (2), power saw #60 (1 unit, to be rented).

(*) Procedure and specifications may vary according to different clones.

(+) Quantities and use of fertilizers, lime and other chemical inputs will vary according to soil analysis, pests, fungal disease and weeds, and following local recommendations. Systemic fungicide/insecticide will normally vary from 1-3ml per litre of water.

Methodology:

Tree trunks of old unproductive cacao trees of any age can be grafted. The basic requirement is that the bark of the cacao tree must be healthy, undamaged, and able to peel easily.

- A panel is opened on the tree trunk, making a horizontal cut (7cm in length) at the height of 40-60cm above the soil, or at a different height depending on the condition of the bark. The cut must be deep enough to reach the cambial zone. A semi-circular cut is then made 5cm above the horizontal cut, descending vertically to join each end of the horizontal line, thereby achieving a semi-circular patch, and the bark is removed. Another cut, 8cm in length, is then made descending vertically from the centre of the horizontal cut.
- The clonal scion (15cm in length, with 4-5 buds) is prepared by cutting a 5cm, gradually tapering wedge at the base, which is then vertically inserted under the bark through the T-shaped incision in the trunk, or through a bark flap that has been opened up in the tree trunk (Figure 6 and 7, Annex 4).
- The entire grafted area is then firmly wrapped with plastic cord to ensure intimate contact of the cambial tissues with the grafted parts.
- A suitable transparent plastic or plastic bag is used to enclose the scion to avoid dehydration.
- Another patch is opened on other side of the tree trunk, at a height of 40-50cm above the soil, or where possible, in the same way as described above.
- In approximately three to four weeks, the successful graft will quickly develop leaves and will require post-grafting care.
- Management will include pest and disease control with the recommended chemicals, especially for every crop of new leaves. Increasing light to stimulate scion growth by progressive pruning of the canopy, in combination with foliar fertilizer applications, are basic requirements for the rapid establishment of newly grafted clones.

- The mother tree crown should be cut away, leaving about 100cm of trunk above the graft. The remaining trunk should be eliminated four to six months later, at 20cm above the graft; graft paint should be applied to the cut as a seal. The whole process for the replacement of the old tree canopy requires about six months.
- Success rates of take are normally low when applied to mature tree trunks (less than 30%), but high (over 70%) when applied to young basal chupons or to high branches (crown grafting) of up to 20-year-old trees, as shown in Table A5.2, Annex 5.

A4.7 Materials and inputs required to plan and manage one hectare of cloned cacao plants through crown grafting

Side grafting on high branches, above the jorquette, in trees of any age, is also known as crown grafting. This technique is normally utilized to quickly restore yield or change varieties in stands of low productivity. In order to be able to carry out this technique, branch bark must be undamaged, healthy and easy to peel. Usually, two to four grafts are performed per tree, especially on branches of young trees, 20-40cm above the jorquette. Assuming an average of three grafts per tree crown, and an average success rate of take of 70%, the following items are required:

- Approximately 1,700 clonal scions with 10-12 buds on each(*) (or about 4,300 scions with four to five buds on each), and each scion measuring 15cm in length(*).
- Inputs: fertilizer (150-250g NPK/tree per application)(+); NPK plus micronutrient leaf fertilizer (about 10ml/100(+) grafts per application, beginning after leaf expansion); fungicide (about 10g/100(+) grafts per application, beginning after bud burst); insecticides (about 10ml/100(+) grafts per application, beginning after bud burst); herbicides (3-5kg/ha per application) (+).
- Supplies: wrapping tape (3-5kg/ha), plastic tags (50 units), about 3,300 transparent plastic bags (15 x 30cm x 8 to10µm).
- Other supplies: notebook, pencil, pen, marker, cotton cloths (3 units, 40 x 40cm), paper knife sharpeners (5 units, No. 400), alcohol gel (1 litre), commercial chlorine (1 litre), soap (1-2 units), paraffin (4 kg), small pan (1), jute (4-6 large bags).
- Tools: 20-litre buckets (2), knives (4), pruning shears (4), manual saws (4), and sharpeners (2).
- Equipment: four mini-sprayers (500cm^3), 20-litre manual sprayers (2), power-saw #60 (to be rented).

(*) Procedure and specifications may vary according to different clones.

(+) Use and dosage of fertilizers and other chemical inputs will vary according to soil analysis, pests, fungal disease and weeds, and following local recommendations. Systemic fungicide/insecticide will normally vary from 1-3ml per litre of water.

Methodology: side/lateral grafting applied to tree crowns (high branches)

- The methodology is the same as described for side/lateral grafting on the trunk (see A4.6 above), but applied to high branches. The scions are grafted at about 40cm above the jorquette of young cacao trees (up to 20 years in age), in branches of good vigour and free of disease (see Figure 6 in Annex 4).
- Normally, two to four grafts can be performed per tree, depending on the number of branches available.
- Post-grafting management includes pruning to increase light in the canopy, which should be carried out progressively after the development of the grafted scion – the process can begin about three months after grafting.
- Application of leaf fertilizer is recommended to stimulate growth and to maintain vigour. Chemical control of pest and disease is also recommended, especially at every crop of new leaves.
- It takes about six months to rehabilitate the tree crown and approximately 24 months to increase production in crown-grafted trees.

- The rate of success of cacao rehabilitation through grafting in high branches depends on time of year, nutrition and vigour of the trees, and post-grafting management of sanitation and overhead shade. An estimate for the success rate of take is provided in Table A5.2, Annex 5.

A4.8 Production of cloned cacao plants through rooted cutting technology

Materials and inputs required:

- Greenhouse facilities and materials similar to those described in A4.1 and A4.2
- Semi-woody sticks (15cm long) harvested from fan branches (or orthotropic chupons) of clonal stock plants. Usually, high rates of rooting success are attained by using young cuttings harvested from the terminal part of the branches. Assuming 60% rates of success at the final selection, 1,400 initial cuttings will be required to produce 1,000 rooted cuttings for planting.
- Inputs: fertilizer (10-15mg/ml of soluble NPK plus micronutrient leaf fertilizer per 100 cuttings per application, beginning after leaf expansion)[(+)]; fungicide (2-3g/ml per 100[+] cuttings per application, beginning after bud burst); insecticides (about 2-3g/ml per 100[(+)] cuttings per application, beginning after bud burst); herbicides (0.3-0.5g/m^2 of nursery floor per application) [(+)].
- Rooting promoter mix: usually the cuttings are treated with 6,000 ppm of IBA (a.i.) powder mix, which is applied to the basal cut. 6 g of IBA in 1kg of talc can treat 10,000-15,000 green cuttings.
- Substrate: mixture of 50% each of composted pine bark (or other) and shredded coconut fibre, plus slow-release complete fertilizer (e.g. osmocote or similar, plus micronutrient fertilizer).
- Equipment: substrate-mixer, vibrating table, wheelbarrow, plant-tray shelves on wheels, manual and motorized sprayers, buckets, watering can, shovel, small sprayer, trucks and pick-ups, basic laboratory equipment [(*)].
- Tools: pruning tools (pruning shears), grafting knife.
- Plastic tubes (288cm^3 capacity), 54-cell plastic trays.
- Highly trained labourers.

(*) Quantities will vary according to project scale (e.g. 1,000 rootstock seedlings will require 1,000 tubes, 0.3m3 of pot mixture, and 19 plastic trays (54 cells per tray for holding 280 cm3 tubes); see for example www.mecprec.com.br).

(+) Quantity and use of fertilizers and other chemical inputs will vary according to soil analysis, pests, fungal disease and weeds, and following local recommendations. Systemic fungicide/insecticide will normally vary from 1-3ml per litre of water.

Methodology: rooted cutting technology

Basic technology utilized in Ecuador

- Semi-hardwood scions are harvested from orthotropic branches of disease-resistant clones in a clonal garden.
- The scions are cut to about 15cm in length, with five buds and three mature leaves on each (leaf area reduced to 1/4 by pruning), and are kept hydrated through intermittent water misting.
- The cut stems are then treated with a root promoting hormone (6,000 ppm Abscisic acid [ABA] powder mix) applied to the basal cut; some projects utilize a mix of Indoyl butric acid and α-Naphthaleneacetic acid (IBA:NAA; 50:50%).
- Following hormone treatment, the cuttings are planted in conventional plastic bags that have been prepared with a mixture of sterilized soil (50%), compost (50%), and appropriate fertilizers. The cuttings are planted in core (filled with sand; size 1.5-2cm diameter, 10cm depth) baskets or pots containing potting mixture.
- The bags are set in nursery beds and, after a thorough watering, are completely enclosed with an opaque polyethylene cover to maintain an appropriately humid environment (Figure 6 in Annex 4). The beds in the nursery will be maintained under reduced light exposure of about 50% of full sunlight.

- Three to four weeks later, the plastic cover is removed for one hour, after which the beds are covered again. The next day, the rooted seedlings remain uncovered for two hours and this process continues over the following days, with the time of exposure slowly increasing until the complete removal of the cover after about a week or so.
- A regime of watering is normally maintained to keep potted soil humidity close to field capacity.
- Leaf fertilizer application is recommended to stimulate growth and to maintain vigour. Chemical control of pest and disease is also recommended, especially at every crop of new leaves.
- After shoot growth, the rooted cuttings are hardened for some weeks before being released to field planting.
- This method has a success rate varying from 50-70%.

Basic mass propagation technology utilized in Bahia, Brazil

- Semi-hardwood bud sticks of clones resistant to witches' broom disease are harvested from a clonal garden.
- Scion sticks are cut to about 15cm in length, with four to five buds and three mature leaves on each (leaf area reduced to 1/4 by pruning).
- Clonal scions are treated with rooting hormone (6,000 ppm ABA powder mix) applied to the basal cut. Usually, 6 g of IBA in 1kg of talc can treat 10,000-15,000 green cuttings.
- The cuttings are then planted in plastic tubes (288cm^3 capacity), in appropriate plastic trays (for examples, see www.mecprec.com.br), and immediately transferred to rooting chamber beds, which are created by stretching wire between metal poles, 90cm above the soil surface. See Annex 4, Figures 5 and 11 for details. The cuttings should be kept continuously hydrated.
- Rooting substrate varies according to humidity/aeration requirements, but a mixture of 50% each of composted pine bark and coconut coir dust (or coarse fibre), in addition to a complete slow-release fertilizer (e.g. osmocote plus micronutrients), has been successfully used.
- The cuttings are managed in a high humidity chamber, equipped with an automated intermittent irrigation system. Application of hydro-soluble leaf nutrients will vary according to time of year and age of cuttings.
- Bud burst and initial leaf development normally occur within two weeks. Foliar pest and disease incidences are preventively controlled with a regime of chemical spraying.
- After about 150 days, including a period of hardening (about six weeks of reduced water and increased light regimes), the rooted cuttings are ready to be planted in the field.
- Rates of rooting success depend on numerous factors, as mentioned in the text, but on average, Biofabrica has registered success rates ranging from 60% to 75% (Table A5.2, Annex 5).

A4.9 Procedure and materials used to propagate cacao through marcotting

The marcotting procedure is very simple and quite similar to the basic method used for rooting many other plant species (see http://www.cropsreview.com/marcotting.html) main steps are as follows:

- Select a vigorous, healthy branch with brown bark in the crown of the cacao tree, preferably not in complete shade.
- Remove strips of bark from around the portion of the stem to be rooted by pressing a grafting knife against the bark just below a node, and cutting around the stem. A similar cut is made generally about 5cm below the first cut, but the strip can be wider for larger branches. The bark is then peeled off and removed; excessive dehydration of the exposed cambial tissue should be avoided.
- Place sphagnum moss or coconut coir dust (or sawdust), slightly wet, around the debarked stem and wrap with plastic sheeting. Transparent plastic sheeting is preferred in order to observe the development of the roots.

- Then, tie the ends of the plastic sheeting securely against the stem, with one end just under the bottom part of the de-barked stem (the lower cut) and the other a short distance above the upper part (the upper cut). It is important that the upper cut should be covered with the rooting medium because the adventitious roots will develop from this superior cut.
- The rooted shoots should be severed from the parent plant when plenty of roots have developed; at this point, the rooting medium will have become hard and rough to the touch. New shoots will also have sprouted from the portion of the stem immediately below the rooting medium. In most plant species, rooting occurs at least 15 days after marcotting, but in cacao it may take more time, around three to four weeks. After six weeks, a well developed root system might be visible, at which stage the marcott is ready to be separated from the parent tree. First, cut halfway through the stem at the lower incision, and then wait for 15 days before completing the cut. Before cutting, the leaf area of the marcott should be reduced to a 1/3 by pruning.
- Do not plant the rooted cutting with a large ball of substrate adhering to the roots. This may give rise to many fungi; try to eliminate as much of the rooting ball as possible without damaging the young roots.
- The rooted cutting is normally planted in good potting mixture and well drained. The top of the roots should be level with or slightly above the ground, and the transplanted cloned plant should be securely staked and lightly watered, under nursery conditions. Before field planting, the rooted cutting must be hardened in the same way as any other propagated planting material.
- *Materials required*: grafting knife, transparent plastic sheeting, sphagnum moss or coconut coir dust, wrapping cord, small sprayer, small plastic clean brush, cotton cloth, and bucket.

A4.10 Methodology for the establishment and maintenance over a three-year period of one hectare of clonal garden designed to produce fan cuttings and scion sticks

Note – the production of orthotropic material follows a similar procedure.

- Plan ahead. Start preparing the field about 10-12 months before planting.
- Select a non-forested plot (in an early fallow stage) - preferably flat or slightly inclined - with a soil depth of less than 80cm, good natural fertility, and proximity to a water source, strategically located in a central area close to the rehabilitation or expansion project.
- Take soil samples from 0-20cm and 20-40cm depths, separately, (10 samples from each depth) and send them to a laboratory for soil analysis. Make sure the samples are taken in different quadrants of the entire selected area.
- Clear field of weeds and bushes; remove this vegetation from the area or heap the biomass to decompose in the firebreak areas. Apply lime as recommended.
- Get stakes and mark the planting sites in a square pattern for both temporary banana shade plants (3 x 3m) and permanent shade trees (24 x 24m, although this can vary depending on the species). Shade tree seedlings are planted in banana rows.
- Dig the planting holes for the banana plants (30 x 30 x 30cm), or the permanent shade plants (30 x 30 x 40cm), and plant them as early as rains permit. The planting holes must be ready for the seedlings a few weeks before planting.
- Carefully handle and transport the planting material (banana plants and permanent shade tree seedlings) to the field for planting. Water the plants before transportation. Cut and remove bags and place plants in holes without breaking the root ball. Fill the space left in the hole with a mixture of soil and fertilizer (as recommended by local technical assistance). Apply mulch around the planted seedlings soon after planting; this will help the establishment of seedlings by

reducing moisture stress, and controlling the weeds. Management of the planted seedlings will include: chemical control of insects and diseases; application of fertilizers; pruning of shade plants for crown formation, and the replacement of low vigour or dead seedlings.

- Order the cloned seedlings according to the recommendations of local technical assistance, at least six to eight months prior to the date of planting. Alternatively, the cloned seedlings can be prepared following the methodologies described above in A4.4, A4.5 and A4.8.
- Stake the field for cacao seedlings after sufficient development of the banana plants, when at least 50% of the area is well shaded (about five to seven months after planting). Stake the holes for cacao using the planting space of 3 x 1m (3m between rows and 1m within plants in the row).
- Stake the cacao holes in the banana rows halfway between the banana pseudo-stems (stalks).
- Dig the planting holes for cacao (40 x 40 x 40cm), and mix the lime and/or fertilizers with the soil removed from the hole, as recommended by the local technical assistance. Refill the holes with the fertilized soil and wait for appropriate time to plant cloned cacao seedlings or rooted cuttings.
- At planting time, re-dig the hole and carefully plant the cacao, repeating the procedure used to plant the shade seedlings, including mulch. It is very important to water the cacao plants before transportation and field planting.

The management of the stand will include:

- Manual weeding during the first six months after planting and chemical weeding thereafter.
- Replacement of low vigour and/or dead plants.
- Periodical pruning of shading plants to remove dry/dead plant parts (sanitary pruning), and to progressively increase light incidence in the cacao canopy.
- Elimination of the banana plants 24-30 months after the planting date; or by 36 months at the latest.
- Chopping of banana leaves and stalks into small pieces to rot into mulch in the cacao rows.
- Elimination of basal chupons of cacao seedlings, especially budded/grated plants.
- Periodical spraying to control insects and diseases.
- Application of fertilizers (NPK, micronutrients, and foliar).
- Harvesting of cuttings and or scions to supply cloning propagation projects; harvesting of plants should be avoided during cycles of growth - flushes of new leaves.
- Use of tags to ensure identification of clones.
- Careful transportation of the harvested material to a shaded area.
- Separation of the distal green to slight brown portions of the scions (first 30-44cm, from the apex) for use in the production of rooted cuttings, with the remaining portions (brown bark) to be used for budding or grafting projects.

Table A4.1 Comparative productivity, success rate of take (%), cost and general considerations for propagation methods utilized by the Brazilian cacao rehabilitation programme.

Methods	Productivity (unit/day or unit/m^2 per year)	Rate of take (%)	Cost[(*)] (US$)	Notes
Grafting (field)				
Field (chupons, seedlings)	70-150	70-90	0.40	Simple installation; cheap and easy to implement; few skills required, favoured by farmers; easily sanitized; low cost.
High branches (crown grafts)	40-60	70-90	0.60[(1)]	As above for selected plantings; trees less than 30 years old.
Old trunks (lateral/side graft)	30-50	30-50	0.40	Rarely used on hybrids over 30 years old.
Grafting (nursery)				
Seedling rootstocks in plastic bags	150-180	70-80	0.74[(**)]	Simple; easy to implement; gaining popularity; easily sanitized; small-scale; medium investment.
Seedling rootstocks in plastic tubes	170 - 200/m^2 [(2)]	80-90	0.76[(**)]	Simple; easy; gaining popularity; good sanitation required; small-scale; medium to high investment; cheap and easy to transport.
Budding (nursery)				
Seedling rootstocks in plastic bags	120-150	60-70	0.60	Simple; easy but requires skill and much care; strict sanitation required; low rate of survival; not favoured by farmers; low investment.
Rooted cuttings (nursery)				
Rooting cuttings in tubes	900-1,100/m^2[(3)]	60-75	0.58[(**)]	Requires costly infrastructure, good management and strict sanitation; medium to low rate of survival; high investment; suitable for mass propagation projects for specific locations; cheap and easy to transport. See other options for non-mist propagators in Figure 12, Annex 3

(1) 2-4 branches above jorquette;

(2) Bom Retiro Nursery, Camacan, Bahia;

(3) Biofabrica Mass Propagation Unit, Ilhéus, Bahia;

(*) by contract

(**) Project cost of production.

Table A4.2 Projected labour and materials required for the establishment and maintenance for three years of one hectare of clonal garden in Bahia, Brazil.

Specification	Unit	Quantity
YEAR I		
I – Preparation of the planting area		
a) Labour:		
Cleaning and removal of vegetation, chemical weeding, marking of planting holes	Days of manual labour (ML days)	81
Liming	ML days	6
Planting of shade trees (permanent shade trees and banana plants)	ML days	25
Management of shade plants	ML days	36
Analysis of soil sample	ML days	0.5
Opening of planting holes and application of basic fertilizers (about six months after planting of shade trees)	ML days	48
Total labour	*ML days*	*196.5*
b) Materials		
Wood stakes (marking)		3,350
Banana planting mats		1,200
Tree shading plants		25
Lime (soil analysis recommended)	Bags	20
Herbicide	Litres	12
Wheelbarrow, bucket, watering can, hoe, shovel, hole digger, rake,		2-4 of each
Machete, grafting knife, pruning shears		2-4 of each
Insecticides/fungicides	Litres	4
Formicide	Kg	2
II - Planting facilities and materials		
a) Labour		
Simple greenhouse construction (before planting time)	ML days	6
Planting of cloned seedlings/rooted cuttings, including application of fertilizer	ML days	64
Management (watering, weeding, sanitation, application of fertilizer)	ML days	6
Total labour	*ML days*	*76*
b) Materials		
Cloned seedlings (budded/grafted) or rooted cuttings		3,600
Urea - cacao seedlings (40 g/plant per application; 2 applications)	Bags	6
Super Phosphate (triple) 100 g/planting hole (cacao seedling)	Bags	7
Potassium chloride 50 g/planting hole (banana plant)	Bags	7
Ammonium sulphate 100 g/planting hole (banana plant)	Bags	15
Micronutrient FTE-Br-12 (40 g/planting hole; cacao seedlings)	Bags	4
III - Maintenance/management		
a) Labour		31
Chemical weeding	ML days	4
Fertilizer application	ML days	5
Intensive pest control	ML days	10
Replacement of dead shading plants	ML days	1
Replacement of dead cacao seedlings	ML days	1
Management of cacao plants (pruning of chupons, dead branches)	ML days	10

Specification	Unit	Quantity
III - Maintenance/management (cont'd)		
b) Materials (inputs)		
Fungicides	Litres	3
Insecticides	Litres	3
Herbicide (3 applications)	Litres	12
Foliar fertilizer 30ml/100 plants per application; 4 applications	Litres	5
Fertilizer NPK 70 g/plant per application; 2 applications	50-kg bags	12
YEAR II		
IV - Maintenance/management		
a) Labour		35
Manual mowing (twice a year), including firebreaks	ML days	4
Chemical weeding	ML days	4
Fertilizer application	ML days	5
Pest control	ML days	6
Management of shade and application of fertilizer	ML days	14
Light pruning (including basal chupons)	ML days	2
b) Materials (inputs)		
Fertilizer NPK (105 g/plant per application; 2 applications (cacao plants); 100g/plant (banana plants)	50-kg bags	15
Urea (40 g/plant per application; 2 applications) - cacao seedlings	50-kg bags	7
Insecticides	Litres	3
Foliar fertilizer 50ml/100 seedlings per application; 4 applications	Litres	8
Micronutrient FTE-Br-12 (40 g/plant); around cacao seedlings	50-kg bags	4
Herbicide (3 applications)	Litres	16
Formicide	Kg	1
YEAR III		
V - Maintenance/management		
a) Labour		38
Manual mowing, including firebreaks	ML days	4
Chemical weeding	ML days	4
Fertilizer application	ML days	4
Pest control	ML days	4
Shading control	ML days	10
Crown formation, chupon pruning	ML days	12
b) Materials		
Fertilizer NPK (140 g/plant per application; 2 applications); cacao seedlings	50-kg bag	19
Urea (50 g/plant per application; 2 applications) - cacao seedlings	50-kg bag	8
Insecticides	Litres	3
Foliar fertilizer 50ml/100 plants per application; 4 applications	Litres	8
Micronutrient FTE-Br-12 (40 g/plant); around cacao plants	50-kg bags	4
Herbicide (3 applications)	Litres	12
Formicide	Kg	2

Specification	Unit	Quantity
Equipment: totals included in Table A4.3		
Manual sprayer (1 unit per 5 ha)		
Formicide applicator (1 unit per 5 ha)		
Motorized sprayer (1 unit per 10 ha)		
Irrigation equipment would add a further US$ 4,500.00, including a water tank (not in the budget)		
Other expenses: calculated values included in Table A5.4.		
Tools (5%) + Transport (8% Mat.) + fuel (5%) of total		
Technical Assistance (5%), Administration (10%)		

(*) = Items will vary from 2 to 4 unities each; m/d = men working day; bag of 50 kg; l = liter.

Table A4.3 Projected budget for the establishment and maintenance of one hectare of cloned cacao in Bahia, Brazil.

Expenses	Cost in US$							Total
	Year 1	*Year 2*	*Year 3*	*Year 4*	*Year 5*	*Year 6*	*Year 7*	
Wages	695	447	357	298	268	268	268	**2,601**
Social charges and benefits	347	223	179	149	134	134	134	**1,300**
Supplies	2,120	362	328	380	345	345	345	**4,226**
Transport	170	29	26	30	28	28	28	**338**
Equipment	47		120	47			47	**261**
Technical assistance	158	52	43	41	37	37	37	**406**
Administration	316	103	43	83	75	75	75	**769**
Total	**3,854**	**1,215**	**1,097**	**1,028**	**887**	**887**	**934**	**9,902**

Exchange rate: US$1 = R$2.25 (27 July 2013).

Table A4.4 Grafting in Malaysia: success rate of take (%) according to methods and seedling age.

Treatment	Grafting method/age	Success rate (%)
1	Chip x 2 weeks	16.25
2	Green x 2 weeks	6.25
3	Chip x 4 weeks	10
4	Green x 4 weeks	18.75
5	Top x 6 weeks	36.25
6	Green x 6 weeks	12.5
7	Top x 8 weeks	42.5
8	Green x 8 weeks	12.5
9	Side x 10 weeks	16.25
10	Patch x 10 weeks	30
11	Side x 12 weeks	30
12	Patch x 12 weeks	45

Source: Kelvin Lamin, Presnetaiton made on Cocoa planting materials – Propagation and distributions in Malaysia. Bioversity International Workshop, 13 – 15 May, 2013, London.

Table A4.5 Variation in cost (US$) of seedling rootstock in various cocoa-producing countries.

Country	Specifications	Cost/unit (US$)	Remarks
Ecuador	2.5-3 months old; plastic bags	0.07-0.17	About 3-4 pairs of fully expanded leaves
Brazil	4-6 months old; plastic bags	0.44-0.58*	About 6-8 pairs of fully expanded leaves
	3-4 months old; plastic tubes	0.53*	About 4-6 pairs of fully expanded leaves
Costa Rica	4-6 months old; plastic bags	0.80-3.00	About 6 pairs of expanded leaves
Ghana	4-6 months old; plastic bags	0.50	About 6-8 pairs of mature leaves Farmers pay US$0.10 per unit
Côte d'Ivoire	3-6 months old; plastic bags	0.50**	
Malaysia	2-6 months old; plastic bags: 2 months old (green budding); 2-4 months old (top grafting); 3-4 months old (patch budding); 3-6 months old (field planting)	0.50-0.70	3-8 pairs of mature leaves Prices vary according to the age of the seedlings
Indonesia	3-6 months old; plastic bags	0.50- 0.70	4-8 pairs of mature leaves Few-weeks-old seedlings may cost a third of the estimated rate
Philippines	3-6 months old; plastic bags	0.50 **	
Nigeria	3-6 months old; plastic bags	0.10-0.20**	

*Rate BR R$2.25 to US$1 (July 27, 2013); **estimated.

Table A4.6 Projected costs (US$) for the production of 100,000 seedlings through grafting or rooted cutting techniques, based on Brazilian experiences.

Projected costs for the production of grafted and rooted cuttings, propagated in farms or commercial nursery units, with a production capacity of 100,000 units/year are presented below. These projected costs can also be used to plan projects of conventional cacao vegetative propagation in various scales, with the necessary adjustments:

Materials/inputs required	Costs in US$ according to methodology		
	Split grafting *in tubes with commercial substrate (commercial nursery)*	**Rooted cutting** *in tubes with commercial substrate (commercial nursery)*	**Split grafting** *In plastic bags with soil, compost and sand mix substrate (farm nursery, southern Bahia)*
Physical structure	23,030.45	23,428.93	13,864.25
Materials/supplies	9,357.25	19,491.58	19,573.08
Labour	25,233.14	7,002.13	23,435.12
Social costs	12,616.57	3,501.07	11,717.56
Technical assistance/ administration	5,618.99	4,273.90	5,487.20
Total	75,856.40	57,697.59	74,077.20

Exchange rate: US$1 = R$2.07 (15 November, 2012).

Table A4.7 Projected budget for the establishment and maintenance of one hectare of clonal garden in Bahia, Brazil.

Expenses	Cost in US$			Total
	Year 1	*Year 2*	*Year 3*	
Wages	4,518.78	521.11	565.78	5,605.67
Social charges and benefits	2,259.39	260.56	282.89	2,802.83
Supplies/inputs	8,372.89	1,216.00	1,376.44	10,965.33
Transport	669.83	97.28	110.12	877.23
Equipment	167.11			167.11
Technical assistance	686.43	91.72	102.62	880.77
Administration	1,667.44	218.67	243.78	2,129.89
Total	18,341.87	2,405.33	2,681.63	23,428.83

Exchange rate: US$1 = R$2.25 (27 July 2013).

Annex 4 - Figures

Patch budding

Patch budded seedling

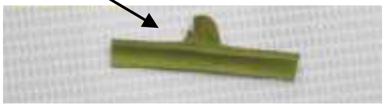
Green patch budding

Green chip budding

Figure 1. Some conventional cocoa budding utilized by farmers in Malaysia.

Source: Kelvin Lamin 2013. Cocoa planting material - propagation and distribution in Malaysia. Workshop on Cocoa Propagation, Bioversity International, London, 13-15 May 2013.

Note active trimmed patch bud.



Sequential steps on patch budding, from left: openning patch window, inserting the patch bud, and wrapping plastic tape on poted seedling rootstock stem.

Sequential steps for top/split-grafting cocoa seedling rootstock stem in a small nursery, from left: scion inserption into split grafted top stem, plastic tape tighten covering the cuts, and below: grafted seedlings with tops enclosed with transparent plastic bags in nursery bed.

Figure 2. Cocoa propagation through conventional patch budding method utilized in Philippines.

Source: Cocoa Nursery Manual Philippines June 2011. © ACDI/VOCA. (http://aboutphilippines.ph/filer/Phils-Cocoa-Nursery-Manual-2011.pdf).


Small and medium scale cocoa nurseries showing rootstocks (left) and top grafted seedlings (right)


Green patch budding on two-week-old seedlings in a large scale project in Java.


Top grafting method (left) and grafted seedlings (right) in a nursery in Indonesia.

Figure 3. Cocoa rootstock nurseries and conventional propagation methodologies used in Sulawesi and Java, Indonesia.

Source: Smilja Lambert, 2013 – personal communication.

Patch budding in Costa Rica.

Field budding in Nicaragua

Nursery top/split grafting in Nicaragua

Figure 4. Cocoa conventional vegetative propagation methods used in Central America.

Source: Phillips-Mora W. 2013. Cacao propagation practices and distribution of planting material in Central America. Bioversity International Workshop on Cocoa Propagation, London, 13-15 May 2013.

Grafted rootstocks enclosed with plastic bag | Growing scions 40 days after grafted | 8-month grafted seedlings after field transplanted.

Figure 5. Split grafting technique used by the medium scale clonal propagation facility at Bom Retiro Farm, Camacan, Southern Bahia, Brazil.

Source: Augusto Roberto Sena Gomes, 2014.

Rooting cutting bed and rooted seedlings in plastic bags in commercial nursery, Ecuador

120-day-old seedling rootstocks, Lagoa Pequena Farm, Ilheus, Bahia, Brazil

Nursery grown 150-day-old seedling rootstocks in plastic bags, Ceplac, Ilheus, Bahia, Brazil

60-day-old seedling rootstocks in Sta Tereza Farm, Urucuca, Bahia, Brazil.

Cabruca system of planting in Renascer Project, Ilheus, Bahia, Brazil

Six-year-old grafted plants of clone CCN51 in Cabruca System, Renascer Project, Ilheus, Bahia, Brazil

Two side top splice graft on field seedling rootstock, Sto. Antonio farm, Ilheus, Bahia, Brazil

Crown side graft on five-year-old Sto. Antonio farm, Ilheus, Bahia, Brazil

Rehabilitation through top-grafting onto basal chupons in Teixeira Freitas, Southern Bahia, Brazil

Figure 6. Cocoa conventional propagation methodologies and facilities used in Ecuador and Brazil.

Source: Augusto Roberto Sena Gomes, 2014.

Side / lateral grafting in the nursery

Splice grafting

Side / lateral grafting
in tree trunk

Figure 7. Cocoa conventional vegetative propagation in Costa Rica.

Source: Phillips-Mora W. 2013. Cacao propagation practices and distribution of planting material in Central America. Bioversity International Workshop on Cocoa Propagation, London, 13-15 May 2013.

Top grafting with plastic top cover

Top grafting with biodegradable plastic

Figure 8. Cocoa vegetative propagation utilized by farmers in Malaysia.

Source: Kelvin Lamin, 2013. Cocoa planting material - propagation and distribution in Malaysia. Workshop on Cocoa Propagation, Bioversity International, London, 13-15 May 2013.

Large scale cocoa rootstock nursery

Nursery facility using bamboo as scaffold, with wire supported shade cloth (saran)

Top/split grafts in small scale nurserygrafting tents

Top/split-grafting tents (10 grafted seedlings each) inside a small scale nursery.

Figure 9. Cocoa propagation facilities, seedling rootstocks, and top-grafting method utilized in the Philippines.

Source: Cocoa Nursery Manual Philippines June 2011. © ACDI/VOCA. (http://aboutphilippines.ph/filer/Phils-Cocoa-Nursery-Manual-2011.pdf)

Scion cut for top grafting

Nursery top grafted rootstocks

Top grafted basal chupon

20-month field grafted plant, clone Cepec 2002

20-month field top grafted plant, clone BN-34

Figure 10. Top/split grafted nursery seedling rootstocks, basal chupon, and field grown seedling rootstock in Bahia, Brazil.

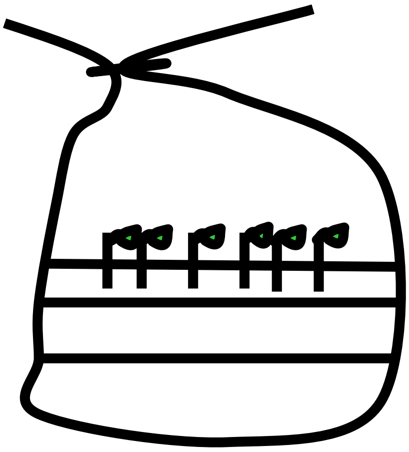
Source: Augusto Roberto Sena Gomes, 2014.

Orthotropic two nodes scion stick after 21-day in a rooting chamber showing callus formation (top left); 40-day-old fan green cuttings showing formation of adventitious fibrous roots (top middle and right); 60-day rooted brown cutting at Nova Felicidade Farm, Ilheus (middle); mass propagation facilities (bottom left) and a 50-day rooted cutting from green tip clonal stick (bottom right) at Biofabrica, Ilheus, Bahia, Brazil.

Six-year-old rooted cutting plants, after field transplanted clones PH16, CCN51, PS1309 (from left), Project Lembrance, Eunapolis in Southern Bahia, Brazil.

Figure 11. Rooting, facilities used for medium-large scale clonal propagation and rooted cutting planting in Southern Bahia, Brazil.

Source: Augusto Roberto Sena Gomes, 2014.



The poly-propagator was designed to be a big poly bag in a wooden box. If it is a watertight / airtight bag, there should be no need spray the cuttings for months, except when you open the lid and let the humidity out

Poly-bag propagator (top)

Non-mist conventional plastic cover propagator unities (left), and planting cuttings (right). Top poly-bag propagator is 100% air-tight; sprayers for period when lid is open; it uses shade of about 30% and ambient irradiance (R.B. Leakey, 2013 - Personal Communication).

Figure 12. Different models of non-mist rooting propagators.

Source: Augusto Roberto Sena Gomes, 2014.

Preparing marcott – ring of bark removed

Debarked area enclosed with plastic sheet (middle);

Marcott prepared with pine compost mixed with shreded coconut fiber and plastic covering the debarked area

Figure 13. Propagation of cocoa clones through marcotting technology.

Source: Augusto Roberto Sena Gomes, 2014.

ANNEX 5 – Summary of the status of application of methodologies

Table A5.1 Status of application of propagation methodologies in cocoa-producing countries.

Country	Propagation methodology	Scale of application	Remarks
Brazil - Bahia	Seeds	Predominant	Rootstocks (Comum variety), own selections
	Split grafting	Very popular	Rootstocks: chupons and field seedlings, and nursery seedlings (since 1999)
	Side grafting	*See remarks*	Onto young trees above jorquette; (rehabilitation)
	Rooted cutting	*See remarks*	In specific locations
Brazil – Pará and Rondônia	Seeds	Predominant	Bi-parental hybrids; own selections
Cameroon	Seeds	Predominant	Bi-parental hybrids; own selections
Colombia	Seeds	Predominant	Bi-parental hybrids; rootstocks, own selections
	Side grafting, lateral budding	Becoming popular	Onto nursery and field seedlings
Costa Rica	Rooted cuttings, seeds	Predominant	Bi-parental hybrids, own selections, rootstocks
	Budding, top grafting	Very popular	Onto nursery and field-planted seedlings
	Side grafting	Popular	Onto tree trunks of various ages (rehabilitation)
Côte d'Ivoire	Seeds	Predominant	Bi-parental hybrids; own selections
	Budding, side grafting	*See remarks*	Onto nursery rootstocks; field experimental trials
Dominican Republic	Seeds	Predominant	Bi-parental hybrids
Ecuador	Seeds	Predominant	Rootstocks CCN-51 and Nacional variety, own selections
	Rooted cutting	Very popular	Mainly CCN-51
	Side grafting	Popular	Onto tree trunks (rehabilitation); nursery, rootstocks, seedlings
Ghana **Ghana (cont'd)**	Budding	*See remarks*	Onto nursery seedling rootstocks for field experimental trials (grafting after 18 months
	Top grafting	*See remarks*	Onto field young trees (rehabilitation); field experimental trials
	Seeds	Predominant	Bi-parental hybrids; own selections
India	Seeds	Predominant	Bi-parental hybrids; own selections
Indonesia	Side grafting	Very popular	Onto nursery rootstocks; mainly tree trunks in the field (rehabilitation)
	Budding	Popular	Onto nursery rootstocks

Country	Propagation methodology	Scale of application	Remarks
Indonesia (cont'd)	Split and top grafting	Becoming popular	Onto basal chupons (gaining popularity); nursery rootstocks
	Rooted cutting	*See remarks*	Selected clones
	Seeds	Predominant	Bi-parental hybrids; own selections
Jamaica	Budding	*See remarks*	Onto nursery rootstocks
	Side grafting	*See remarks*	Onto tree trunks (rehabilitation)
	Seeds	Predominant	Selected hybrids; own selections
Malaysia	Side grafting	*See remarks*	Onto nursery seedlings; tree trunks in the field (rehabilitation)
	Budding	Very popular	Patch budding onto nursery seedlings
	Grafting	Popular	Top grafting onto nursery rootstocks
	Seeds	Predominant	Bi-parental hybrids; own selections
Nicaragua	Seeds	Predominant	Bi-parental hybrids; own selections
	Grafting, side grafting	Popular	Onto nursery rootstocks; tree trunks (rehabilitation)
	Budding	*See remarks*	Onto nursery rootstocks
Nigeria	Seeds	Predominant	Bi-parental hybrids; own selections
	Budding, grafting,	*See remarks*	Onto nursery rootstocks; field experimental trials
Papua New Guinea	Seeds	Predominant	Bi-parental crosses
	Clones	Small-scale to date	
Peru	Seeds	Predominant	Hybrid rootstocks; own selections
	Split, side, brown budding	Becoming popular	Onto nursery and field seedlings
Philippines	Seeds	Predominant	Bi-parental hybrids; own selections
	Side grafting	Popular	Onto tree trunks of various ages (rehabilitation)
	Budding, top grafting	Becoming popular	Onto nursery rootstocks
Sierra Leone	Seeds	Predominant (large - in relation to size of industry)	Bi-parental hybrids; own selections (Seed garden rehabilitation proposed)
Togo	Seed	At least some from bi- or poly-parental seed gardens	
Trinidad and Tobago	Rooted cutting	*See remarks*	Most TSH clones
	Seeds	Predominant	Bi-parental hybrids, selections

Table A5.2. Main characteristics of propagation by seeds.

Technology	Main advantages	Points for consideration, challenges and opportunities
General Seed is planted directly into the ground at its final growing position ('at stake') or raised in a nursery before planting out as a three- to seven-month-old seedling.	• Low-cost, efficient technology that is easily understood. • Seedling habit and structure (taproot, main trunk/jorquette/branches) is familiar to most farmers, including those in West Africa. • Seed is not known to transmit viruses, including cocoa swollen shoot virus (CSSV).	• Unless the mother tree is homozygous, or cross-pollination is carried out using pollen from a known pollen parent, seed-derived trees may be highly variable in their characteristics and performance due to both genetic and environmental factors. • Careful pruning may be required to prevent the tree from becoming too tall. • Care must be taken to avoid spreading pests/diseases present on pod husks when whole pods are distributed; good sanitation and/or phytosanitary treatments and training may be required to ensure that only healthy pods are distributed.
Locally sourced seed: Farmers use seed from their own or neighbouring farmers' trees for planting at stake, or raise seed in small local nurseries before planting.	• Seed is collected locally at no/minimal cost. • Minimal facilities, skills and resources are required. • Seeds/seedlings are easy to transport and distribute, and are only moved over short distances.	• Unless true-breeding traditional varieties are used, seed-derived trees may not have the desirable characteristics of the mother trees, and are likely to be highly variable in performance. • The benefits of using improved planting materials, instead of locally available types, need to be clearly demonstrated to farmers, and support mechanisms may be needed to cover the additional costs of the materials and/or loss of yield following replanting, before farmers are willing and able to adopt improved planting materials.
Seed from seed gardens: Pods are collected from clonal mother trees (open-pollinated) or, preferably, manual pollination is used to produce bi-parental seed. Seed/seed pods can be supplied directly to farmers, or seedlings are raised in an associated nursery.	• Recommendations for bi-parental crosses have been made in many countries/regions following local field evaluation. • Propagation by seeds is often the only method tested over the long term for locally adapted varieties, particularly in West Africa. • Once established, seed gardens can remain productive for over 40 years and their output can be improved by introducing new pollen parents for existing mother trees. • Costs are very low (open-pollination) to low (manual pollination). • Skills required for field/nursery operations and manual pollination are easy to acquire.	• Open-pollinated seed may not have the desired characteristics since even normally self-incompatible types can occasionally set seed with their own pollen. • Carefully managed mass manual pollination can result in high quality output. Molecular marker technology can be used to monitor seed purity. • Careful planning is needed to ensure that seed garden capacity matches demand especially in new cocoa-producing areas, since seed gardens require suitable land and may take up to seven years to come into full bearing. • Full integration with breeding programmes can ensure that seed gardens are ready to produce seed of new crosses as soon as the recommendations are made. • Seeds are typically only viable for up to three weeks after harvesting, and the time of seed availability and the natural seasonality of cropping do not coincide with the period of maximum demand in West Africa with its bi-modal rainfall pattern.

Technology	Main advantages	Points for consideration, challenges and opportunities
	• Seeds/ pods are easily distributed. • Seeds can be grown in plastic bags in associated nurseries for supplying to farmers when they have time, and the weather conditions are favourable for planting.	This can impact on transportation and distribution. • It may be possible to manipulate the seasonality of cropping, for example by frequent manual stripping of cherelles. In cases where the resulting crop develops through the dry season, irrigation may be required. • Enhanced awareness on the potential of seed storage, and better knowledge of seed storage practices, is recommended, as affordable short-term storage could make seed more accessible.

Table A5.3. Main characteristics of vegetative propagation methodologies.

Technology	Main advantages	Points for consideration, challenges and opportunities
Conventional vegetative propagation – general	• Plants will be genetically identical to the source material. • Recommended clones are available in Latin America and the Caribbean, and in South-east Asia. • Clones can be selected which have an architecture offering advantages of lower canopy height, and thus easier management, when propagated using plagiotropic material. • Clonal gardens can be established and managed to provide a ready source of vegetative materials for propagation in the nursery or in farmers' fields. • Rootstocks used for budding and grafting can be chosen to confer resistance to some diseases (e.g. *Ceratocystis).*	• Although genetically identical, the phenotype will vary to some extent with the propagation technique used, in addition to environmental factors. • Since the tree architecture and growth characteristics of a clone differ according to whether it is has been propagated from plagiotropic material or orthotropic material, it is important to select and evaluate clones which have been propagated using the same method as that which will be used for the eventual large-scale propagation for supply to the farmers. Very few clones have been fully evaluated for their performance in West Africa. Selection work to develop clones for use in West Africa is underway but it will be many years before a range of sufficiently well-proven clones is available. • Good sanitation is required in the clonal gardens and nurseries to increase propagation success rates and prevent the spread of pests/diseases. • Since vegetative materials can transmit viruses, including CSSV, regular screening/indexing of the source materials should be undertaken in areas where CSSV occurs, to reduce the risk of spreading the virus.
Seedling rootstocks: Seed to be used as rootstocks for budding/grafting may be produced in seed gardens (open pollinated or using controlled crosses) or obtained locally.	• Materials are low cost and easy to obtain. • A number of parental types are known to provide some disease control (e.g. *Ceratocystis* spp., damping off agents). • Indications thatsome scion-rootstock combinations improve establishment ability.	• Issues concerning variability and availability could be improved by controlled pollination and the development of seed storage technology/out-of-season production (as discussed in the seed garden section of Table A5.2 above). • Research could lead to an enhanced understanding of rootstock-scion interactions, and therefore better rootstock selection to optimize yield of the scion whilst maintaining beneficial characteristics such as disease resistance of the rootstock.
Top and side grafting: Grafting of orthotropic or plagiotropic scions, either onto a seedling in a nursery or directly onto field-grown material	• Generally high success rates for a range of clones and rootstocks. • Can be used on a variety of rootstocks, including seedlings, chupons, stems, trunks and	• These techniques use graftwood sticks with four to five buds per graft, so budding, which only uses a single bud per propagated plant, can be more efficient in terms of use of source material providing that success ('take') rates are

Top and side grafting (cont'd): (full details of the different grafting techniques and their applications are provided in Chapter 3).	branches. Relatively easy to carry out, at low to medium cost compared to other conventional vegetative propagation methods. • Grafting can be carried out in the field if graftwood can be obtained from a local clonal garden, and the rapid growth of the scion can lead to early improvements in the yield of rehabilitated trees. • Grafting can be carried out in nurseries with only basic facilities, and the rapid growth of the scion can reduce the time required in the nursery before planting.	comparable. • Plastic bags or biodegradable grafting tape are required; plastic cord may also be needed (e.g. side grafting in mature trees). • Well trained labour required.
Budding: A form of grafting where a single bud is grafted onto a seedling in a nursery or onto a field-grown plant (full details of the different budding techniques are provided in Chapter 3).	• Very efficient use of source materials since only a single bud is required and therefore can be used where the quantity of clonal material available is limited. • Minimizes wastage of seedling rootstocks since procedure can be redone if first budding fails. • Green budding, where single buds from young shoots are grafted onto very young seedling rootstocks, can be very efficient in terms of time in the nursery and use of source material. • Budding can be used to propagate clones using older, field-grown rootstocks (old trunks and high branches). • Technique can be used for virus screening (test plant is budded onto a rootstock such as Amelonado, which is known to show symptoms of some economically important viruses).	• Variable rates of take (low to medium rates) and slow post-graft growth, especially for some genotypes, can make the production costs higher than for other vegetative propagation techniques. However, where the systems have been optimized, including the management of the stock plants in the clonal gardens, and particularly where green budding can be used, budding can be highly efficient. • Training may be required to develop the necessary budding skills.
Rooted cuttings: Clonal plants are produced from orthotropic or plagiotropic cuttings, which are generally harvested in clonal gardens.	• No rootstocks involved so no possibility of graft incompatibility or outgrowth from the rootstock. • Procedure relatively easy to carry out; medium to high success rates noted for many clones. • Can be implemented in simple, low-cost, non-mist facilities on a small scale.	• Mass propagation through rooted cuttings technology demands large investment in facilities, inputs, trained workers and good administration. • Plants derived from orthotropic cuttings may develop a very low first jorquette, and if so, pruning is required to ensure a second jorquette forms at a more manageable height. • Plants derived from plagiotropic cuttings

Rooted cuttings (cont'd)	• Larger facilities, capable of producing several million cuttings each year, can be set up. • Once optimized for a particular clone, mass propagation systems can offer higher multiplication rates and lower costs than some other conventional vegetative propagation techniques.	of some genotypes may develop open and spreading crowns that require formative pruning. Clones which show this type of architecture are normally eliminated at an early stage in the selection process where the intention is to use plagiotropic cuttings as the method for mass propagation. • The development of the rooting system of plagiotropic and orthotropic cuttings is probably affected by genetic factors in addition to rooting conditions soon after the cuttings are taken, whilst the plants are in the nursery and after field planting, though many of these factors are yet to be elucidated • Cuttings that only form fibrous root systems, with little or no development of taproot-like framework roots (due to either genetic factors or rooting conditions) are less suitable for steeply sloping sites but may still be useful for flat sites with deep, well-drained soil. Further breeding/selection work is needed in order to develop clones that have the appropriate rooting characteristics and architecture appropriate for the intended mass-propagation method.
***Marcotting/air layering*:** Partially cut stems or branches are encouraged to root by covering in damp media and wrapping.	• Simple methodology. • Reliable for propagating hard-to-root priceless trees. • Can multiply ontogenetically mature shoots. • No special facilities are needed. • Low level of skills required. • Suitable for small-scale use on farm.	• Low rate of 'take'. This method is expensive, due to the high labour involved and slow multiplication rate, and is not suitable for large-scale operations. • Applicable only when other more efficient propagation technologies cannot be utilized (see Chapter 3 for detailed information on several other propagation techniques and their applications).

Table A5.4. Main characteristics of vegetative propagation using tissue culture (somatic embryogenesis).

Methodology	Main advantages	Points for consideration, challenges and opportunities
***Vegetative propagation using tissue culture (somatic embryogenesis)*:** Production of clonal plants with a seedling habit through floral tissue culture.	• Plants will be genetically identical to the source material. • Recommended clones are available in Latin America and the Caribbean, and in South-east Asia. • Trees grow uniformly with a consistent jorquette height. • Trees propagated using this method usually come into bearing more quickly than seed-raised material. • Method has been shown to work for a number of clones that are recommended in Latin America and the Caribbean, and in South-east Asia. • Method is useful for rapidly increasing stocks of plants where only a limited amount of material is available. • Tissue culture can be performed year round in a laboratory setting. • Method can be used for the production and testing of disease-free materials. • Somatic embryogenesis may reduce risk of CSSV transmission.	• Although genetically identical, the phenotype will vary to some extent according to environmental factors and the propagation technique used. • Since the tree architecture and growth characteristics of a clone are likely to differ according to whether the tree has been propagated using plagiotropic or orthotropic material, it is important to select and evaluate clones that have been propagated using the same method as that which will be used for the eventual large-scale propagation for supply to the farmers. • Very few clones have been fully evaluated for their performance in West Africa. Selection work to develop clones for use in West Africa is underway but it will be many years before a range of sufficiently well-proven clones is available. • Some genotypes are less likely to form somatic embryos than others – the technique may have to be optimized for specific clones. • Care must be taken to reduce risk of somaclonal variation (which is more likely to occur in older cultures or certain genotypes). • Laboratories with tissue culture facilities, and nurseries suitable for acclimating plantlets involve high capital costs/running costs. • The trees need to be producing flowers to be cultured. • Technique requires highly skilled technicians.

ANNEX 6 – References for further reading

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ISBN: 978-92-9255-009-7

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