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Cocoa propagation, technologies for production of seedlings

George Andrade Sodré[1] & Augusto Roberto Sena Gomes[2]

Abstract- The vegetative propagation in cacao has been used in Central America since the beginning of the last century. However, only from the end of the century this technique has been intensified in the state of Bahia, where vegetative propagation changes have increased the yield of cocoa growing areas, especially those using other technologies associated with clonal seedlings. The use of clonal garden to collect stems in nursery can reduce by 80% the space required for maintenance of stock plants. On the other hand, the buds obtained from orthotropic and plagiotropic shoots are used to improve the performance protocols and reduce production costs of seedlings. Among the current and future challenges in the production of cocoa seedlings emphasizes the somatic embryogenesis and rootstock with disease resistance. These technologies to increase the productivity of the crop and permit Brazil return to the exporter condition of cocoa beans, which does not occur for more than two decades.
Index terms: *Theobroma cacao* L, clones, grafting, nursery.

Propagação de cacaueiro e tecnologias para produção de mudas clonais

Resumo - A propagação vegetativa em cacaueiro foi iniciada na América Central no início do século passado. No entanto, somente no final do século essa técnica foi intensificada no estado da Bahia onde se verificou que mudas de propagação vegetativa elevam o rendimento de áreas de cultivo do cacaueiro, especialmente aquelas que usam outras tecnologias associadas às mudas clonais. O uso de jardim clonal para coletar hastes em viveiro pode reduzir em até 80% o espaço necessário para manutenção das plantas matrizes. Por outro lado, estacas de uma gema obtidas de ramos plagiotrópicos e ortotrópicos melhoram os protocolos de enraizamento e reduzem custos de produção das mudas. Dentre os desafios atuais e futuros da produção de mudas de cacaueiros destacam-se o uso da embriogênese somática e a escolha de porta-enxertos com resistência a doenças. Essas tecnologias buscam elevar a produtividade do cultivo e fazer o Brasil retornar a condição de exportador de amêndoas e derivados do cacau, o que não ocorre há mais de duas décadas.
Termos para indexação: *Theobroma cacao* L, clones, enxertia, viveiros.

Corresponding author:
gasodre@hotmail.com

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[1]Researcher MAPA- Ceplac, Itabuna-BA, Brazil E-mail: gasodre@hotmail.com(ORCID 0000-0003-0711-8637)
[2]Researcher MAPA- Ceplac, Itabuna-BA, Brazil - E-mail: arsenag@hotmail.com (ORCID 0000-0003-2963-5985)

Introduction

In the early 20th century, when the cocoa plantation initiated a stronger cycle of worldwide expansion the cocoa planting was performed by the method called "beak of machete." In this form of planting, a small planting holes were open using the machete and, after open the fruit and the partial withdrawal of the pulp, three seeds were sowed and covered up with soil. After germination a seedling was cut and after12 months removed another leaving the most vigorous to be conducted and form the new cocoa plant (SODRÉ, 2013).

Even in the last century, during the decades of 1970 and 1980 the Brazilian producers implanted more than 150 thousand hectares of cocoa using seminal seedlings in polyethylene bags, following recommendations of Executive Comission for the Cocoa Farming Plan CEPLAC, due to the results of research work showing that it was possible to prepare vigorous seedlings in less time. According to Sodré (2013), the recommendations for preparing the seedlings of hybrid seeds included the utilization of protected nurseries, polyethylene bags, fertilizers, foliar fertilization and pest control.

The vegetative propagation in cocoa has been little disseminated in Brazil due to good productions obtained by hybrids and varieties propagated by seeds (DIAS, 2001). However, due to sanitary problems since the 1990s, there was a need for increased planting of cocoa Trinitarians, more resistant, and fix the genetic characteristics in a short time.

It has been consensus among researchers in cocoa plantation which would not be possible to replant large areas of cocoa, in many countries, using a single technology. In general, the majority of the producing countries utilize conventional propagation (seeds and grafting) for the rehabilitation and or to expand the planting areas. However, it is quite clear that some methods are more preferred than others.

Although the technology of rooted cuttings is limited to countries such as Brazil, Ecuador, Nigeria, Philippines and Indonesia, it should highlight the use of different methods in the same country or region as has been observed in Brazil, for example, intensive planting of seminal seedlings are utilized in Southern State of Pará, where clonal planting and grafting on cocoa plants in the field are preferred by producers in the Southern State of Bahia.

The research in genetics and plant breeding in Brazil and in other producing countries should continue to produce new varieties of cocoa, for attending the growing increase demand. Currently, there is already a large demand for seedlings in Brazil in the, which clearly indicates the need for large-scale propagation and it has been estimated by millions of seedlings annually. Thus, production of seedlings will be increasingly necessary. In this context, and disease resistant clones, in addition to techniques for production of seedlings will also be improved.

Vegetative propagation of cocoa

According to Mooleedhar (1998), vegetative propagation of cocoa tree was propagated with success in Jamaica, by the budding method in 1902. Later, Pyke (1933) developed the technique for the production of rooted cuttings, method that was also used by Evans(1951), Alvim (1953), Alvim and Ovidio (1954), and Murray (1954). Also, improvements in propagation methods were developed by the College of Agricultural Sciences (IICA / OEA) in Turrialba, Costa Rica and Research Station in Agriculture Pichilingue, Ecuador.

Other important contributions for the vegetative propagation of cocoa were made in West African countries, especially Ghana, by Richards (1948), Archibald (1955), MacKelvie (1957) and Hall (1963). Evans (1953), observed variation in the ability of rooting between clones and the influence of mineral nutrition and health of mother plants and also pointed out that the position of the cutting (apical or subapical) and the length are important factors in rooting, especially because interfere on yield and quality of the seedlings roots.

According to Leite (2006), the ability of rooting in cocoa cuttings change with the season of the year in which they were collected. SENA-GOMES et al. (2000) tested types of cuttings (woody and semi-woody) in clones resistant to the witch's broom and the results showed that the genotype has a strong influence on rates of rooting and survival of the plants. These authors also identified clones with average rates of rooting above 70% while others were lower than 30%.

Works performed by Sacramento et al. (2001) and Sacramento and Faria (2003),on rooting of clones, in mist chambers and treated with indolbutyric acid (IBA), showed that the rates of rooting for some clones were greater than 87%, and also observed that the beginning of the emission of roots occurred between 20 and 30 days after the treatment.

Rootstocks in vegetative propagation of cocoa

The rootstocks are used in many species to minimize restrictive effects on the production of plants in the field, for example, soil compaction and infection by pathogens from soil. In addition, the rootstock can affect the fruit quality and plant height (Hartmann et al., 2002). In the case of cocoa tree, it should be emphasized that the majority of the producing countries uses as rootstock seedlings grown from seeds of open pollination. The rootstocks are usually obtained from pre-germinated seeds which are sown directly in the field, or in polyethylene bags filled with soil or agricultural substrate and remain for 6 to 8 months until they are grafted (Figure 1).

The purpose of rootstocks on seedlings in cocoa may vary with clone and environment. For example, experimental results in Trinidad indicated that there was no evidence of incompatibility, caused by the interaction rootstock / graft, as mentioned by Mooleedhar (1998). By contrast, Cheesman (1946) has been found that rootstocks consistently reduced the average yield in clones ICS (International College Selection). In conventional cultivation during five-year Efron et al. (2003) reported that the grafted on rootstock with dwarfism (MJ12-226) resulted in plants with short internodes, size of multiple stems, reduction of roots and leaves and the vigor of the trees in the early years, however, this effect is of short duration and the grafted plants reverted to a pattern of normal growth after some time.

Twelve families of cocoa used as rootstocks were evaluated by Yin (2004) in Sabah, Malaysia, using three commercial clones. The results showed that the yields on the rootstocks pure genetic type "Scavina" were about 10% above the average of clones "Amelonado" and also that the rootstock did not influence the bark weight, number of seeds per fruit and the uniformity of the trees.

The purpose of rootstock on plants in the field was reported by Irizarry and Goenaga (2000) in Puerto Rico. The results indicated that, on average, only 45% of the initial yield of cocoa was a result of grafting and the difference of 55% was attributed to genetic and environmental factors, and possibly the degree of incompatibility rootstock graft. The authors also added that the relationship between the canopy and rootstock in selections of the family TSA x SCA-12 does not favored the increase in production in the field, in comparison with selections of other families.

Rootstocks can influence the rate of survival of grafted seedlings as reported by González et al. (2003). These authors showed that rootstocks of ICS-39, Pound-4, Pound-7, Pound-12 and Catongo from seeds, grafted with descendants of UF-677, significantly altered the survival rates of seedlings in greenhouse. A higher percentage of survival was recorded for rootstock Pound-4. The authors concluded that the survival rates of rootstocks were influenced by several factors, including the genotype, type of graft, practices of grafting and environmental conditions.

Marinato et al. (2006) testing four rootstocks of cocoa four months age, grafted with a single clone and two methods of grafting in a greenhouse at CEPLAC in Espírito Santo State, Brazil, found that the survival rates of grafted plants were not influenced by the method of grafting. However, these authors found that the rootstock ESFIP-02 significantly increased in the vegetative growth of grafts of the clone TSH-1188.

Another important effect of rootstock in cocoa seedlings is related with the transport of nutrients. Sodré et al. (2012) found that the combination canopy/ rootstock interfered in the transport of nutrients from root to shoot. The clone Salobrinho-2, grafted on a selection of Forastero cocoa "common" used as rootstock, accumulated significantly less N, P and K in leaves when compared to the clones CCN-51, ICS-1, CEPEC-02 and CP-49.

In the American countries it is common to use rootstocks for control of soil borne pathogens. For example, rootstocks of TSH 1188 and varieties of "Cocoa" are used by Brazilian producers to control *Fusarium spp*. Cocoa farmers in Colombia using the clones PA 46, PA 121, PA 150, IMC 67, and the hybrid seeds of PA 46, to control of root rot caused by *Phythopthora spp*. and *Roselinia* (Florez and Calderon, 2000). In Nigeria farmers use rootstocks selected and, according to Adewale et al. (2013) the genotype group Amazon is more used due to resistance to diseases.

Sena Gomes and Sodré (2015) commented that in the state of Bahia, Brazil, during the 1990s the use of rootstocks not tested for diseases caused considerable losses of cocoa plants. Several areas planted with rootstocks from clones CCN-51, PH-16, PS-1319, and others from crosses involving ICS-1, have similar risk. Recent research with Forastero variety "cocoa jaca" showed excellent results for resistance to *Ceratocystis spp*. (SILVA et al., 2010).

Rootstocks of cocoa can be propagated by seeds, cuttings and tissue culture, as mentioned by Westwood (1993). However, the vast majority is still obtained from seeds of open pollination. This is because it is easy and low cost to obtaining seed production areas of the farmer himself, as well as for the preparation of seminal seedlings. Although there are many studies published on the topic rootstock, this issue is not yet well understood and particularly the mechanisms involving the physiology of the graft and the rootstock in cocoa. Thus, long-term studies that show the purpose of rootstocks on the agronomic performance of grafted seedlings need to be expanded and include more field experiments, since the majority of reported results on this question refer to short-term studies, conducted mainly in the greenhouse.

It is also important to investigate and determine which variables can be used to selecting rootstocks, including dwarfism. Rootstock dwarfism can control part of the size and shape of the plant in the field, as well as improve productivity. In a recent review on the subject, Lockwood (2013) mentioned that the effect dwarfism has attracted the attention of researchers and this is important for two reasons: a) when associated with the improvement of production efficiency the rootstocks can maintain or increase commercial production, b) dwarfism let's make crops "friendlier", with all the fruits of a tree in easy reach and even simplifying the management of pests.

Changes in stomatal resistance and sap flow have been associated with the effects of force of rootstocks, as suggested by Simons (1986). There is considerable evidence, in perennial species, that the root systems have qualitative differences in the anatomical structure of the xylem and this may be related to the potential of the root system of rootstock dwarfism in relation to graft, perhaps by reducing the flow of water in the trunk as emphasized by Beakbane and Thompson (1947). Root systems in dwarf plants have a low xylem to and phloem ratio, while the opposite is true for systems that promote the growth of canopy, which have xylem tissue with more and larger vessels (BEAKBANE; THOMPSON, 1947). The consistency of this reasoning suggests that it can be used in protocols for selecting rootstock in cocoa.

Because of the wide variation in growth, vigor and disease resistance, rootstocks of half-sib cocoa seedlings, especially collected in a mix of hybrid plants were not selected, as an option to minimize overall risk. Thus, it would be safer to obtain seeds from trusted sources, such as seed gardens of government and or accredited private organization seed which produce proven combinations of hybrid seeds or selection families, especially for the control of major diseases in certain regions.

In general, the production costs of rootstocks of cocoa (seedlings) vary greatly between countries and in accordance with the scale and protocol of production, cost of labor, time in the nursery, size of plastic bags, substrates, irrigation system, management procedures and administration. To produce rootstocks on a commercial scale, Brazil requires certification by the Ministry of Agriculture Livestock and Food Supply (MAPA). In addition to legal procedures of production, the seed garden administration has to pay attention to important other aspects of production projects, such as: synchronization between demand and production volume, supply and quality of irrigation water, calendar and rates of foliar fertilizers, pest and disease control, elimination of atypical and sick seedlings, as well as quality of grafting. Table 1 shows the advantages and restrictions on use of the graft with seminal seedlings

Budding graft

The budding graft is one of the most important methods of propagation of plants and refers to the transfer of a tissue containing a single bud plants (variety selected) for rootstocks. In order to ensure the success of budding the bud must be placed in contact to allow the development of functional links between the vascular tissues of the bud with the corresponding tissues of rootstock. As discussed by Hartmann et al. (2002), the physiological processes of recognition of cells, the formation of the callus, as well as the differentiation of vascular tissue and parenchyma are critical steps in the formation of the graft. The budding technique makes use efficient of graft material, because it needs only a single bud to graft a rootstock, contrasting with traditional methods of grafting that requires several buds (Figure 2).

The first experience with grafting budding in cocoa was held in Jamaica in 1902 (Mooleedhar, 1998). Today it is still used in countries as: Peru, Haiti, Costa Rica, Jamaica, Ecuador, Brazil, Malaysia, São Tomé, Nigeria, Ghana, Ivory Coast and Indonesia. In general, the budding graft has been applied to multiply parents selected in breeding programs and becomes very important when the amount of material of a given clone is limited, for example, during the introduction of the new botanical material in quarantine.

The results of the budding in cocoa vary according to stage of maturation of the bud, quality of rootstock, environment and management of the process. In Malaysia (LEE, 1998) obtained good rates of survival with the use of budding in seedlings of four months of age. However, according to Yow and Lim (1994) budding applied to rootstocks seedlings of three months of age, takes at least six months to produce seedlings able to go into the field. These authors also emphasized the high costs to prepare and maintain grafted seedlings in plastic bags for a long time.

Researches aiming to reduce costs of conventional budding and the time that the seedlings remain in the nursery were performed by replacing it with budding green. For example, Rosenquist (1952) and Jacob (1969),tested successfully budding graft in cocoa seedlings in cotyledons while Giesenberger and Coester (1976) describe the green bud method applied to hypocotyls of from two to six weeks of age with successful from 90 to 100%. Also compared the methods "T" and inverted "U" noting that the latter was more suitable. Yow and Lim (1994) made extensive studies on budding in cocoa and reported good results for budding green in rootstocks from two weeks of age in a nursery in Sabah. In contrast, Are (1967) in Nigeria, compared rootstocks of seedlings from 18 months of age and found that the clones tested in canopy had better performance in rootstocks older.

Techniques of grafting involving rootstocks young (seedlings) have shown good results. For example, Ramadasan and Ahmad (1986) have budding below of cotyledon with success ranging from 70 to 80%. It is a method especially promising because there is a drastic reduction in the age of the rootstock for only 10 to 20 days. These authors also emphasized that the reserves of nutrients available in the cotyledons of rootstock would allow earlier bud to develop making the grafts more vigorous. Emphasize, however, cost increase to the extent that the grafted plants require the humidification intense until the union of the graft.

The bud grafting was tested by CEPLAC until mid 1990 and the success rates ranged from 50 to 60%. However, this modality has not been used commercially as a methodology of clonal propagation in Bahia or in any other producers in Brazil. This occurred because the method requires skill of graft and good sanitary condition and nutritional of the bud. The restrictions also include the low rate of survival, especially due to contamination before and after budding and very slow start of seedlings. Among the general guidelines to ensure success for seedlings produced by budding include: a) Rootstocks aged from sixteen weeks produced better results than the rootstocks younger, b) bud ranging in size from 1.5-2.5cm length were ideal, c) the watering should be in the form of fine mist in order to avoid any disturbance to the seedlings budded.

It is important to mention that the deterioration of buds in the budding method in cocoa is caused by a complex of fungi. A transfer of accesses of cocoa to the quarantine station in Miami, Florida, USA, were identified five fungi associated with buds in cocoa as: *Botryodiplodia theobromea*, *Fusarium decemcellulare, Fusarium oxysporum*, *Pestalotiopsis spp*, and *Phomopsis spp.*, which can trigger death of grafted seedlings of cocoa (Purdy, 1989). Table 2 presents advantages and restrictions on use of budding in the production of cocoa seedlings.

Split grafting

The split grafting in cocoa refers the manner which a branch containing several buds is inserted in a fraction of top of the plant or laterally under the bark of rootstocks. It can also be performed in basal shoots and branches of young trees in the field and in the nursery (Figure 3). The same principle to the budding also applies to all forms of graft, i.e., intimate contact of tissues and external protection against dehydration. The contact of tissues favors the development of functional links of xylem tissue and phloem of the canopy with the rootstock (HARTMANN et al. 2002). These authors also point out that the physiological processes of recognition of cells, the formation of the callus, as well as the differentiation of vascular tissue and parenchyma are critical processes for the formation of the graft.

It is considered that there are many methods of grafting, however, only the methodologies of commercially important are discussed in this review. The split grafting, for example, can be done both in basal stems of cocoa adults as in rootstocks seedlings in the nursery (5 to 8 months of age). The grafting is the most used by producers to propagate clones in Bahia, Brazil, as well as in other regions producing cocoa in South America, Central America, Caribbean, West African and Asian countries. Sena Gomes et al. (2000) Using split grafting obtained rates of survival from 55 to 92%, varying with clone, place of grafting and season of the year and found that grafting has been more successful during the months of september to march in years when weather conditions typical of the region.

Some applications of the method of grafting are not able to provide acceptable results. For example, studies in Malaysia by Ramadasan and Ahmad (1986) using rootstocks of 2 to 4 weeks of age and side grafting showed reduced rates of survival. The authors observed that the connection was very difficult because the wedge tends to slip out, due to the presence of viscous fluid along the fissure tended to "squeeze" the graft. These results were also reported by Giesenberger and Coester (1976), as well as by Rosa (1998), in Bahia, Brazil, which recorded success rates in the grafts of 3 weeks of only 35%.

The cleft side is the method in which the graft is cut obliquely (laterally) wedge-shaped thin and pushed down and inside the stem bark of rootstock. The lateral grafting performed on the trunk of mature trees of cocoa, as described by Yow and Lim (1994), was used at the beginning of the program in clonal Bahia (Pinto, 1998; Sena Gomes et al., 2000) to replace plants with high rates of infection by the witches' broom disease of witch (*Moniliophthora perniciosa*). The rates of survival, however, were low when applied on the trunk (<30%), but high (> 70%) when applied in basal shoots. The low rate of survival made the grafting in trunks of cocoa trees was not longer recommended in Bahia, Brazil (SENA GOMES and SODRÉ 2015).

A variation of the conventional method of grafting was used to propagate clones in Ecuador, where grafts in wedge-shaped double-face and length similar to the depth of the side are performed in seedlings of 3-4 months of age, being inserted laterally and tied with a strip of plastic. In Espírito Santo state, Brazil is common to carry out the grafting by cleft side with two subsequent cuts the canopy after 21 and 40 days. The rate of survival is 80% and the average income man day is 450 grafts (Figure 4).

Grafting on canopy

It is grafting in the main branches of the canopy, above the "jorquette", known as a replacement of the canopy and aims to rapidly restore the yield of cocoa trees susceptible to diseases. Only the branches located above the grafting point are removed by pruning, after about six months from date of grafting, time when the clonal foliage is partially established. The crown grafting is commercially suitable for the replacement of the tree foliage and part of branches under specific conditions and considering the age of the tree. This method has been well accepted by farmers in Bahia as a way to improve the production of cocoa trees under 15 years old. On branches of the canopy, the technique follows the same grafting protocol, but using 2-4 branches per cocoa tree.

The technique of side grafting of canopy on cocoa was successful in Indonesia, Malaysia and the Philippines, with methodology described by Yow and Lim (1994), in rehabilitation programs of plants. In Indonesia, for example, plantations of cocoa that in the period 1998 - 2003, In South and Southeast Sulawesi had productivity of about 1,200 kg/ha/year in 2011 this productivity has dropped to 120 to 240 kg/ha/year. Several factors have been associated with this decline, especially age of the tree, lack of crop management (weed control, fertilizing, pruning and insect pests and diseases). To overcome the decline a government program for rehabilitation of plants was carried out using the technology side grafting applied in shoots of old trees, as described by Yow and Lim (1994). The field observations showed that the success of side grafting reached approximately 60% of farmers.

The acceptance by farmer of the grafting in canopy, also called “technique of salvation” is related to the fact that the process of flowering in fruit species is not interrupted, only being reduced during the replacement of the old canopy of the trees, once that occurs in a overlapped manner with the development of new leaves from the clonal scion (SENA GOMES; CASTRO, 1999). This is so because parts of the former canopy (branches and foliage) are only eliminated about six months after grafting, and this time the leaf area of the new canopy is already well developed. The grafting of canopy in general takes 12 to 16 months to rebuild the new leaf area of cocoa tree (SENA GOMES and SODRÉ, 2015).

Characteristics of the rooting in cocoa tree

The production of seedlings by rooted cuttings is a technology universally recognized for clonal propagation of numerous species of plants, including the cocoa. The facilities and methods for rooting vary from simple to highly sophisticated; however, the anatomical and physiological principles involved in the process are common to all species. For example, growth of adventitious roots from the base of a bud sticks, in an environment of high humidity, are developed from the pericycle (region of vascular cylinder) at the base of the stem, just above the cut, as shown in Figure 5 B.

The main advantage of the cuttings is that the plants are identical in all respects to the unique individual, or parent plant, as emphasized by Kramer and Kozlowski (1979), and they can be planted directly without use of rootstocks. Details of anatomy, physiology, and the environmental factors involved in the rooting of forest species and vegetables were discussed by Hartmann et al. (2002) and Leakey (2004).

The production of cocoa seedlings by cutting was described and tested in the state of Bahia for the first time by Fowler (1955). Recently, technical characteristics and methodologies were also described by SENA GOMES and CASTRO (1999), MARROCOS et al. (2005), SODRÉ and MARROCOS (2009), SODRÉ (2013) and Sena Gomes and Sodré (2015). According to HALL (1963), clones of the “Alto Amazon” and the Trinitarian root better than the “Amelonado”, while TOXOPEUS (1970) emphasized seasonal differences of rooting among clones. WOOD and LASS (1985) mentioned a list of factors that affect the rooting of cuttings, with emphasis to genetic type, management of nurseries, plant growth regulators, environmental factors (temperature, light and humidity) and means of rooting.

Hartmann et al. (1997) emphasized that in general the cutting of the apical position of woody species, due to the intense meristematic activity, are those that present higher levels of rooting. When it comes to cocoa, CHEESMAN (1935) and EVANS (1953) observed that younger mother plants root better. In this context, SODRÉ, (2007) found that cuttings of cocoa from the apical positions improve the ability of rooting of the juvenile material. Consequently, it was observed a greater productivity per plant matrix, increased levels of rooting, as well as reduction of cost of production of seedlings.

It is also important to mention that although the majority of studies of the cocoa propagation have been developed in plagiotropic branches, the methods in general are also applicable to the orthotropic material, since there is no evidence of real difference between the two types of growth, as mentioned by CHEESMAN (1935).

The technology of rooting of cocoa in Brazil has been advancing in terms of environment, facilities and logistics. At the very beginning, sand was the basic substrate for rooting of cocoa. Started with the use of using sand as substrate for rooting (ALVIM, OVIDIO, 1954; PYKE, 1933).Thereafter, the method was evolved to utilization of bag and cutting planted in pots containing a mixture of sand and organic matter and covered with canvas of polyethylene bags (MURRAY, 1954; BURLE, 1957; MACKELVIE, 1957; LEWIS, 1960). More recently, for the production of seedlings of type “mudão”and mini clonal garden in pots (SODRÉ, 2013). An important advancement for large-scale propagation of cuttings was obtained with the rooting in plastic tubes, use of commercial substrates and mist chamber equipped with fertigation system as described by SENA GOMES et al. (2000).

The success for production of cutting in cocoa tree is influenced by environmental factors, among which stand out the temperature, humidity and means of rooting. Light intensities are generally not associated with rooting, although cuttings with leaves can produce roots in the darkness and the high intensity of light causes the leaf fall. The temperature normally interferes with the quantity and quality of callus, as well as the quality of the root system. Considerable variation in rooting occurs among clones, seedlings of the same clone, parts of a same plant and places in the same nursery. ARCHIBALD (1953), suggested that the photosynthetic efficiency of the leaf seems to determine the survival of the cuttings. However, as mentioned by LEAKEY (2004), the interactions between factors (plant, environment and management) guarantee a good rooting.

Amoah et al. (2006),observed physiological factors affecting the rooting in cuttings of cocoa tree. Among the relevants, this author emphasized the importance of the anatomical components, the nutritional status of the cutting and the position in the nodal plant stock. The results suggested that rooting of cuttings is marked by a well-differentiated vascular region, high level of sugar content and the C:Nratio.

Research conducted in Cocoa Research Institute in Ghana (CRIG) shows that the roots were significantly affected by the nodal positions of the bud stick.. The best rooting happen with material harvested in nodal positions 4-6 from the apex of 15-year-old tree and cocoa type “Amelonado”. Report showing differences in the performance of rooting in cuttings of cocoa in relation to the cutting position in plant has also been published by RAMADASAN and AHMAD (1986) that also highlighted the diameter of internodes, lignifications of the stems, nutrients and carbohydrate content, as important criteria for the selection of cuttings in clonal gardens.

Dimorphic characteristics of growth during the rooting of cuttings of cocoa were recorded in Trinidad by PYKE (1933), CHEESMAN (1935) and EVANS (1951). Since then, the producers have recognized some negative aspects of the plantation of cocoa rooted of plagiotropic branches. In general, the cocoa trees from plagiotropic branches develop a dense canopy and require repeated pruning to form the canopy and facilitate the management and harvesting. MILLER (2009), mentioned that plagiotropic branches develop characteristics such as susceptibility to lodging and moisture stress, possibly due to the combination of formation of canopy unbalanced and inaccurate root system development. LEE (1998) added that the lack of taproots in plants of plagiotropic branches is considered a disadvantage, especially during the dry season. In addition, it is well known that a system of fibrous roots and shallow can limit growth in the production and the survival of cocoa in conditions of water stress.

Murray (1961) in Trinidad and Tobago noted root systems of plants cultivated in the field from cuttings which were similar to those produced from seed. In another study, this author verified the performance of plants of clones ICS- 95 (cuttings) and ICS-1 x SCA-12 (seminal seedlings) cultivated in rectangular boxes of 0.5 m width and length of the base and 1,07m high, and noted that 15 months after planting, the mean of the fresh weights of clonal or seminal seedlings were similar, however, the clone had a higher proportion of roots and leaves in relation to the total weight.

In the absence of a taproot in plants propagated by cuttings, two or three adventitious roots tend to develop. Normally, lateral roots working as “anchor roots” then arise from those adventitious roots. It should be noted that often the lack of taproot in rooted cuttings can stop the growth and productivity of the plant during the dry season, especially in shallow soils and with high levels of aluminum, resulting in losses of crops and mortality of trees.

It is technically important the production of rooted plants from orthotropic shoots. This is so because the trees from this material will develop plants in a similar manner to a seminal plant, with the same structure of branches(GLICENSTEIN et al., 1990). These authors reported that the roots produced in orthotropic branch sustain the tree making them less sensitive to water stress. MILLER (2009) emphasized that orthotropic cuttings require minimum demand in pruning by reducing labor costs during the establishment in the field. This author argues, however, why researches on orthotropic branch have not expanded as expected. It is well know that the main reason is the lack of material for large-scale production. However, same studies on the subject have been published. For instance, the production of cocoa clonal seedling from orthotropic material, using rooting cuttings method has been studied in Bahia (SODRÉ, 2013). Although the results in Trinidad and Malaysia have shown similar yield potential of clones grafted with orthotropic or plagiotropic bud stick, technologies of dissemination using orthotropic material must still be tested with large number of recommended clones available in different countries.

Some initiatives to overcome the shortage of orthotropic seedlings were initiated in Ghana since 1984. BERTRAND and AGBODJAN (1989) published methodologies to increase the number of orthotropic bud stick production, including severing the trunk of the tree and bending branches. These authors observed a good production of sprouts orthotropic after five months, on both methods cutting and bending. In the same line of work, GLICENSTEIN et al. (1990) and MAXIMOVA and GUILTINAN (2012) at Pennsylvania State University, USA and SODRÉ (2013) in Bahia, Brazil, presented results showing that the bending of cocoa tree branches in bow, tying the tops to the ground in a greenhouse, produced large number of orthotropic shoots on top and on the side of the warped trunk (Figure 6).

Production of cocoa seedlings by cuttings in state Bahia Brazil

In the state of Bahia, the mass propagation of clonal Cocoa seedlings using rooted cuttings is in operation since 1999. For the first time in the world, a unit of cocoa propagation produced millions of rooted cuttings using plastic tubes of 288 cm3,filled with substrates, fertilizer and acclimatized before planting in the field (SENA GOMES and SODRÉ 2015).

The production unit, known as the Biofactory Institute of Cocoa IBC, is located 45 km north of the Ilhéus city, Bahia and since 2000 has been propagating cocoa clones recommended by CEPLAC. The project was initially planned to meet the rehabilitation of 300,000 ha of plantations of cocoa, heavily infected by the witches’ broom disease and with low productivity. Currently, the unit has an annual capacity to provide up to near eight million of rooted cuttings.

The CEPLAC and State University of Santa

Cruz (UESC), ensure the scientific and technical support for the IBC project, since the year of 2000. The project has 20 units of rooting propagation "chambers", measuring 2,500 m^2 each, covered and protected laterally with polypropylene mesh (50% of interception of light), with automated system of irrigation. Each unit of rooting may contain 216,000 cutting in rooting for a period of six months (86 cutting/m^2/6 months). It is important to highlight a new initiatives projecting to increase the capacity of each chamber to about 700,000 cutting (280 cutting/m^2/6 months), what will be done with the reduction of the cutting length, utilizing smaller plastic tubes and by using lower density substrate (Jackson Oliveira Cesar, 2013 - Personal Communication).

The rooting of cuttings is the more costly method of production of cocoa seedlings, especially if compared with the seminal propagation. The costs of production are normally associated with the needs of labor and inputs and vary widely between countries. In Table 3 are presented the advantages and limitations of the propagation by cuttings in cocoa.

Minicutting in vegetative propagation of cocoa

As much as the researches on cocoa propagation by stem cuttings are concern, types and sizes of cutting have been investigated for more than a century. Historically, the studies began with cutting measuring 20 cm in length and only one whole leaf (FOWLER, 1955; PYKE, 1933). More recently, GUILTINAN et al (2000) found successful results experimenting cuttings with a single orthotropic bud, measuring 2 cm in length, working with somatic embryogenesis.

Considering only the length of the shoot, the term minicutting has no practical use in studies of plant propagation. Additionally the length is a variable according to species, mother plant, nutrition and age of the branch. In the case of cocoa, the term minicuttings was initially used to differentiate from the standard length of the cuttings with 16 cm,used by Cocoa Biofactory (MARROCOS; SODRÉ, 2004).

The substitution of the conventional length of cuttings of 16 cm by those of the minicuttings of 6 to 10 cm, has the important advantage of being using more herbaceous material, which has intense meristematic activity, therefore, promoting a raise on the rates of successful rooting (SODRÉ, 2007), (Figure 7 A,B). On the other hand, cutting of smaller size increase the yield per mother plant with means lower the costs of production. SODRÉ (2007) compared the growth of herbaceous cuttings of 4 and 8 cm in length and concluded that there were no differences between the two lengths for the final height and dry mass of roots of six-month-old seedlings. Other research was performanced in year 2016 to evaluate the rooting of orthotropic cocoa cuttings with a single bud. This assay required adjustment of several items, such as new rooting substrates, nursery, dosages of AIB and sprouting promoters and the results indicated that this technique is very promising, since rooting of cuttings with a single bud produced 5 to 10 times the number of seedlings grown in relation to the standard system of cutting length of 16 cm (Figure 7 C,D).

To large-scale production of seedlings, the minicuttings boosted the levels of rooting in plants and allows, for example, the utilization of pre-rooting trays in chambers of nebulization, and transfer them thereafter for larger plastic tubes, between 40 and 60 days. The adoption of the technology of pre-rooted cuttings reduces considerably the loss of substrates, especially in clones hard to rooting. On the other hand, this technology will facilitate the certification of nurseries for the production of cocoa clonal rooted seedlings, required by the Brazilian legislation.

Mitigation of risks in the production of cocoa seedlings by cuttings and grafting

Although the results of production of seedlings are markedly influenced by the plant, environment and factors of management, technology of the vegetative propagation of any kind will not improve yields of unproductive progenies or clones as emphasized by SENA GOMES and SODRÉ (2015). According to HARTMANN et al., (1997) this occurs because the genetic characteristics of the mother or progeny plants, such as are transferred to the plant being propagated. Therefore, field performance of low production, susceptibility to diseases, growth habit, and low quality of seed, for instance, will also be transmitted to new generations of plants produced by vegetative propagation.

The spread of viral diseases also represents a risk in plants den replicated by any methods of vegetative propagation. Diseases such as bacterial wilt and in some cases witch broom in Latin America and the Caribbean countries can also be disseminated via propagation material. To minimize these risks is recommended the adoption of sanitary eliminating any material with symptoms of diseases.

In general, among small producers, there is a trend in propagate a single or few clones by cuttings or grafting, generally the more productive. However, it should be noted that this represents a risk due to the lack of genetic diversity on account of new diseases. A project of clonal propagation should include not only clones tested for each specific region, but, also maintain high genetic diversity for agronomic traits, such as the production, resistance to disease, early maturity, format of the canopy, and seed quality. This diversity in plantations of cocoa in practice can be obtained by planting several clones as proposed by MANDARINO and SENA GOMES (2009), but in a monoclonal planting model.

This proposed model of planting cacao, monoclonal planting or simply one clone per block, is now in current use by the cocoa producers in Southern Bahia, with self-pollinated clones only. The innovation has also become very popular in Bahia, especially among new planting projects which are been established on the new cocoa planting frontier, in the semiarid regions of the far southern of the State, as well as in the very North area of the neighbor State of Espirito Santo. In these new planting projects, cocoa is planted with rooted cuttings seedlings, at full sun light and fertigated.

Experience with cocoa produced by cuttings in Bahia Brazil

The seedlings of rooted cuttings were initially used in Bahia to increase planting density of the areas grafted with resistant clones to diseases. Currently, the rooted cutting seedlings are being very important for large-scale plantations, instead of been utilized for increase the planting density in areas of low population density.

Project Nova Redenção, Bahia Brazil.

Experimental data of production collected in clonal planting, located in municipality of Nova Redenção, Western Region of Bahia, showed high productivity. The region is characterized by semiarid climate, with a average annual temperature of 26 ^{o}C, precipitation of 600 mm/year, with irregular distribution and a relative humidity under of 60%. In this project, the cocoa rooted cuttings seedlings were transplanted to the field in March 2003, with temporary shade of banana trees. The plants were maintained managed with fertigation drip irrigation and pruned regularly. According to Leite et al. (2012), the productivity of the clone CCN-51, at 52 months after planting in the field, reached 2,260 kg/ha/year of dried cocoa beans, with the excellent performance for most agronomic characteristics, especially vigorous growth, early production and pest free.

Project Lembrance, Bahia Brazil.

The establishment of cocoa plantations, in southern Bahia, replacing areas of papaya in decline has been successful so far. This is the case of the Project Lembrance, a clonal planting of initially 250 ha, established in 2007, using partial shading of papaya and banana trees. From the second year until today, the plants were kept in full sunlight conditions and with winds breaks of *Eucalyptus tolleriana*.

Currently, the project Lembrance, has expanded to 250 ha of cocoa and uses only rooted cuttings of eight clones, managed in a semi mechanized system with chemical weeding control, spraying pesticides and pruning, as well as the utilization of windbreaks and drip irrigation (Figure 8). The area is located 760 km South in Bahia State, the soils have medium texture, are flat lands of low natural fertility. In the year of 2014, the following clones were in production: CCN-10 and CCN-51, CEPEC 2002, 2004, 2005 and 2006, CP-49, PH-16, PS-13.19 and SJ-02, all arranged into monoclonal blocks.

The Lembrance's project has registered yield above 3,000 kg/ha/year of dried beans and can be considered as a reference to be replicated in large areas along coastal regions of Southern Bahia, as well as in other producing regions in Latin America, Africa and Asia.

Clonal garden

The majority of Brazilian cocoa farmers still produce the seedlings in their own properties and, in general, they receive vegetative material from clonal gardens or from neighboring farmers. Normally, the amount of grafting material obtained from the first introduction is sufficient to provide propagation for next generations of grafts or to form clonal gardens of varieties.

Clonal gardens are designed for the production of bud sticks for grafting on adult plants or on cocoa seedlings in nurseries. A general protocol for the production of quality bud sticks requires an intensive management of the nursery, including: weeding and fertilizing, pest and disease control, wind break, pruning, irrigation and shading control.

The spacing and the genetic material influence yield (number of cuttings) of the clonal garden. For example, in Ghana, the number of bud sticks taken in a clonal garden, established on planting spacing of 2.6 x 1.3 m, varied from 100 to 60 per plant per year, for selections of "Amazon cocoa", and only 20 to 40 for the "Amelonado" selections, as discussed by Dias (2001).

Whereas, the Cocoa Biofactory (IBC), Bahia, has recorded production of bud sticks, in number of cutting/ per plant/ year, ranging from of 56 for the clone IP-01, 63 for Cepec 2002 and 112 for the CCN-51.The clonal garden plants were field planted using space of 3.0 x 3.0 m, and the bud sticks sizes varied from 0.6 to 1.0 m.(Jackson Oliveira Cesar, Agricultural Engineer, 2013 - Personal Communication).

Projections made by SENA GOMES and SODRÉ (2015), for the production of bud sticks from a clonal garden established with 3,333 plants/ha, with intensive management of fertigation, pruning, pest and weeding, wind break, disease and shading control, indicate productivity ranging from 300,000 to 500,000 green cuttings/ha/ year, considering 6 to 9 harvest per year.

Simple technologies for production cocoa clonal seedlings

Simple and inexpensive units of plant propagation, designed to produce seedlings of clonal cocoa, can be used by small farmers, anywhere. An example of this is the propagation units utilized in Ecuador, for the production of rooted seedlings of cloneCCN-51, in nursery planting beds that are covered with plastic blanket, forming a high humidity chamber.

This type of propagation showed in Figure 9, is quite simple and it does not require running water or electricity, therefore it is suitable for family farming projects, especially located in remote places. LEAKEY et al. (1990), claim that the methods shown in Figure 9, in addition to being low cost technology and highly effective, are less prone to problems, compared to large and more sophisticated systems. According to LEAKEY, the efficiency of rooting in these systems is dependent on the genotype and time of year, but average of success usually varies from 60 to 75%.

Advanced technology for cocoa clonal propagation

Among advanced technologies applied for cocoa propagation, the somatic embryogenesis (ES) deserves special attention. It is a system of tissue culture that uses flora parts (petals and staminoids) to develop embryos genetically identical to those of the matrix plant (Figure 10). These embryos have the advantage of growing with both dimorphisms of tree growth, orthotropic(like a normal cocoa tree) and plagiotropic (fan like type of growth).

Despite some losses due to the production of abnormal embryos it is theoretically possible to produce more than 4,000 plants of secondary embryos derived from a single flower in about a year. Somatic embryogenesis has been developed and tested by several research institutions around the world, such as Ceplac, Brazil (Sandra Queiroz, personal communication, 2015), Pennsylvania State University, USA (LI et al., 1998), GUILTINAN et al (2000), MAXIMOVA and GUILTINAN (2012), CRIG, Ivory Coast (TRAORE; MAXIMOVA; GUILTINAN, 2003), CIRAD, Montpelier, France (ALEMANNO et al., 1998) and CATIE, Costa Rica (SOMARRIBA et al., 2011).

Graft One

The Research on “Grafting One technology” in cocoa started in the year 2012, in Brazil, and preliminary data show successful results, for the control of soil borne pathogens, as well as on the production of reduced plant size.

The “Grafting One” is an adaptation of the methods of rooting and cleft grafting which is performed at the same time. According to SODRÉ (2013), the main advantage of this method is that two different technologies are applied in a single operation, rooting and grafting, during the process of production of clonal seedlings.

The bud sticks size used for “Grafting One” method varies from 15 to 18 cm in length, same conventional size utilized for rooting (Figure 11). The concentration of plant growth regulator (AIB) and substrates are the same used in traditional cuttings methodology. Immediately after grafting the rootstock, it must be transferred to the rooting chamber, where the budded stick is treated with rooting hormone, and kept with appropriated humidity to avoid dehydration.

The first shoots growth normally occurs around 30 days after the grafting, and the plants must be removed from the rooting chamber between 60 and 90 days. At this time, the growing plants have to be replanted to polyethylene bags, for hardening. Seedlings will be ready for field planting around 8 months later.

Currently, the technology “Grafting One” is in an experimental scale. It requires special care with general sanitation, especially the cleanup of rooting facilities (chamber and mist system, propagation flats, benches, plastic containers and tools). It is also necessary to keep the graftings in a high humidity environment, equipped with fog system, to ensure successful rooting of the budded bud stick. This method of propagation works better for easy rooting clones.

Use of grafting filters

The cocoa seedlings produced through the “intergrafting technique” are obtained by inserting a small section of a bud stick or green stem, approximately 10 cm in length, via two graft unions, between the scion graft and the rootstock. The double “intergrafting” may be done at the same time, or the scion graft can be done at a separate time, approximately 3 months later from the date of the “intergrafting”. It should be noted that the stem diameter of the components, rootstock, “intergrafting” and scion, must be of similar size to ensure the success of grafting.

According do HARTMANN et al. (1997), there are specific reasons for using interstock in propagation, most of them related to avoidance of incompatibility of scion and rootstock in many clonal combinations within species, disease resistance, reduction of vegetative growth and enhancement of reproductive growth. Westwood (1993), mentioned that when compatibility bridge “intergrafting” is requires, the double budding technique is the best approach to be selected, as with pear on quince, that can be done in a single operation. In this context, it is worthwhile to mention that the variety of cocoa, known as “jackfruit cocoa”, has great potential for to be used as “intergrafting”, due to its high resistance to the important pathogen, *Ceratocystis cacaofunesta* (Figure 12), which occurs in cocoa areas of Southern Bahia.

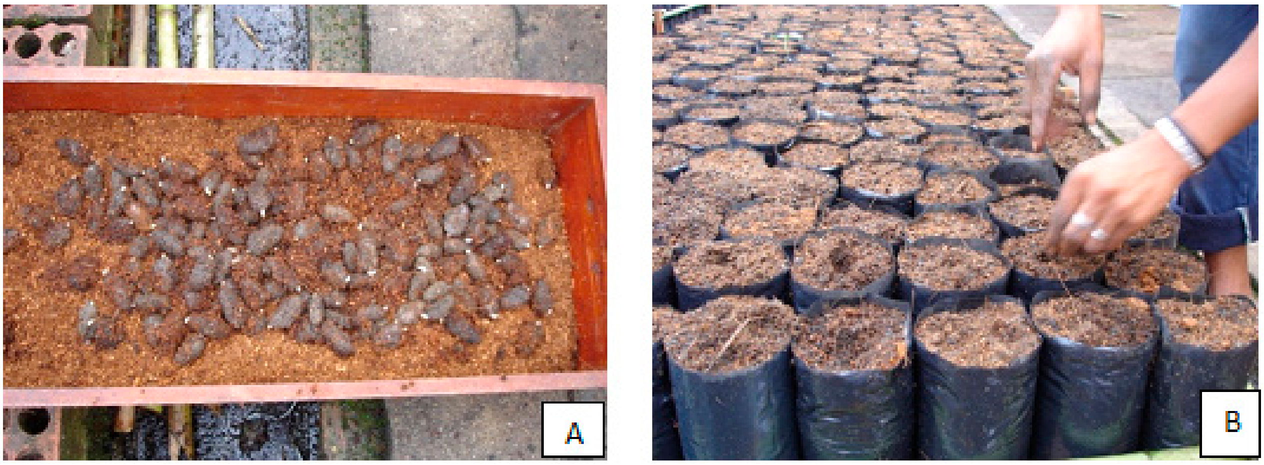

Figure 1. Pre-germinated cocoa seeds (A). Transplanting seeds in polyethylene bags for later grafting (B).

Table 1. Advantages and restrictions on use of the graft with seminal seedlings in cocoa.

Advantages
Seeds are free from dormancy, low cost and easy to obtain.
Simple methodology adopted by farmers.
Resistance to major diseases such wilt of Ceratocystis.
Can increase the income of some clones.
Restrictions
Bad in some combinations canopy/rootstock.
Seeds viability is short time “recalcitrant” .
Can reduce the performance of some clones duo to low resistance to diseases.

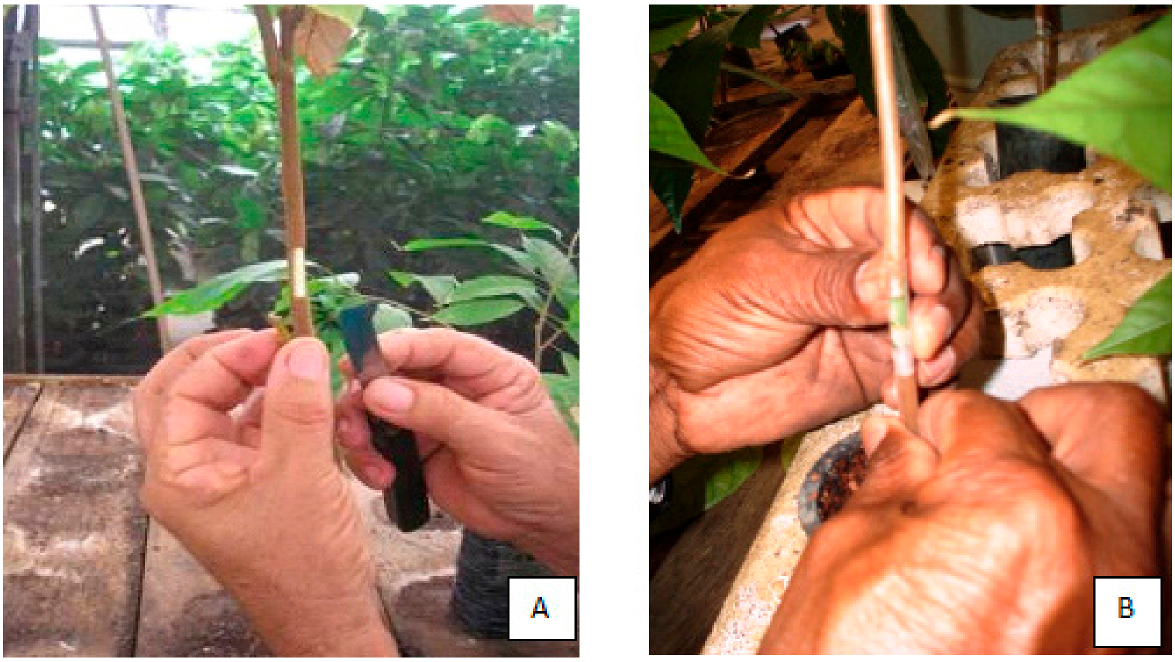

Figure 2. Stages of grafting by budding on cocoa trees. Removal of the bud (A). Inserting in rootstock (B)

Table 2. Advantages and restrictions of using budding in the production of cocoa seedlings.

Advantages	Restrictions
• Uses a single button to graft a rootstock;	• Requires highly trained grafters ;
• Minimizes waste of rootstocks seedlings that can be redone if the first budding fails ;	• The high cost of production;
• Important when there is limited amount of clonal material and also in quarantine services ;	• In large part dependent on reorganization measures;
• Propagate clones that cannot be propagated by other techniques;	• Variable rates de survival;
• Can be used as auxiliary technique for detecting the presence of viruses in plant material "Indexing".	• The slow start after budded .

Figure 3. Split grafting in cocoa. In basal shoots (A). In basal shoots after six months (B). Preparation of the branch (C). Grafted seedlings and protective chamber (D).

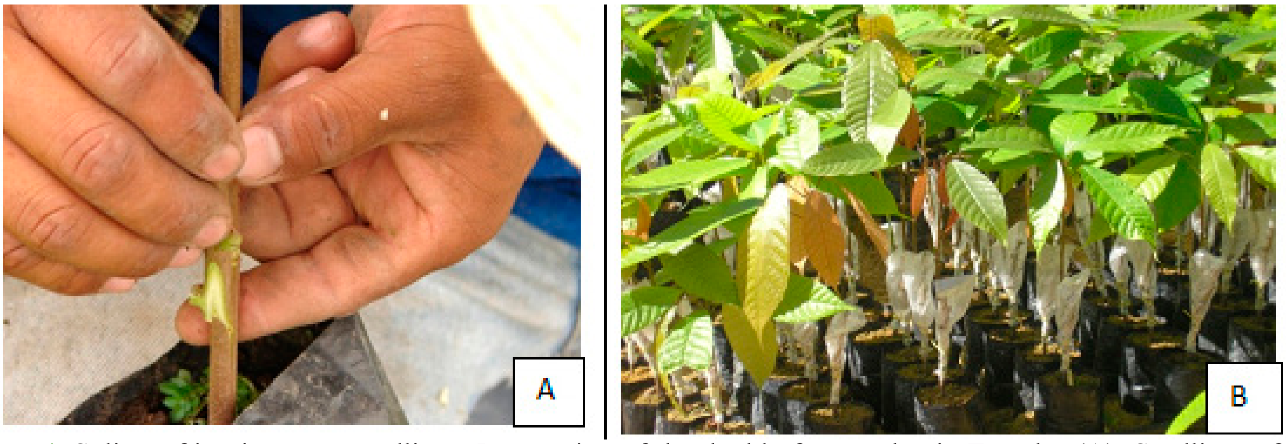

Figure 4. Split grafting in cocoa seedlings. Preparation of the double face wedge in Ecuador (A). Seedlings after grafting and before cut canopy in the Espírito Santo state, Brazil (B).

Figure 5. Rooting of cocoa cuttings. Rooting environment (A). Detail of the wedge and the adventitious roots (B).

Figure 6. Orthotropic seedlings in cocoa. Bending plants to produce orthotropic branches (A). Seedling formed from orthotropic branch (B).

Table 3. Advantages and limitations of the propagation by cuttings in cocoa trees.

Advantages	Limitations
• Higher rates of multiplication . • Easy, free from problems of incompatibility of the graft. • Plants which are identical in all respects to the unique individual or parent plant . • Propagation can be done with simple rooting and cheap. • Is possible propagate material plagiotropic and orthotropic. • Often medium to high rates of success.	• Project on a large scale requires high investments in facilities, equipment, clonal and logistics of supply of materials. • Demands trained workers. • Recommended for specific locations (well drained soils and not incline) . • The lack of taproot, especially in plagiotropic branches, can reduce productivity of plants during the dry season, in shallow soils. • Risk of infection by pathogens of soil especially in susceptible clones.

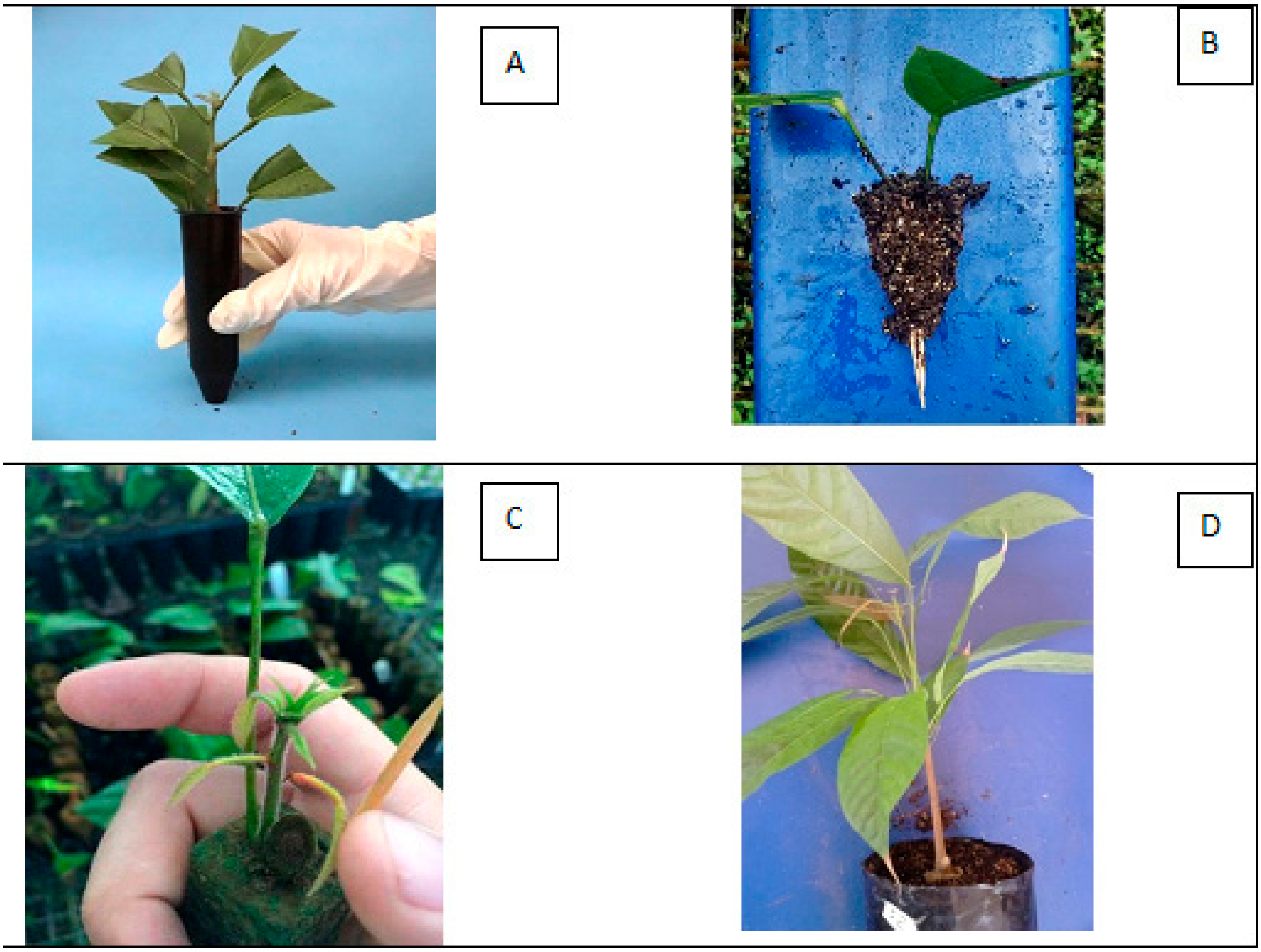

Figure 7. Minicutting in cocoa seedlings. Rooting in tubes (A). Seedling rooted at 50 days (B). Cutting of an orthotropic bud root rooted in phenolic foam (C). Seedling formed by single bud (D).

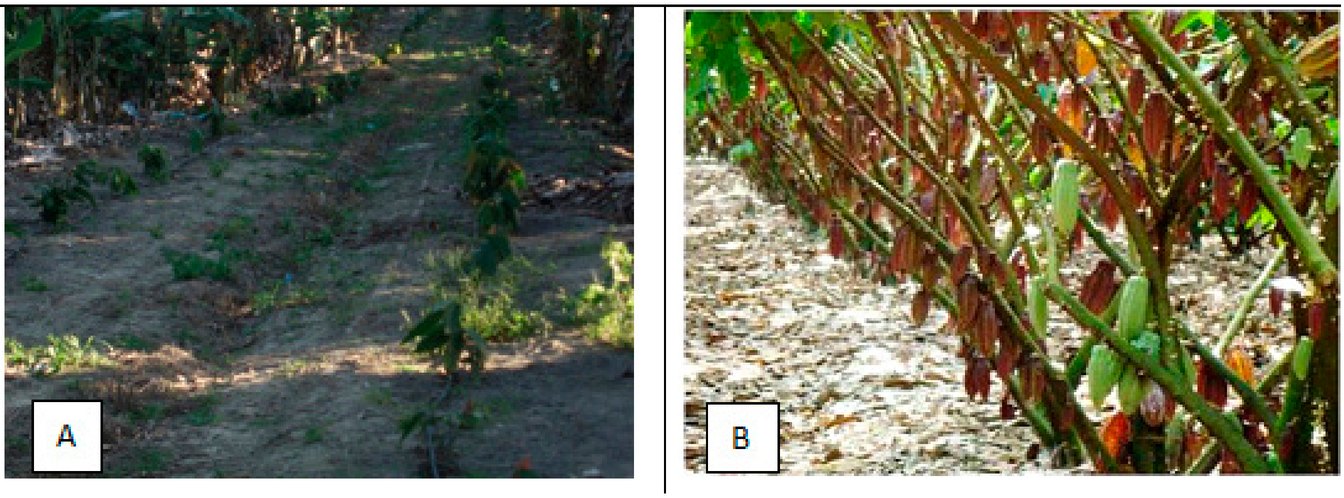

Figure 8. Lembrance project. Cocoa trees implanted with cuttings and drip irrigation (A). Production area (3,000 kg/ha/year) after five years (B).

Figure 9. Technologies for production of clonal cocoa seedlings. White plastic huts (A). Shelter used in Ecuador (B).

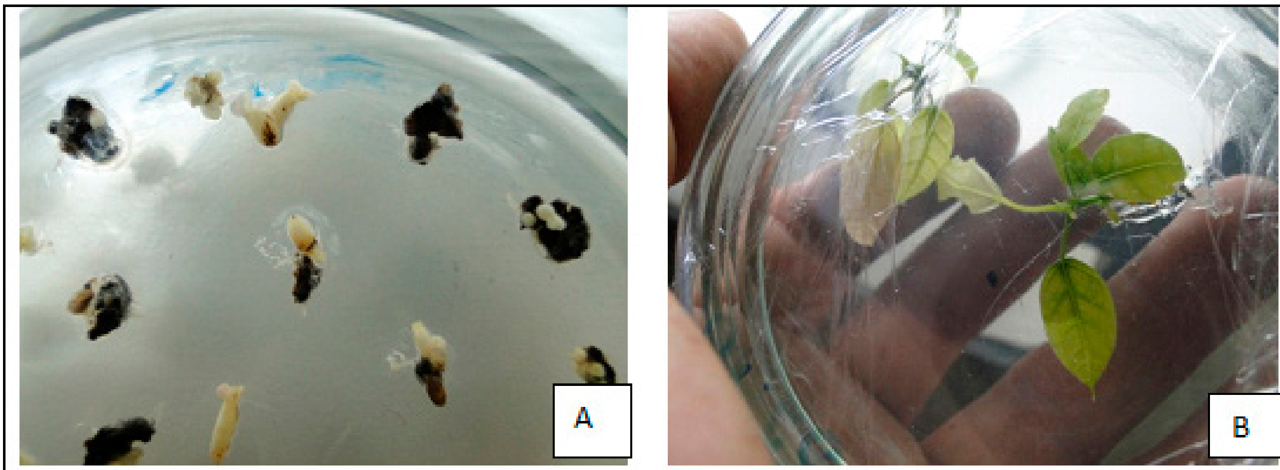

Figure 10. Somatic embryogenesis in cocoa tree. Somatic embryos obtained from petals (A). Embryogenic seedling in pre-acclimatization (B).

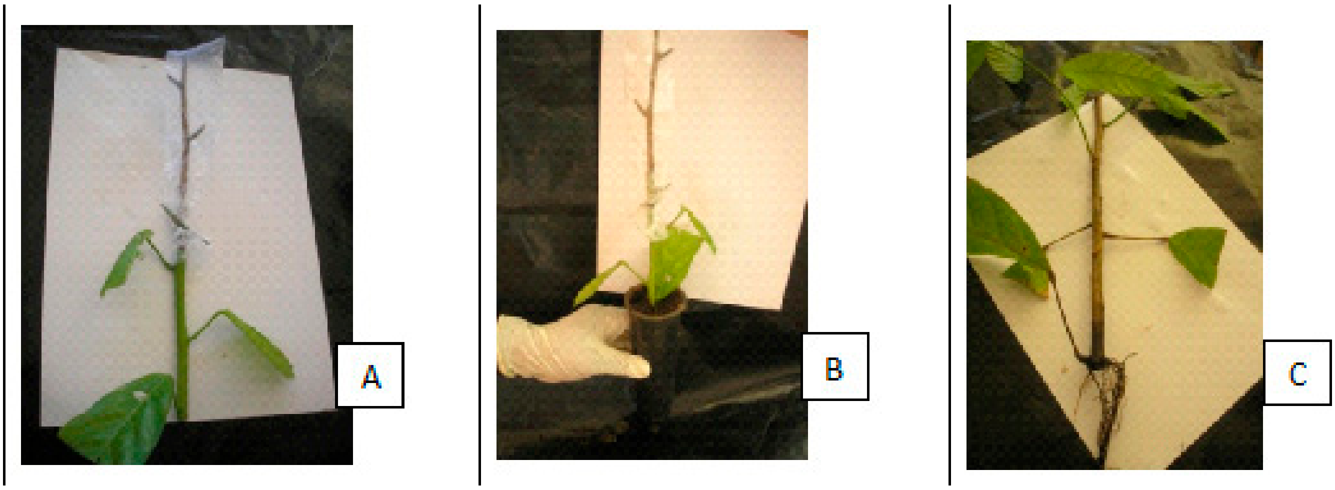

Figure 11. Seedling type “graft one”. Union of the rootstock and graft (A). Joint inserted to be rooted (B). Seedling ready after eight months (C).

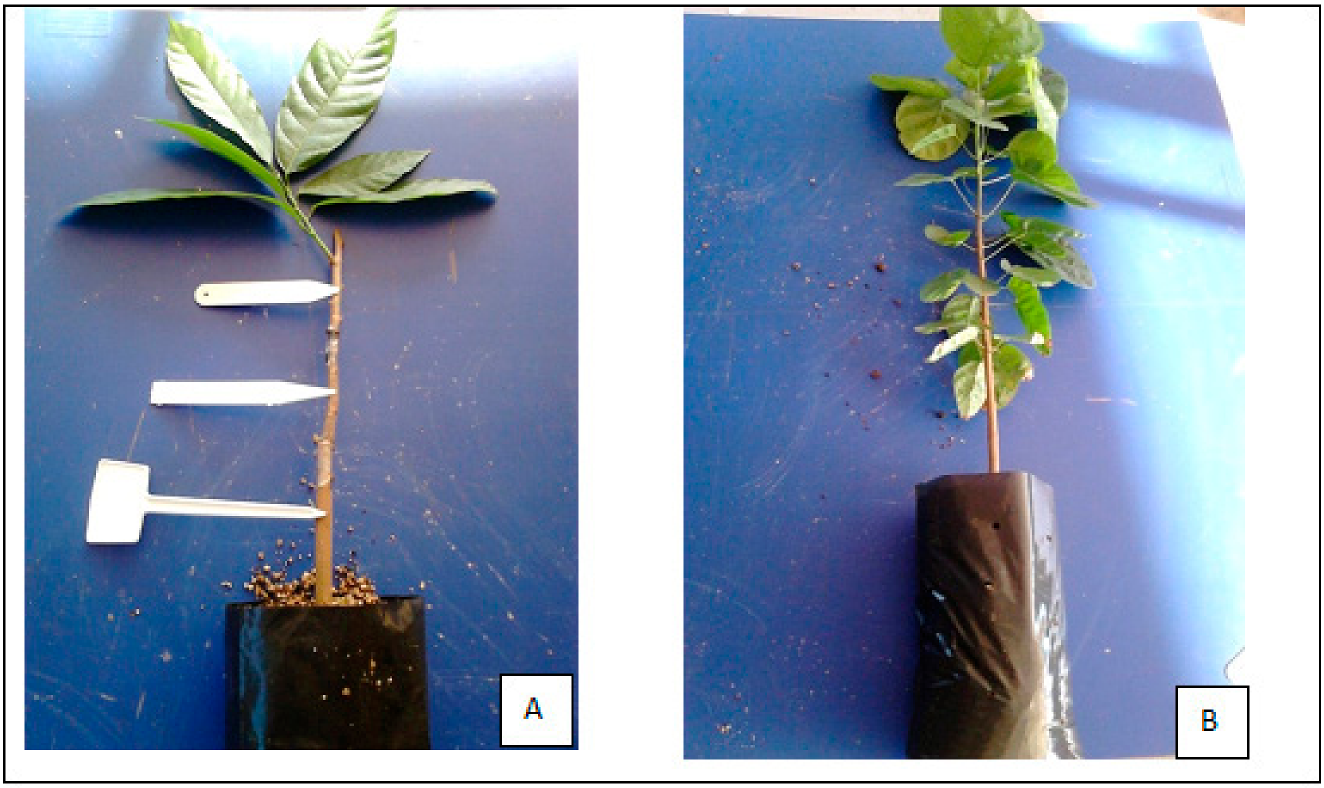

Figure 12. Cocoa seedling with graft filter. Parts of the seedling (rootstock, filter and canopy) indicated by arrows (A). Variety resistant to the fungus *Ceratocystis cacaofunesta* “cocoa jackfruit” (B).

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Final considerations

Even considering that the production of cocoa seedlings in nurseries accredited is an activity incipient in Brazilian producers. It is very likely that in a few years, the production of seedlings is subject to legal process of certification. The regional and national potential for the production of clonal seedlings of cocoa can be an attraction for new nurseries. These companies will require production technologies increasingly efficient to produce seedlings with quality and reduced costs.

It is important to highlight that the certification of cocoa seedlings is a requirement of the Brazilian Ministry of Livestock Agriculture and Supply (MAPA, 2003).The aim of certification is to ensure especially the genetic quality and health of seedlings produced.

Among new challenges for research on the cocoa propagation, emphasis the adoption of the technique of clonal nurseries, because as verified by (Sodré, 2007) in 5 ha of clonal garden in nursery conditions it would be possible to produce material for production of up to 4 million seedlings of cuttings per year. Additionally must be added the gains with quality and overall production costs of cocoa seedlings.

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