

ADVANCES IN GUAVA PROPAGATION¹

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ABSTRACT - Guava (*Psidium guajava* L.) can be propagated by seed, layering, air layering, grafting (budding or grafting), cuttings (root or shoot) or tissue culture. Propagation by seed is used for rootstock production and for raising populations for screening at early phases in the breeding programs. Vegetative propagation methods are used to clone selected genotypes from these programs and commercial orchards because it perpetuates all characteristics of each cultivar. This review addresses different methods that can be used to propagate guava, the methods commercially adopted and the progress obtained in recent years. There are several propagation technologies available, however, the adoption levels are rather different between producing countries. Needs for improvement on the production of guava trees will be discussed.

Keywords: *Psidium guajava*, seeds, grafting, cutting, air layering, tissue culture.

AVANÇOS NA PROPAGAÇÃO DA GOIABEIRA

RESUMO - A goiabeira (*Psidium guajava* L.) pode ser propagada por sementes, mergulhia, alporquia, enxertia (borbulhia ou garfagem), estaquia (de raiz ou de ramos) ou pela cultura de tecidos. A propagação por sementes é utilizada na produção de porta-enxertos e na fase inicial do melhoramento genético. Os métodos de propagação vegetativa são utilizados para clonar os genótipos selecionados nas fases mais adiantadas dos programas de melhoramento genético e nos plantios comerciais, pois perpetua todas as características das cultivares de interesse. A presente revisão bibliográfica tem por objetivo abordar os diferentes métodos que podem ser utilizados para propagar a goiabeira, os comercialmente adotados e os avanços obtidos nos últimos anos. Diversas são as tecnologias de propagação disponíveis; entretanto, os níveis de adoção são bastante diferentes entre os países produtores. Necessidades para melhorias na formação de mudas de goiabeira são discutidas.

Termos para indexação: *Psidium guajava*, semente, enxertia, estaquia, alporquia, cultura de tecidos.

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INTRODUCTION

Guava, *Psidium guajava* L., belongs to the family *Myrtaceae*, which has more than 80 genera and 3,000 species, distributed in the tropics and subtropics, especially in the Americas, Asia and Australia. The genus *Psidium* consists of approximately 150 species of shrubs with persistent leaf and *P. guajava* is the best-known and distributed specie worldwide (PAULL; BITTEMBENDER, 2006). Guava is one of the 50 best-known edible fruits of tropical and subtropical climate worldwide and has commercial importance in more than 50 countries (YADAVA, 1996). Guava is grown successfully in a wide range of climatic and edaphic conditions, from sea level to altitudes of 2,100 m. However, temperatures between 20 and 30°C, rainfall from 1,000 to 2,000 mm well distributed throughout the year, soils with good drainage, high levels of organic matter and pH between 5 to 7 are the optimum growth conditions (YADAVA, 1996; PAULL; BITTEMBENDER, 2006). The world's major guava producing countries are India, Pakistan, Sudan, Brazil, Egypt, Mexico, Indonesia and Bangladesh. The technological levels adopted for nursery trees production, orchards and post-harvest management are quite different among countries. **Table 1** summarizes information on production, productivity, main cultivars and propagation methods used in guava trees in some producing countries.

The morphology of flowers of guava tree favors self-pollination; however, cross-pollination is estimated at 35%, carried out mainly by bees and other insects (PEREIRA, 1995; YADAVA, 1996; PAULL; BITTEMBENDER, 2006). However, the proportion of cross pollination may vary among genotypes, with environment of the production area and the availability and efficiency of the pollinizing agents. Therefore, propagation by seed features individuals that are genetically heterogeneous, which can be observed between orchards and plants of the same orchard (MARTÍNEZ-DE-LARA et al., 2004). Hence, seed propagation is not recommended in commercial orchards of high productivity (PEREIRA, 1990). Methods of vegetative propagation for guava have been highly studied and their adoption, on a commercial scale by nurserymen and fruit growers, is directly related to propagation facility, costs, technology transfer and mainly the organization of the production chain at regional level, in addition to the interest in adopting new technologies.

The main advances in plants cloning have occurred in the last 100 years (PREECE, 2003).

Although the types of grafting and cuttings currently used (hardwood, semi-hardwood, herbaceous, leaf or root cuttings) were already well known at the beginning of the last century, notable contributions have emerged since then, mainly in terms of health and disease control, reduction or elimination of water loss in cuttings, the discovery of the growth regulators, knowledge on the role of juvenility in propagation, advances in knowledge on chimeras, micropropagation and the use of vegetative propagation to avoid diseases caused by viruses and other pathogens (PREECE, 2003; HARTMANN et al., 2002).

The International Society for Horticultural Science (ISHS) held five international symposia on guava or species belonging to the family *Myrtaceae*. The events were held in Curitiba-Brazil (1996), Lucknow-India (2005), Mérida/Aguascalientes-Mexico (2008), Petrolina-Brazil (2012) and Cairns-Australia (2016). Of 213 papers published in *Acta Horticulturae*, resulting from these five symposia, only 15 papers (7%) addressed guava propagation and tissue culture was the main method studied. In a complete bibliographical review in The “*Revista Brasileira de Fruticultura*”, the main Brazilian scientific journal on fruits, of 2,433 papers published in 2000-2014 period, only 11 papers (0.45%) addressed to guava propagation, and cutting was the most studied method (7 publications).

This review aims to address the different methods for guava propagation, adopted commercially, and the progress achieved in recent years.

GUAVA PROPAGATION AND RESEARCH ADVANCES IN THE AMERICAS

Propagation by seed

Seeds of guavas for industrial purposes are generally considered a waste product. Their use in propagation is restricted to breeding programs or rootstock production for grafting of scion cultivars. However, in countries that do not use high technology, guava is still propagated by seed, without grafting. Plants that are produced by seed are referred to as seedlings, which is the simplest propagation method of guava trees. As a disadvantage, the use of seeds results in plants with great variability. It is an undesirable feature in commercial orchards, as it causes low productivity and fruit quality (PEREIRA, 1990). In addition, seedlings have a higher juvenility period, delaying entry into production, because they need between two and four years to bloom. Therefore, the use of seeds is recommended only in genetic

improvement programs, where variability between plants is desirable and a necessary feature. Flavor and aroma, for example, vary widely among populations of seedlings (PAULL; BITTEMBENDER, 2006). In Brazil, several cultivars were obtained by selecting seedlings from open pollination. On the other hand, the continuous use of seeds virtually eliminated some cultivars, such as 'Guanabara', whose genetic characteristics are no longer the same as the originally selected material (PEREIRA; NACHTIGAL, 2009).

In some guava producing countries, which adopt grafting for propagation of the scion cultivars, seed is used for rootstock production. In this case, in addition to the genetic variability of the plant root systems, especially differences in vigor, another downside is the increased time required for the production of seedlings (1.5 to 2 years), compared to propagation by stem cuttings of scion cultivars (six to eight months).

In the method of propagation by seed, ripe fruits are harvested from selected plants. Then, seeds are removed, washed in running water and dried in the shade for 10 days, without the need for rip fence or stratification. The seeds can be sown in nurseries or directly on plastic bags, containing substrate with good drainage. Germination can be higher than 90% and usually occurs between 15 to 20 days. For propagation by grafting, seedlings need to have 12-20 mm of diameter to 20 cm of the lap.

In Brazil, there is no official recommendation provided by Rules of Seed Analyses for germination test on guava seeds (BRASIL, 2009). However, Alves et al. (2015) proposed to collect the seeds manually from the fruits, wash them in running water on fine sieve, dry them at room temperature for three days and use paper roll, on paper or on sand as a substrate, alternating temperature of 20-30°C, with final count at 23 days after sowing. Chemical analyses of guava seeds indicate 16% of crude protein, 18% of crude oil, 20% of crude fiber and 44% of carbohydrates, based on dry weight. However, the oil of the seeds has 77% of linoleic acid and may have application in production of edible oils and inks (PAULL; BITTEMBENDER, 2006).

Vegetative propagation

In Florida, until 1948, 160 ha of guava crops were grown, and all trees were obtained from seed (KUPERBERG, 1953). With the emergence of more productive cultivars and the need to perpetuate their characteristics, studies on vegetative propagation were carried out in California using root cuttings (WEBBER, 1944) and in Florida using layering (RUEHLE, 1948), herbaceous cuttings from shoots

under intermittent mist system (KUPERBERG, 1953) and grafting (NELSON, 1954).

The guava tree can be propagated by vegetative methods (SINGH, 2007). Guava trees propagated vegetatively are uniform and present a shorter juvenile period, compared to plants multiplied by seed. For use in vegetative propagation, mother trees must be clones of the original plant cultivar, be healthy, young, vigorous and grown in separate blocks (SINGH, 2007).

The main methods of vegetative propagation adopted in guava trees are presented as follows:

a) Grafting: in the literature, Webber (1944) already mentioned grafting success in propagation for guava tree at the beginning of the last century. To accomplish grafting, the apical portion of 3-4 months-old branches should be used. The forks must be prepared with 3-4 buds, 15-18 cm long and 8 mm diameter. The selected branches should be clipped and defoliated on the mother tree, between 5-7 days before they are detached. This practice helps in swelling of the buds, which will be ready to sprout once the grafting is accomplished. This is considered essential for the success of grafting (SINGH, 2007). The rootstock is cut between 15-18 cm above the lap and the grafting of full crack is performed. After knotting, the fork should be bagged with transparent polyethylene to prevent dehydration and increase the success percentage. Sprouting is initiated between 9-12 days after grafting and the bag must be removed. In greenhouse conditions, grafting success rate may be between 70-92% (SINGH, 2007). In Brazil, the grafting of guava trees (grafting or different budding types) in rootstocks propagated by seeds was the most widely used method during 1980's to early 1990's. However, with improvements in the method of herbaceous shoot cuttings under mist system, use of grafting or budding was also ceased in the main Brazilian nurseries (PEREIRA, 1990).

b) Air layering: it is a simple, fast and efficient method to clone guava trees and may be the cheapest method. Ruehle (1948) highlighted these advantages and mentioned that the nursery trees could be developed in 4-5 months in Florida with 100% success using this method. In Venezuela, air layering has been studied in guava tree genotypes tolerant to *Meloidogyne* spp., observing rooting percentages of 50% in the selection of AgroLUZ-42, without the need for ringing or growth regulators (VILCHEZ et al., 2011). The physiological condition of the branches, the application form, concentration of growth regulators, type of auxin and substrate used are important factors to be considered when propagating guava trees by air layering (URDANETA

et al., 2009). However, as main disadvantage, air layering presents low yield per mother tree, compared to shoot cuttings or grafting.

c) Shoot cuttings: the guava tree can be propagated by root or shoot cuttings (herbaceous, semi-hardwood or hardwood). Webber (1944) highlighted its adoption in colder regions like California as the main advantage and reported the possibility of propagating guava trees by root cuttings. Therefore, when intense cold caused the freezing and death of tree canopy in orchards, it was possible to use the new shoots emerged from the root system to develop a new plant, without need to re-grafting or over-grafting, because the root system is genetically identical to the scion.

Kuperberg (1953) tested different types of mist chambers and carried out pioneer studies on guava tree propagation with herbaceous cuttings under intermittent mist system. He also optimized need for fertigation during rooting, the types of branches to be used, the size of the cuttings and established the main variables for evaluation. The use of herbaceous stem cuttings shortens the period for nursery tree formation (six months) and ensures genetic uniformity of the plants to eight obtained from both the root and the shoot systems (PEREIRA, 1995). Herbaceous stem cuttings must be held under an intermittent mist system, which is a device installed in screen house, greenhouse or outdoors that offers a set of nebulizers that spray water on the surface of cutting leaves, keeping them constantly moistened. The system is controlled by a timer, which commands the solenoid valve. Thus, the system requires water availability and daily inspections to check for possible problems.

To have success in vegetative propagation of guava trees by herbaceous cuttings, it is crucial to carry out drastic pruning, which consists of removing all the branches of mother trees at approximately 1 m from ground level. Therefore, along with adequate fertilization and pesticide treatments, it is possible to obtain herbaceous shoots (green, not yellow) ideal for stem cuttings at 70-80 days after pruning. The cuttings should be prepared with two nodes, keeping the pair of leaves (intact or cut in half) at the top node, by removing the basal leaves. Vermiculite (thin or medium) is the rooting media recommended. By following all the recommendations at different stages and with favorable environmental conditions, it is possible to obtain rooting percentages greater than 95%, with excellent quality of roots, in 60 days after set cuttings. Before removing them from the mist chamber, it is recommended to shut down the system 5-10 days for acclimatization, a process known as

rustication of cuttings, which increases the chances for success of the method in plastic bags. Because of the excellent results obtained with the herbaceous stem cuttings under a mist system, semi-hardwood or hardwood cuttings are not used on a commercial scale in Brazil.

Distribution of the root system of a guava tree with 12 years-old of cultivar Rica, propagated by herbaceous cutting, was evaluated under Oxisol soil field conditions without irrigation. The results showed that, because this propagation method, the plant had no pivoting root. However, the primary roots exhibited appropriate number, distribution around the plant, satisfactory development and abundant branching, which ensure adequate anchoring and plain conditions to explore large volume of soil in search of water and nutrients (FRACARO; PEREIRA, 2004).

With respect to *in vitro* clonal propagation, this method has not been widely used in the guava crop in the Americas. This topic will be discussed in detail in section for the Asian countries.

Research advances in Brazil

The Brazilian guava production amounted to 349,615 t from 14,982 ha, in 2013. All regions in Brazil have commercial production of guava; however, the main areas are found in the states of São Paulo (4,472 ha), Pernambuco (3,261 ha), Ceará (1,197 ha), Minas Gerais (950 ha), Bahia (699), Rio Grande do Sul (601 ha) and Rio de Janeiro (569 ha) (AGRIANUAL, 2016). The preference is for red-flesh guava, whose price at Ceagesp/SP market is 32-46% higher than the price of white-flesh guava (AGRIANUAL, 2016) and 'Paluma' is the main cultivar, cultivated in 70% of guava orchards (PEREIRA; KAVATI, 2011).

Guava is currently cropped commercially in Brazil from the equator line to the latitude of 32°19'S, in Jaguarão-RS (60 m a.s.l.), highlighting wide adaptation of the cultivars to diverse edaphoclimatic conditions, mainly 'Paluma' cultivar, as well as the technical feasibility of own-rooted scion cultivars propagated by herbaceous cuttings (without rootstocks). In terms of costs, nursery trees, at unit price of R\$ 4.30 (≈ US\$ 1.20), respond for 10.39% of the total costs in the first year for the formation of the 'Paluma' guava orchard at spacing 7.0 m x 5.0 m in the reference region of Vista Alegre do Alto – SP (AGRIANUAL, 2016).

The guava culture raised commercial interest in Brazil in the 1950's, following decline of the quince (*Cydonia oblonga*) culture and jam production in the states of São Paulo and Minas Gerais. Food

companies in the region of Jaboticabal-SP began to produce guava paste on an industrial scale and quickly became the most consumed sweet in Brazil (PEREIRA; KAVATI, 2011). Pioneer studies on guava breeding had already been conducted such as the doctoral thesis of Soubihe Sobrinho, at “Escola Superior de Agricultura Luiz de Queiroz” (ESALQ/USP), in late 1940’s. At that time, guava propagation was essentially by seed, which produced plants with great genetic variability and non-uniform orchards. Important studies on guava culture were also carried out by Brazilian fruit growers of Japanese origin, such as Tadao Ogawa and Sinishi Ogawa, who still contribute to the selection of genotypes for *in natura* consumption and development of techniques, such as grafting (PEREIRA; KAVATI, 2011).

At “Faculdade de Ciências Agrárias e Veterinárias” (FCAV/UNESP), Campus Jaboticabal-SP, a guava breeding program was started in 1976 with the introduction of seeds from several countries, selection and controlled crossings, which allowed obtaining ‘Paluma’ and ‘Rica’ cultivars (released in 1984) and ‘Século XXI’ (released in 2002) (PEREIRA; NACHTIGAL, 2009; PEREIRA; KAVATI, 2011). Guava propagation presents a close relationship with species improvement, since, in parallel to the breeding program, it was also necessary to develop an economically viable technique of vegetative propagation of guava trees due to high costs and required time for the formation of grafted nursery trees (PEREIRA; KAVATI, 2011).

Thus, research studies were carried out using herbaceous stem cuttings under an intermittent mist system (PEREIRA et al., 1983; PEREIRA, 1990; PEREIRA et al., 1991; BACARIN et al., 1994), which led to significant changes in the production system of guava nursery trees in Brazil. In this regard, we highlight the learning desire, technology adoption and investments in infrastructure by nurserymen, in special the pioneer nursery at Hero Farm, in São Carlos-SP, whose managers responsible were the agronomic engineer Ângelo A. Oioli. Mr. João Mateus and Mr. José Mauro da Silva, owners of the Paluma Nursery (Sítio São João), in Taquaritinga-SP, agronomic engineers Alberto Samaia and Arlindo Piedade Neto, technical responsible of Taperão Farm in Brotas-SP also played a very important role. In addition to other nurseries located in Petrolina-PE, in Juazeiro-BA, and in Linhares-ES, which made this technology a reality for guava propagation until the present time.

Due to the advantages of easy implementation, short time to obtain the nursery trees (8 months on average), high yield per mother tree and excellent

quality of nursery trees, guava propagation by herbaceous cuttings under an intermittent mist system has been the most widely used in Brazil (Table 1, Figure 1), since the end of the 1980’s (PEREIRA; NACHTIGAL, 2009). In this production system, after the rooting phase (≈ 60 days), rooted cuttings are transplanted to plastic bags, tubes or small pots, and marketed in these packages. Until the early 2000’s, soil or soil mixes with manure, sterilized or not, were used to fill these containers. Due to the high weight for transport, the banning of methyl bromide and, especially, the suspicion that guava nursery trees were leaving the nurseries infested with *Meloidogyne enterolobbi*, there was the need to use lighter commercial substrates, free of nematodes and weeds and of recognized origin. This need, along with the remodeling of nurseries (concrete floors, countertops and employee training) to prevent nematode infestation, was the main advances in the production of guava nursery trees on a commercial scale in Brazil in this century. Adequate infrastructure and the use of available technology allow producing guava trees in the nursery free of nematodes.

The main phytosanitary issue faced by the guava culture in Brazil in recent years, which deals directly with the nursery tree production system, is the guava root-knot nematode (PEREIRA et al., 2009), first recorded by Carneiro et al. (2001) in the state of Bahia and identified as *Meloidogyne enterolobbi* (syn. *M. mayaguensis*). Pereira et al. (2009) estimated a reduction in productivity between 30 and 70%, direct losses of at least R\$ 112,700,000.00 (\approx US\$ 59,315,000) and 3,703 unemployed workers in five Brazilian states because nematode parasitism in guava trees. Thus, several studies have been conducted using other species botanically similar to guava aiming to find resistance sources to be used as rootstocks. Some resistance sources were found in *Psidium* spp., *P. cattleianum*, *P. friedrichsthalianum*, *P. rufum*, *Acca sellowiana* and *Eugenia stipitata* (ALMEIDA et al., 2009; FREITAS et al., 2014; BIAZATTI et al., 2016).

However, in studies conducted in Brazil, these accesses were graft incompatible with scion guava cultivars (FREITAS et al., 2014; ROBAINA et al., 2015). Guava sub-grafting with strawberry guava genotypes (*Psidium cattleianum* Sabine) resistant to *M. enterolobbi* was proposed as an alternative to replace the root system of nursery trees or mature trees; however, a vascular connection was not established successfully (ROBAINA et al., 2012). Costa et al. (2012) carried out interspecific hybridizations between *P. guajava* and *P. guineense* and identified some off springs with resistance

to *M. enterolobbi* that, when grafted with the 'Paluma' guava, did not show symptoms of graft incompatibility in the first four months in the field.

In recent years, still on an experimental basis, guava mini-grafting has also been proposed as a way to reduce area and costs in nurseries and to give support to genetic breeding programs or production of certified nursery trees (MARINHO et al., 2009). In addition, it was tested seriated mini-grafting from mother trees placed in clonal mini-gardens to strengthen or increase the rooting percentage of some clones and improve the quality of the root system (FREITAS et al., 2013).

In a little more than 60 years of commercial production of guava in Brazil, the propagation and nursery tree production systems have evolved significantly, along with the availability of new plant varieties, the need for modernization of nurseries and increased yield. The system was started with the use of seeds (1950's to 1970's), shifted to grafting in rootstocks propagated by seed (1980's) and evolved into the own-rooting of scion cultivars by herbaceous cuttings under an intermittent mist system. In the case of fruit tree propagation methods used in Brazil, the herbaceous cuttings under an intermittent mist system has on guava tree its main example of commercial use. In the light of the serious damage that *M. enterolobbi* has been causing to commercial orchards since the beginning of this century, the guava propagation system requires a new change, because there are no registered nematicides for guava crop in Brazil and scion cultivars available in the country are susceptible to this nematode. However, this change still depends on nematode-resistant rootstocks, which are easily propagated vegetatively, give satisfactory quality fruits and are graft compatible with the available scion cultivars. Interspecific hybridizations seem to be the likely path to be followed in order to achieve these objectives.

GUAVA PROPAGATION AND RESEARCH ADVANCES IN SOUTH AFRICA AND OTHER AFRICAN COUNTRIES

Due to its easy cultivation, high nutritional value and popularity of processed products, guava is an important produce in international trade as well as in the domestic economy in South Africa and other African countries, such as Egypt and Sudan. Guava cultivation in South Africa dates from 1688 when Jan van Riebeeck (BOLT, 1984) brought the first guava trees to the country from the Madeira Island (Portugal). As there were no other guava trees in the country at that time, the plants that were multiplied by

seeds kept genetic fidelity. Different types of guavas were introduced in Cape Town between 1830 and 1835 and natural hybridization occurred. Seeds of these hybrids were sown and only the best plants were selected for cultivation. Farmers made selections and one of the current commercial cultivars, named 'Fan Retief', arose and was grown throughout South Africa. The main producing regions of guavas in South Africa are Mpumalanga, the Limpopo province and Western Cape (**Figure 2**). Restrictions faced by the South African guava industry in relation to cultivars are related to Guava Wilt Disease (GWD) susceptibility, nematode infestations, high number of seeds and short shelf life.

Guava arrived in Egypt in 1830 through India (EL-TOMI, 1953), and since then, it has been a popular fruit, mainly for *in natura* consumption. Furthermore, processed guava products were developed and currently several processing establishments arouse in Egypt, producing juice of guavas and purees. The main producing regions of guavas in Egypt are divided into three zones, referred to as Lower Egypt, Middle Egypt and Upper Egypt. These zones are not similar in terms of climatic characteristics. Lower Egypt, represented by the city of Alexandria, has 28°C average maximum temperatures and average minimum temperatures 10°C in winter. The second zone, Middle Egypt, is represented by the city of Cairo, with average maximum temperatures 33°C and minimum average 7°C. Most guavas produced in Egypt are originated from Alexandria and, secondly, in Cairo. Upper Egypt enters the Dakhla oasis, which features only a few guava trees in cultivation in this area. In Egypt, the cultivar called 'Bassateen El Sabahia' has been the standard commercial guava (MORTON, 1987). Efforts were made to improve this cultivar and seedlings were planted. Of these seedlings, the most promising selection was tested and introduced into the cultivation system in 1975, under the name 'Bassateen Edfina'. The fruit has a pear-shape; the flesh is thick and white and has a higher content of ascorbic acid than their parents do. Productivity is also high throughout the season from early spring to late fall.

A remarkable variety of food crops are grown along the width and the length of Sudan. Guava is a tropical fruit very common in Sudan. The plants are grown commercially in all regions and production is continuous throughout the year (SALIH; ELBASHIR, 2000). The main producing regions of guavas in Sudan are Khartoum, Blue Nile (Sennar, Singa, Shandi, Kassala), Northern Kordofan (Rahad, Um Rawaba, El Obeid) and Darfur (BABIKER, 2010). The main problems faced by the

industry are GWD, large number of seeds, small size and the various fruit shapes and low productivity. Fruit growers in Sudan have adopted several old cultivars named after the intensive production areas, namely 'Shendi', 'Shambat', 'Sinja', 'Ganib', 'Sudani' and 'Musaid'. However, there are no distinct cultivars, since the only propagation method used is by seeds, which produce seedlings that are not true copies of the parent plant. Studies were successfully conducted to establish vegetative propagation by root cuttings (HUSSEIN, 2008), however, it is not known if growers are using this method in Sudan.

In South Africa, nurseries are using three methods of propagation namely cuttings, grafting and air-layering. In Egypt, several varieties have been propagated by seed to improve quality and yield. Orchard establishment by budded or veneer grafted varieties has been initiated, however, budding is not commonly used in guava propagation.

Shoot cuttings: propagation through shoot cuttings was not very important in the past; however, more recently, this method has become popular. The best time to prepare the cuttings is from September to March (Southern hemisphere) when the plants are in active growth. Shoots of all kinds can be used for making cuttings provided they are growing actively and have young leaves. The cuttings should have four nodes and three internodes. Leaves of basal nodes are removed, while leaves from the apical nodes are cut in half to reduce transpiration as discussed previously. Depending on the length of the internodes, the cuttings size can be reduced to ensure that only one node remains within the rooting media. The cuttings are placed in steam sterilized rooting substrate to produce basal leaves with approximately 10 mm above the substrate .

Cuttings are treated with root promoters (synthetic auxins such as indole-butyric acid-IBA or naphthaleneacetic acid-NAA) and placed in a container at the propagation unit with mist (**Figure 3a**). The cuttings are rooted when temperature of the propagation unit is maintained at 28°C with regular water spray (intervals of 5 sec every 5 min.). The cuttings will be ready for establishing in the field after approximately three months. Cutting developed from young and juvenile branches give better rooting compared with cutting developed from mature and old branches.

Grafting: plants can be grafted when they reach a height of 200 mm and diameter of approximately 5mm to 7mm. The whip grafting technique is used commonly. The plant material to be used in the grafting process should be treated against fungal diseases, before handling. For the

graftwood, it has to have exactly the same thickness of the rootstock or be substantially thinner than the rootstock at the level where the grafting is performed. When the graftwood is cut from the mother plant, leaves should be removed immediately. The rootstock and the graftwood to have straight and smooth cuts made with sharp grafting knife to ensure a perfect fit.

Once the rootstock and the graftwood have been properly attached, the graft union is covered with a perforated PVC grafting tape. Care should be taken to make sure that graft between the rootstock and graftwood coincide with one another. The cutting is then entirely covered with biodegradable material – Parafilm®. After approximately 2-3 weeks, the buds begin to swell. When the buds break the Parafilm® and have about 6-8 nodes with hardened leaves, the grafting can be removed. All shoots developing below the graft union must be removed regularly to prevent competition of rootstock growth.

Air layering: in the past, air layering (**Figure 3b**) was the most important method used to propagate the guava tree. However, due to the development of GWD-resistant rootstocks, this method is no longer used in nurseries on a commercial scale. Air layering can, however, still be used to propagate the guava tree. Care should be taken to ensure that the propagating material was obtained only from the best plant with no Guava Wilt Disease symptoms. The most appropriate time for air layering is from August to February (Southern hemisphere). A suitable strong, straight shoot on a mother tree must be selected for air layering. The selected shoot must have 500 mm of length from the tip to where the branch is to be ring-barked. A piece of bark, about 25-40 mm wide, is removed around the selected branch. The cambium layer (slimy tissue) between the bark and the wood must be either scraped off or left open for 2 days to dry.

The wound is covered with damp sphagnum moss, moist and sterile, or a mixture 50:50 of peat moss and sterilized decomposed humus and then tied with special PVC film for layering. As there are no rooting problems in this method, the use of rooting hormones is unnecessary. The roots become visible within 2-3 months, depending on the climatic conditions of the region. Once they reach approximately 50% of the roots developed, the layers are removed from the mother tree and planted in a 5-liter plastic bag, until they are strong enough to be planted in the orchard.

Root cutting: it was used successfully in the past. The roots are cut at approximately 0.5 to 1 m from the trunk of a mature tree. Shoots that develop from the chopped roots are removed with their roots

and planted in 5-liter plastic bags. However, in this method there is possibility of GWD entrance through wounds in the roots and thus it is not recommended.

Topworking of old trees: old trees can be successfully top-worked in the orchard. The tree must be sawn off at no less than 0.5 m from the ground. The top of the trunk must be treated with fungicide and the rest of the trunk, painted white with PVA diluted paint. The shoots initially arise from the top of the sawn trunk. These shoots should be removed to force the sprouts below (200 mm) to develop. The strongest shoots are then selected to obtain the desired format. Six to eight shoots are then whip-grafted. All other shoots should be removed to reduce competition for nutrients. This technique is preferred to directly grafting into the main trunk, since the shoots will form a strong graft and will not break easily with strong winds. Once an acceptable format of 4-5 branches have been formed, all additional grafts and other branches are removed. At this stage, the grafting tape must also be removed from the grafting region. The old trunk that is protruding above the new format of the plant can then be sawn and treated with a fungicide sealant.

Budding: it is done immediately before or during the growing season. However, some species can be budded during winter, while they are dormant. It is necessary to make sure that the scion and the rootstock are compatible, the scion is mature, and the cambia of the scion and rootstock match. In guava, the “T” or shield budding is the most commonly used budding technique. The incisions are made in the rootstock bark to present the letter “T” shape, with a horizontal and a vertical cut, which originates from the center of the first cut. A bud piece or a shield containing a bud is prepared with a cut upward, which includes a thin layer of wood of about 1.25 cm (0.5 inch) underneath the bud. A horizontal cut is then made with about 2 cm (0.75 inch) above the bud to remove the shield of the budding branch. This piece of bark has an elongated shape, with a bottom end and a horizontal curved top. The shield, usually a thin layer of adhesive wood, is inserted into the T-cut, from the horizontal cut, downward. It is necessary to tie the area around the bud with a thin (2 mm) polyethylene tape, but without covering the bud.

Seed propagation: seeds are removed from the guava fruit and rinsed to remove all the fruit flesh around the seed adhered. The seeds are sown in trays containing a mixture of compost and placed in a bed with mist to begin the germination process.

Challenges and perspectives

There are three potential serious threats to the guava industry worldwide, which can lead to a

dramatic decrease in production. Firstly, Guava Wilt Disease (GWD), caused by a soil fungus, which has had a destructive effect on guava orchards since the beginning of 1930's (GUPTA et al., 2010). Currently, there are no chemical control measures available; therefore, the only solution is the development of disease-resistant cultivars. This lack of GWD-resistant varieties is a major concern.

In South Africa, Guava Wilt diseases caused by *Nalanthamala psidii* and is a serious disease of guava trees in two of the main guava producing regions, Mpumalanga and the Limpopo province (SCHOEMAN; LABUSCHAGNE, 2014). The Institute for Tropical and Subtropical Crops of Agricultural Research Council (ARC-ITSC) released a cultivar of GWD-resistant guava tree, named ‘TSG2’, in 2000. This is the main cultivar produced commercially in South Africa. However, in 2009, new outbreaks of GWD occurred and resulted in the identification of a GWD mutant strain. Although cultivar ‘TSG2’ is still resistant to the original GWD strain, unfortunately it is not resistant to the mutant strain. Therefore, a greater effort is needed to identify new resistant cultivars.

Nematodes, more specifically the root-knot nematodes (*Meloidogyne* spp.), are also a major threat to the guava industry, since they can destroy the root system of plants and, therefore, inhibit the effectiveness of nutrients and water uptake, resulting in poor yields. In severe infestations, plants can be killed by the nematode. Although chemical control measures are available to control these organisms, it is an expensive method, highly toxic and, in many countries, chemicals are not easily available. The third constraint is the lack of cultivars with excellent internal fruit traits, including red color, small number of seeds, longer shelf life and high yields.

As the guava is classified as minor crops in Africa, not much research has been carried out on this tropical fruit, except in South Africa and Sudan, where investigation is being conducted on genetic improvement, propagation and disease management. It is not known whether researchers in Egypt are also running a research program on guava. A great advantage is that there is a wide range of aspects of guava that can still be investigated, because many research trials have not been conducted on this culture.

One example is the development of micropropagation methods for the guava tree. Although *in vitro* cultivation of seeds and young seedlings is relatively easy and very successful, it is not particularly useful due to the genetic variation of seedlings (YASSEEN et al., 1995; HANNWEG,

personal communication). The establishment of growing shoots from selected adult guava trees in the field is hard (AMIN; JAISWAL, 1987). The challenges include not only the excess of secondary metabolites (phenols, for example), leaching by mature tissues that prevent the culture establishment (BROODRIJK, 1989; CONCEPCION et al., 2005), but also the difficulty in removing surface and endogenous contaminant microorganisms even when mitigating treatments are applied (ZAMIR et al., 2004). In addition, genotypic differences also play a role in the establishment of shoot cultures (JOSHEE et al., 2004). Indirect methods of propagation, such as somatic embryogenesis (JAISWAL; ACHTAR, 1993; 1994) and/or organogenesis of plant organs (leaves and stem sections, for example) can be an alternative to micropropagation of recalcitrant genotypes; however, these methods also have their own challenges, including the development of an entire plant through embryogenesis and/or organogenesis. A successful technique for tissue culture in guava would facilitate the multiplication of plants identified as superior genotypes for further evaluation, as well as provide disease-free plant material to growers.

Guava is one of the fruits with the highest amount of vitamin C per gram of fruit. The expansion of commercial guava orchards in Africa may aid in the need and availability of food and nutritional food products as well as in the increase of income for poor African families. Furthermore, export of processed guava products to Asian countries increased substantially during the last two years. Only white varieties are produced in those countries; therefore, fruit pulp and concentrates from pulp of red varieties are becoming more popular and are highly demanded by Asian countries. If pink/red, Guava Wilt disease-resistant varieties with excellent fruit features are becoming available, guava will expand significantly and nursery gardeners, growers and processors across Africa will benefit and grow toward a brighter future.

GUAVA PROPAGATION AND RESEARCH ADVANCES IN PAKISTAN AND OTHER ASIAN COUNTRIES

Asian countries account for most of the world's tropical fruit production, which is 76% of guava, mango and mangosteen. The main Asian guava producing countries are India, Pakistan, Indonesia, Bangladesh, Thailand and Taiwan (Table 1). Although Pakistan takes the second place, after India, in guava production, the average productivity is only 7.5 t ha⁻¹ and it is the lowest among the major Asian guava producers, like Thailand and Taiwan,

which produce > 23 t ha⁻¹ (Table 1). The potential for increase in productivity from 13 to 14 t ha⁻¹ in countries with smaller capacities, such as Pakistan, should be considered. This gap is presumably due to little focus on research for the selection of best native guava genotypes, lack of new cultivars with high yield, absence of a commercially acceptable method of clonal propagation, use of low density of planting and needs for more efficient breeding and biotechnology programs. In Pakistan, guava is the fourth most widely produced fruit in the country, after citrus, mangoes and dates. Approximately 80% of the guavas is produced in the Punjab province, with only 7 t ha⁻¹ (Figure 4). In recent years, the area cultivated with guava increased markedly, while production decreased, due to the development of new foci of GWD or replanting in affected areas (Figure 4). Other factors for the low productivity in Pakistan are the use of seedling populations for planting, particularly in Punjab (BUTT et al., 2013), biotic factors such as GWD presumably caused by *Fusarium* sp. (USMAN et al., 2014), and fruit fly *Bactrocera dorsalis* (SARWAR, 2006), besides abiotic factors, including drought and salinity.

Conventional non-sanitized nurseries have been the main source of soil pathogens dispersion in all guava orchards in Punjab (USMAN et al., 2014). Inappropriate management practices have also contributed to the decline of the guava culture. The nursery trees production system of guava trees in sanitized containers was started at the University of Agriculture in Faisalabad, Pakistan, and has helped to mitigate the problem (Daily Times, January 20, 2013). The seedlings are produced in plastic containers after seed sterilization and scarification. These containers are filled with steam-sterilized substrate for better plant growth and development (USMAN et al., unpublished data).

Four types of guavas are grown in Pakistan, including round (Gola) of white (RW) and pink flesh (RP) and Pyriform (Surahi) as white (PW) and pink flesh (PP). Consumers prefer white-flesh cultivars, which are thus grown in large areas, compared to pink-flesh guava, which are preferred for processing. Amateur gardeners have selected different genotypes in different localities and the 'Sadabahar' cultivar produces for 7-8 months a year. However, these cultivars have also been propagated sexually similar to other cultivars, which leads to varietal degradation. The establishment of compact orchards is becoming popular (SINGH, 2008) and fruit growers are likely to adopt ultra-high density (1,500–2,500 plants per ha), indicating high demand for healthy nursery trees in the coming years. The guava tree has been

multiplied sexually in Pakistan and asexually (different cutting and grafting methods) in other Asian countries.

Sexual propagation: this method has been a dilemma for the guava culture due to low rate of seed germination and it is still practiced commercially where success rates of asexual methods are low (USMAN et al., 2012). The resistant coating of seeds, seed dormancy and presence of tannins can lead to poor, unequal and delayed seed germination (ALI et al., 2007). Unfortunately, the guava culture in Punjab, in Pakistan, is based on the use of seedlings. Seed germination was increased to 90%, with the seed treatment with 10% HCl for 12 h. (USMAN et al., 2012; BUTT et al., 2013). Among different growth regulators, GA₃ increased seed germination compared with other treatments, such as hot water and thiourea application (KALYANI et al., 2014). Sexual propagation leads to degradation of the plant variety; however, it also increases heterozygosity and provides heterogeneous populations for effective selection. Thus, several cultivars primarily selected by the amateur growers in round (Gola) and pyriform (Surahi) varieties are available under different names based on their shape, size and growth habit, in different growing regions in Pakistan. There is great need to select promising materials on morphogenetic basis, introduce exotic cultivars to germplasm collections, their assessments of fruit quality attributes and establish effective clonal propagation systems in promising native and exotic varieties.

Asexual propagation

a) *In vivo*: asexual propagation using conventional techniques has been hindered by the long juvenile phase, seasonal dependence, longer time length and requirement of more plant material for propagation (JAISWAL; AMIN, 1992; USMAN et al., 2014). Propagation by herbaceous cuttings has been more effective in comparison with other methods such as layering, budding or grafting in major guava producers in Asia (**Table 2**). The mixture of substrates, including sand, vermiculite, perlite and peat moss increased rooting with or without the use of auxins, such the IBA and NAA (GAUTAM et al., 2010; CHENG et al., 2011). However, the key factors to induce roots on cuttings are the plant age, cuttings collection season, temperature and humidity. Air and mound layering are also used as propagation methods in different countries; however, these methods cannot be used for mass propagation because the processes are slower and less efficient. The mixture of rooting substrates and soil has been used in layering (SAHA, 2015). In different grafting methods, patch

budding and veneer grafting had the highest success rates in propagation of different guava cultivars in Bangladesh, India and Taiwan. This can have a particular importance if GWD-resistant rootstocks could be identified and used for grafting or budding in elite scion guava cultivars.

In budding methods, patch budding has been more popular and ‘Allahabad Safeda’ was propagated using this method (BHATT et al., 2013). In Pakistan, guava is propagated by air layering and herbaceous stem cuttings in the Sindh province, on limited scale (Figure 4). The success rate is higher presumably due to mild temperature and higher humidity, which are adequate to rooting compared to the hot and dry climate of the Punjab province in Pakistan. However, few reports show rooting success using herbaceous cuttings of guava and grafting has limited success, in the Punjab province in Pakistan. Growers and other stakeholders are searching for clonal guava nurseries, despite the commercial technology models presented by public institutions linked to the sector (HAFEEZ et al., 1991; KAREEM et al., 2013).

b) *In vitro*: tissue culture of plants offers several advantages compared to conventional tools to propagate guava. For instance, efficiency and cost-benefit analysis for plant propagation throughout the year, the use of less plant material for the establishment of cultivars, greater success, better growth and development of plants due to the predominance of an aseptic environment (CHANDRA; MISHRA, 2005; USMAN et al., 2006; 2012). Micropropagated plants may also yield higher than their parent as in the case of banana. Clonal propagation via direct organogenesis has been established in guava cultivars like ‘Allahabad Safeda’ (SINGH et al., 2002) and cultivars of types “Round” and “Pyriform” (USMAN et al., 2012). The increased production through genetic transformation also requires an efficient *in vitro* regeneration system (RAI et al., 2007). Thus, the future of the guava industry lies in the development of an efficient clonal method that produces healthy plant materials through micropropagation.

Problems of guava clonal propagation and promising approaches

There are two recognized *in vitro* regeneration patterns, organogenesis and embryogenesis, which use *in vivo* plant explants or *in vitro* raised seedlings. However, tissue regeneration of guava trees from *in vivo* sources has been hindered by excessive microbial contamination, greater exudation of plant tissues by phenols, leading the explant to darken, and the recalcitrant nature of plant tissues (own

observations). The shaking and soaking of plant tissues in antioxidants, such as polyvinylpyrrolidone (PVP), solution of ascorbic acid and citric acid and the use of PVP in nutrient medium have significantly minimized phenolic exudation in the medium and aided in the establishment of plantations with explants from *in vivo* sources (LIU; YANG, 2011; USMAN et al., unpublished data). Explant sources from the seedlings established *in vitro* or from plantlets grown in the greenhouses (low light and low temperatures), has resulted in less contamination and little phenols due to reduced synthesis of phenolic compounds in young juvenile plants (CHANDRA; MISHRA, 2005; USMAN et al., 2012). Microbial contamination was also reduced when the explants were collected from plants kept in the greenhouses, compared to the plants grown in the open environments. The spraying of appropriate fungicides at 3-4 days before transferring the plants to the greenhouse has also minimized fungal contamination chances in the explants. Applications of sodium hypochlorite 5-10% followed by mercuric chloride 0.5-1.0% have reduced microbial contamination of the explants, harvested from adult woody plants (ALI et al., 2007; USMAN et al., 2012). Donor plant and explant age, position of shoot on the mother plant, and the season also play a critical role in the success of crop establishment. Explants harvested at 3 to 5 nodes in distal young shoots emerging from the main stem base have shown higher rates of establishment of *in vitro* cultures, compared with mature branches and woody branches growing outer side of the canopy of mango trees (KRISHNA; SINGH, 2007) and guava trees (USMAN et al., unpublished data). Explants of juvenile seedlings responded better to the establishment, when compared to adult woody plants (SHAH et al., 2008). Recent reports have shown responses of *in vitro* regeneration via organogenesis and embryogenesis.

Organogenesis: the production of adventitious shoots from callus or differentiated tissues is called organogenesis. In guava, organogenesis has been reported both in juvenile seedlings raised *in vitro* (USMAN et al., 2012; MISHRA et al., 2014) and *in vivo* (SHAH et al., 2008; USMAN et al., unpublished data) and in adult woody plants grown in an open environment (MEGHWAL et al., 2010; LIU; YANG, 2011; ALI et al., 2007). Responses of *in vitro* regeneration are mainly controlled by the type of explant, medium composition and type of plant growth regulators (PGRs), as well as organic and inorganic additives in the medium and cultivation conditions. In highly recalcitrant species, such as guava, the efficiency of

in vitro clonal propagation depends on the quality of the genotype, explant, physical state of the tissue and the season. Organogenesis has been reported in cultivars Allahabad Safeda, Banarasi, Safeda and Beaumont in India, Pakistan, Malaysia and Iran (**Table 3**). Regeneration of shoots was more efficient in explants harvested from the young plants kept in the greenhouse and *in vitro* cultivation (USMAN et al., 2012) compared to the explants harvested from adult woody plants for later multiplication. However, in cultivars Allahabad Safeda and Beaumont, regeneration of shoots has been established using nodal explants of adult plants (MEGHWAL et al., 2010; LIU; YANG, 2011). The composition of the environment plays a crucial role in *in vitro* plant morphogenesis. Regeneration of shoots in cultivars of guava trees has been reported mainly in MS medium (MURASHIGE; SKOOG, 1962) with 6-Benzylaminopurine BAP (**Table 3**) in explants collected from adult plants and *in vitro* seedlings (SHAH et al., 2008; LIU; YANG, 2011; USMAN et al., 2012; MISHRA et al., 2014), indicating higher metabolism rate of BAP in guava tissues compared to other cytokinins (MALIK et al., 2005; RAI et al., 2008b; RAI et al., 2010). Kinetin and zeatin have occasionally been used in combination with BAP (SHAH et al., 2008; LIU; YANG, 2011). Other additives to enhance induction of buds and higher growth rate include adenine sulfate and sucrose, while activated carbon has improved the frequency of rooting (THOMAS, 2008; USMAN et al., 2012). The development of roots in excised shoots from explants of adult plants require supplements of IBA and NAA to the medium for induction. In case of shoots excised from *in vitro* established seedlings roots developed in MSO medium, devoid of growth regulators (RAI et al., 2010; USMAN et al., 2012). Among the different gelling agents, agar has been widely used in the medium of organogenesis in different cultivars and the use of liquid medium has not been reported.

Embryogenesis: somatic embryogenesis is a useful tool of plant biotechnology and it is widely used for mass multiplication of elite cultivars. In embryogenesis, somatic cells produce masses that develop into mature embryos under conducive cultivation conditions (NIC-CAN et al., 2015). Somatic embryogenesis also assists in studies of plant differentiation, totipotent expression at cell level and it has been widely used for genetic modification in woody plants. Somatic embryogenesis has been widely explored (RAI et al., 2009a; 2012; KAMLE et al., 2014) in cultivars Banarasi and Allahabad Safeda, using zygotic embryos as explants. Other

explants such as leaf disks, internodes, petals and mesocarp were also explored for embryogenesis; however, only the induced mesocarp developed embryos (CHANDRA et al., 2007; BUTT et al., 2013). Immature zygotic embryos obtained after 8-10 weeks of development, induced embryogenesis in cv. Banarasi (RAI et al., 2010). Embryogenesis was induced in MS medium supplemented with 2,4-D (RAI et al., 2008c; RAI et al., 2012; KAMLE et al., 2014) compared to the combination of 2,4-D and other auxins or cytokinins. Nitrogen sources including ascorbic acid and L-glutamine effectively induced somatic embryogenesis, while L-proline and PEG increased maturation of somatic embryos (RAI et al., 2012). Among the different carbon sources, 5-6% sucrose were better for induction and maturation of somatic embryos while the addition of fructose, glucose, maltose, mannitol and sorbitol in the medium showed inhibitory effects (RAI et al., 2008c). The medium solidified with agar (0.7-0.8% weight/volume) showed better induction of embryos, compared to the liquid medium. Germination of embryos can be improved by the reduction of salts (half) and sucrose (3%) in the medium (RAI et al., 2007). Conclusively, the progress on clonal propagation of guava trees has been highly

encouraged with the use of organogenesis and somatic embryogenesis in elite cultivars; however, its marketing is still incipient.

In Punjab-Pakistan, the main limiting factors for clonal guava propagation in field conditions involve a prolonged summer season, with high temperatures and dry weather. Little interest by nurserymen in clonal propagation methods may be attributed to low success rates. Success in the asexual propagation methods has been reported since the 1990's; however, few commercial nurseries adopt clonal methods. Guava grower have been able to select genotypes with better fruit quality and higher yields; however, their clonal propagation has been a dilemma. Expansion of *in vitro* clonal propagation may facilitate rapid clonal propagation; nevertheless, its cost effectiveness in a time of energy crisis needs to be addressed. Other issues of the guava culture, such as GWD, fruit fly, short shelf life of fruits and the greater sensitivity to abiotic stress, like frost, also needs to be considered. Molecular biology tools like marker assisted breeding (MAB), genetic transformation for stress tolerance, use of genomics, bioinformatics and molecular physiology maybe used for a better understanding of the plant physiology under changing climatic conditions and the development of resistant cultivars.

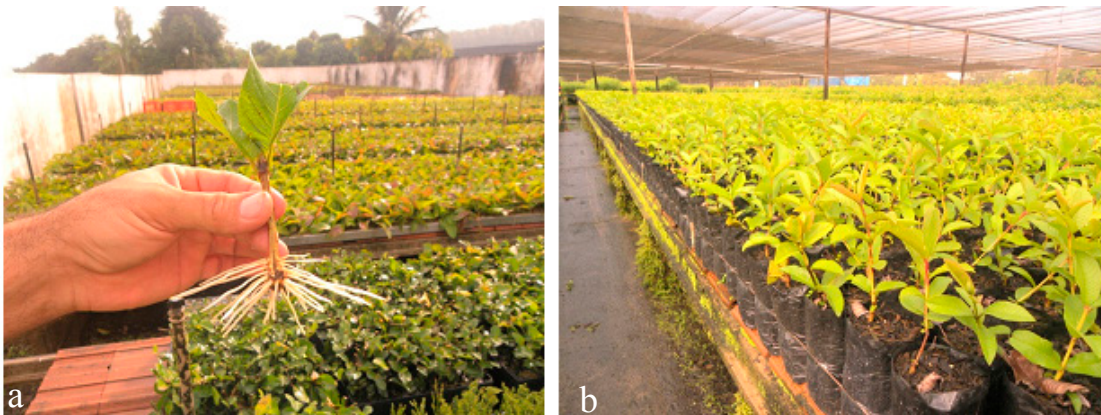


FIGURE 1- Propagation method of guava tree prevalent in Brazil: **a)** rooting phase of herbaceous cuttings under intermittent mist; **b)** acclimation and growth of rooted cuttings in plastic bags containing commercial substrate and kept on countertops, in shaded nursery with concrete floor.

Photos: Newton Alex Mayer.



FIGURE 2- Guava production regions in South Africa.



FIGURE 3.a) Guava tree cuttings in rooting bed under mist; b) air-layering on mother tree of guava in the orchard.

Photos: Oscar Maphanga.



FIGURE 4- Main producing areas of guavas in Pakistan, where GB represents Gilgit-Baltistan, KPK represents Khyber Pkhtunkhwa and AJK represents Azad Jammu and Kashmir.

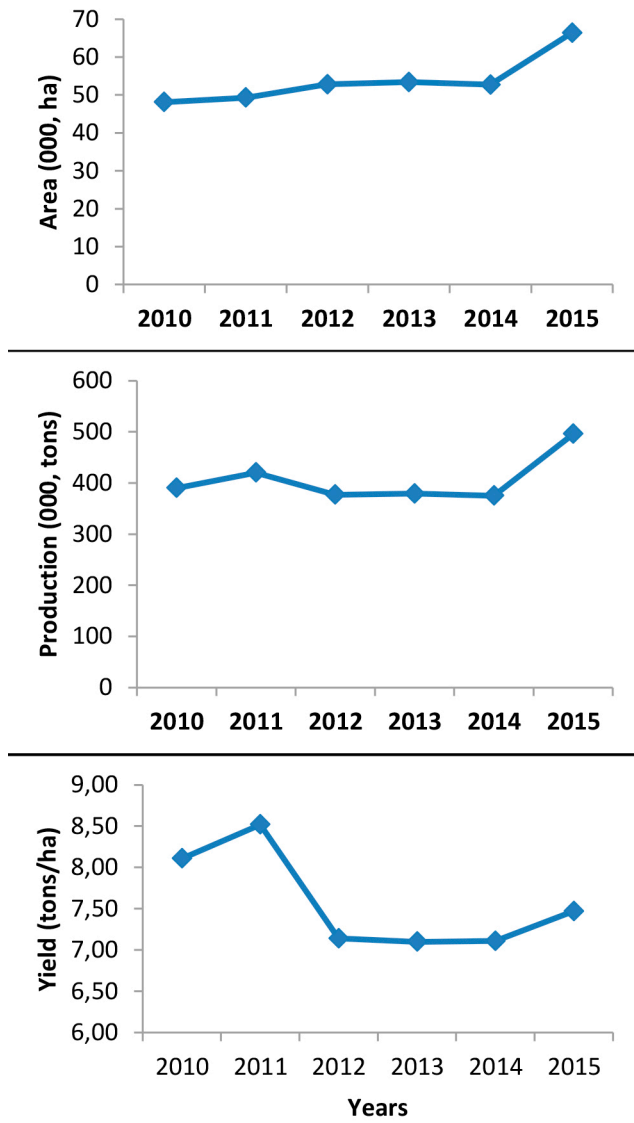


FIGURE 5-Data on guava culture in Pakistan.

TABLE 1-Area, production, productivity, main cultivars and propagation methods used commercially in some guava producing countries.

Country	Area (x1000, ha)	Production (x1000, t)	Productivity (t ha ⁻¹)	Main cultivars	Propagation methods	References
Bangladesh	18.0	200.0	11.11	Kazi Peara (guava), BARI Peara, BAU Guava 1-8, Kazi, Swarupkathi, Mukundapuri and Allahabad	Stem herbaceous cuttings, air layering (>95%)	BBS (2012); Rahim et al. (2011); Saha (2015)
India	255.0	4102.0	16.08	Allahbad Safeda, Sardar (L49), Lalit, Banarasi and Shweta	Stem herbaceous cuttings, chip budding and grafting (>95%)	NHB (2015-16); Mitra (2014)
Indonesia	9.4	208.0	22.12	Indonesian white, Indonesian seedless, ARP9409 and JBT001	Stem herbaceous cuttings, grafting (>95%)	BPS (2014); Nasution; Hadiati (2014).
Malaysia	4.0	65.0	16.25	Red Malaysian, GU 8 (Jambu Kamopochea), GU 11 (Giant guava), GU15 (Jade Seedless), Batu Arang, Subang 6 and Sitiawan	Stem herbaceous cuttings, grafting and air layering (>95%)	Pommer and Murakami (2009); MAM (2013)
Pakistan	66.38	496.0	7.47	Sadabahar Gola (Round), Sadabahar Surahi (Pyriform), Large Pyriform, Larkana Pyriform and Safeda	Seed (>80%), cutting and air layering	MNFSR (2014); Usman et al. (2013)
Thailand	7.94	168.0	21.15	Klomsali, Salithong and Kimchu	Soft wood cuttings, grafting (>95%)	Siwabungson et al. (2013)
Taiwan	7.12	168.0	23.59	Century, Pearl and Crystal	Soft wood cuttings and wedge grafting (>98%)	Agriculture and Food Agency (2014).
Egypt	17.5	315.0	18.0	Bassateen El Sabahia, Bassateen Edfina and Allahabad Safeda	Soft wood cuttings and mound layering (>85%)	Pommer and Murakami (2009); EMALR (2014); El-Shereif (2016)
South Africa	0.5	12.5	25.0	TSG2, Fan Retief, Fredene, Dimple, Jonelle, Welheim, Frederika, Frank Marlhebe, Van Zyl, Rousseau and Du Preez	Soft wood cuttings, air layering and grafting (>80%)	DAFF (2015); HORTGRO (2015)
Sudan	22.0	473.0	21.5	A-H-180, A-H-132, A-H-157, Pakistani and Beamonal	Air layering, semi-hardwood cuttings	Agriculture in Sudan (1999)
Cuba	7.31	47.88	6.55	Enana Roja Cubana	Grafting on seedlings, cutting	Pérez-Rodríguez et al. (2013); Valdés-Infante et al. (2012); Pommer et al. (2006)

Mexico	20.44	302.7	14.81	Wild types named "China" and "Media China"	Seeds and root cuttings	Martínez-de-Lara et al. (2004); SIAP (2016)
Brazil	14.98	349.6	23.34	Paluma, Rica, Século XXI, Pedro Sato, Sassaoka, Kumagai and Ogawa	Stem herbaceous cuttings (> 95%)	Agriannual (2016); Pereira & Nachtigal (2009)
Colombia	8.34	69.1	8.28	Regional Blanca, Regional Roja, Ráquira Blanca, Guavatá Victoria, Pera and Manzana	Seeds (in home backyards), grafting or cutting for commercial orchards	Ocampo & Núñez (2007); Parra-Coronado (2014)
Venezuela	3.50	55.7	15.9	Criolla Roja, San Miguel and Rio Chiquito	Seeds	Aular & Casares (2011)

TABLE 2- Summary of clonal propagation of plant varieties of guava tree in the field.

Propagation method	Media	Growth regulators/ nutrients/ treatment	Cultivars	Country	References
Cuttings (soft wood and semi-hardwood) 10 to 15cm of length	-	-	Red Malaysian, GU8 (JambuKamopochea), GU 11 (Giant guava), GU15 (Jade Seedless), BatuArang, Subang 6 and Sitiawan	Malaysia	Pommer; Murakami (2009)
	Vermiculite, soil, sand	Hoagland solution Girdling and ringing of sprout conditioning	-	India	Gautam et al. (2010) Pandey; Bisen (2010)
	1:2:1 (peat moss, perlite, vermiculite or sterilized sand)	IBA, NAA	Century, Pearl and Crystal	Taiwan	Cheng et al. (2011)
	Soil	IBA, NAA	Safeda	Pakistan	Kareem et al. (2013)
	-	-	Klomsali, Salithong, Kimchu	Thailand	Siwarungson et al. (2013)
	Soil	-	Allahabad Safeda	Egypt	El-Shereif (2016)
	Soil	-	Indonesian white, Indonesian seedless	Indonesia	Nasution; Hadiati (2014)
	Sand	-	-	Iran	Sardoei (2014)
Air layering	Sphagnummoss	IBA/NAA	Century, Pearl and Crystal	Taiwan	Cheng et al. (2011)
	Soil, vermicompost, chicken manure, Sphagnum peat, coconut peat	IBA	Allahabad Safeda	India	Maurya et al. (2012)
	Soil, manure, composting	-	BARI Guava-1, 2 e 3	Bangladesh	Saha (2015)

Mound layering	Soil	-	Bassateen El Sabahia; Bassateen Edfina;	Egypt	Pommer; Murakami (2009)
	Soil	-	L-49 (Sardar)	India	Dadas et al. (2015)
Cleft grafting, Veneer grafting	Soil	-	Kazipiara, Swarupkhati, polypiara	Bangladesh	Rahim et al. (2011)
Wedge grafting	1: 1: 3 (sand, compost, rich black soil)	-	Century, Pearl and Crystal	Taiwan	Cheng et al. (2011)
	Soil	-	Arka Amulya Hisar Surkha Hisar Safeda Allahabad Safeda Lalit Pant Guava L-49 and Local guava (roostock)	India	Mamta et al. (2014); Disket et al. (2014)
Patch budding	-	-	Allahabad Safeda	India	Bhatt et al. (2013)

TABLE 3-Summary of organogenesis and embryogenesis studies in guava tree cultivars.

Explant		Media formulation				Cultivars	Country of study	References
Source	Type	Salts and vitamins	Growth regulators/others					
			Callogenesis/ somatic embryogenesis	Shoot induction	Rooting			
Mature trees	Nodal explants	MS, WPM	-	BA	-	White & Red fleshed cultivars	Iran	Shekafandeh; Khosh-Khui (2008)
		MS	-	BAP, sodium alginate	IBA	Banarasi	India	Rai et al. (2008a)
		WPM	-	BAP	IBA, NAA	Allahabad Safeda	India	Meghwal et al. (2010)
		MS	-	BAP, KIN, PVP	NAA	Beaumont	USA	Liu; Yang (2011)
	Axillary buds, shoot tips	MS	-	BAP, IAA	IBA	Allahabad Safeda	Pakistan	Zamir et al. (2007)
Seeds	Zygotic embryos	MS	2,4-D, Sucrose, ABA,sodium alginate	-	-	Banarasi	India	Rai et al. (2008c)
		MS	2,4-D,	PEG, glutamine, proline	-	Banarasi	India	Rai et al. (2009b; 2012)
		MS	2,4-D	-	-	Allahabad Safeda	India	Akhtar (2013)
		MS	2,4-D	glutamine, BAP,	NAA	Allahabad Safeda, Lalit, Sardar, Shweta)	India	Kamle et al. (2014)
	Mature seeds	MS	-	HCl, H ₂ SO ₄	-	-	USA	Ali et al. (2007)
<i>In vitro</i> Seedling	Seedlings	MS	-	BAP, zeatin, GA3	-	Safeda	Pakistan	Shah et al. (2008)
		MS	-	BAP	-	Lalit	India	Mishra et al., (2014)
	Leaf disc	MS	2,4-D	-	-	Safeda (cvs. Round, Pyriform)	Pakistan	Butt et al. (2013)
	Shoot tips	MS	-	BAP, sodium alginate	IBA	Banarasi	India	Rai et al. (2008b)
		MS	-	BAP	-	Beaumont	Malaysia	Abdullah et al. (2009)
		MS	BAP	-	-	Safeda (cvs. Round, Pyriform)	Pakistan	Usman et al. (2012)

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