

Recent Trends In Guava Propagation- A Review

Gurpreet Singh¹, Homraj Sahare^{2*} and Maninderdeep¹

Department of Horticulture, School of Agriculture, Lovely Professional University, Punjab, India.

²M.Sc. Student, Lovely Professional University, India.

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The propagation of the guava (*Psidium guajava* L.) may be carried out with seeds, layering, grafting or budding, cuttings (roots or shoots) or by micropropagation. The guava propagation by seeds is carried out for the growing rootstocks and for growing plants to be detected in the early stages of the cultivation of guava trees. The asexual methods for propagation are used to clone chosen genotypes of breeding methods and to establish commercial fruit orchards, as they provide all the distinctiveness of each cultivar. This review article deals with various methods used in the propagation of guava, which are commercially adopted, and the recent progress and trends related to it. The various propagation techniques are available, but the levels of adoption are quite different in guava producing areas.

Keywords: *Psidium guajava*, vegetative propagation, seeds, clonal propagation, cutting, layering, grafting, tissue culture.

Guava, *Psidium guajava* L., belongs to the family Myrtaceae, which comprises 3,000 species under 80 genera. It is well-distributed in the tropical and subtropical regions of the world, especially in South America, Asia and Australia. The genus *Psidium* has about 150 shrubs and *P. guajava* is well-known and grown worldwide (Paull & Bittenbender, 2006).

The flower of guava is self-pollinated; cross pollination, however, is estimated to be around 35% and pollination mainly carried-out of by bees and other insects (Yadava, 1996, Paull and Bittenbender, 2006). Therefore, seed multiplication will result in genetic heterogeneity that can be observed in orchards and plants in the same orchard (Martínez-De-Lara *et al.*, 2004). Therefore, seed propagation in commercial orchards to increase

productivity is not recommended (Pereira, 1990). The methods of vegetative propagation have been studied for the purpose of the production chain. In addition to the propagation structure, the costs of new technologies must also be taken into account.

Progress in guava propagation has taken place in the last 100 years (Preece, 2003). It is important to know how to use the same methods of graftings and cutting. Detection of growth regulators, information on role of the juvenile stage in reproduction, advances in knowledge of chimeras, micro-propagation and application of vegetative propagation to prevent diseases caused by viruses and other pathogens (Preece, 2003 Hartmann *et al.*, 2002).

One can propagate guava by buddings (Gupta and Mehrotra, 1985, Kaundal *et al.*, 1987),

*Corresponding author E-mail: homraj.19822@lpu.co.in



air layering (Sharma *et al.*, 1978, Manna *et al.*, 2001), stoolings (Pathak and Saroj, 1988) and inarching (Mukherjee and Majumder, 1983) are not yet commercially feasible due to different success rates, the lack of a tap root system and burdensome processes. The purpose of this review is to discuss the diverse methods of propagating guava which are used commercially and the advancement made in recently.

Recent trends in guava propagation

Propagation by seed

The seed propagation is now limited to raising the rootstock materials. Guava seeds for commercial use are normally considered waste. Its use for propagation is limited to breeding plan or multiplication of rootstocks for scion varieties.

In some countries where modern technology is not used, guava is still propagated with seeds. Plantlets made from seeds are called seedlings. This is the simplest technique for the propagation of guava trees. The disadvantage is the use of seeds in plants has great variability. This is an undesirable feature in profitable orchards as it results in low productivity and low fruit quality (Pereira, 1990). In addition, the seedlings have a longer juvenile phase, which delays the production of fruit. Therefore, the use of seeds is recommended only in breeding programs where variability among plants with essential characteristics is required. For example, aroma and flavour vary considerably among young plants (Paull and Bittenbender, 2006).

In some areas of guava production using grafting technology, the seed is used for the cultivation of rhizomes. In this situation there is a genetic variability in root systems, in particular there is a variation in the strength. Another disadvantage is that more time is needed for the production of seedlings (1.5 to 2 years) compared to the propagation of seed varieties (6 to 8 months) by cuttings.

In the seed propagation technique, ripe fruits of selected plants are harvested. Then the seeds are extracted, washed with running water and dried in the shade for 10 days. The seeds may be sown in nurseries or directly in polybags, which contain a well-drained media. Germination can exceed 90% and generally occurs during 15-20 days. For grafting purposes, the seedlings should have a diameter of 12-20 mm.

The germination of guava seeds was improved by immersing it in tap water before planting it. Cold water also had a beneficial effect, but immersion in hot water had been detrimental (Haq *et al.*, 1973). Bhanuprakash *et al.* (2008) stated that dipping the guava seeds in distilled water for 48 hours had 96% germination. Alves *et al.* (2015) recommended the manual collection of fruit seeds, washing them under running water in a fine colander, drying them for three days at room temperature and using a roll of paper, sandpaper or filter paper at 20- 30°C.

Seed coating, seed dormancy and tannin can cause poor, irregular and late germination (Ali *et al.*, 2007). Germination of seeds was improved to 90%, with seed-treatment with 10% HCl for 12 hours (Usman *et al.*, 2012, Butt *et al.*, 2013). GA3 has improved seed germination among various growth regulators as compared to other treatments, such as the application of warm water and thiourea (Kalyani *et al.*, 2014).

Sugahara and Takaki (2004) observed that “The germination of the seeds of *Psidium guajava* L. is controlled by the phytochrome. The guava seeds can sprout with at least one hour a day of irradiation with high red light: the distant red preceded or followed by the light shade, which indicates that the phytochrome B controls the germination under these conditions. At alternate temperatures, in an interval of at least 5 ° C, the seeds will germinate in the darkness, suggesting that in the cracks of the foliage, when the seeds are covered by a thin layer of soil, will sprout once the alternating temperatures are experienced. Under these conditions, phytochrome A is responsible for controlling the germination of guava seeds”

The effect of different periods of water and acid soak on germination of seeds in guava cv. Allahabad Safada The seeds immersed in water for 36 hours showed a higher percentage of germination (90%) and a reduced time for the appearance of seedlings compared to the seeds soaked in H₂SO₄, HCl and HNO₃. (Pandey and Singh, 2000).

Essien (2004) reported that the maximum percentage of germination was recorded with conc. sulfuric acid (98%) followed by nitric acid (93%) and hydrochloric acid (78%) on control (20%). Manoj *et al.* (2013) observed that the guava seeds scarified with 10% hydrochloric acid for 2 minutes

were higher than the control with the highest percentage of seed germination, the seed strength index and the survival rate of the seedlings. B. Propagation by vegetative method

Traditional asexual propagation techniques have been hampered due to juvenile phase of longer duration, season reliance, long life span, and increased plant propagation material (Jaiswal and Amin 1992, Usman *et al.*, 2014). The mound and air layering are also used as a multiplication method in various countries. However, these techniques can't be used for large scale because the procedures are less effective and slower. The mix of soil and rooting media were used in layers (Saha, 2015). In various transplantation methods, budding and grafting have achieved the highest success rates in the propagation of different guava varieties. This can be particularly important if the wilt-resistant rootstock can be identified and used for grafting or budding of improved cultivars.

The main asexual methods of propagation adopted in guava are described below:

Cutting

Stem cutting

The propagation via stem cuttings was not very significant in older days. Recently, this method has become popular. September to March is the appropriate time for the preparation of cuttings (southern hemisphere), when the plants are in full growth. One can use stem of all kinds to make cuttings and have young leaves that grow actively. Cuttings must have three internodes and four nodes. Due to the length of the internodes, the size of the cuttings can be reduced to ensure that only one node will be immersed in rooting media. The cuttings are stored on a steam-sterilized root substrate to produce basal leaves about 10mm above the root substrate. Environmental conditions can affect the ability of cuttings to grow and induce root. The most common external factors are light, seasons, temperature, humidity, moisture level of the cutting medium and rooting (Hartman and Kester, 2002). Evans (1992) and Singh (2018) have argued that the best time to make cuts in the field probably at the beginning of the rainy season.

“Propagation by cuttings has a significant advantage because, in addition to obtaining plants with the same type of tree, it guarantees the production of economically important plants in a single period of growth” (Tavares, 1994). “Rooting

between methods of vegetative propagation is undoubtedly the most evolved and expanded method” (Manica, 2002), but information on rooting cuttings in guava is very poor. It has been found that the application of auxin improves the histological features such as the formation of callus and tissues and the differentiation of vascular tissue (Singh 2018, Singh, 2017).

Abdullah *et al.* (2009) reported that cuttings had 60% and 70.9% survival rooting rates in the mist-free propagator when treated with a concentration of 4000ppm IBA.

In the absence of treatment with Indole-3-Butyric acid, the cuttings had not induced root. The diameter of the maximum average stem, the number of leaves, the number of roots per plant, the length of the root and the root weight observed in the IBA treated cuts at 1000ppm. The root cuts can give good rooting and survival when the cuts are made in July (Singh *et al.*, 2017).

It would be 2,500 ppm IBA shown the best performance (73.3 to 83.3% success rooting, 5.82 to 7.16 primary roots and 101.2 mg to 112.4mg dry weight of roots) regardless of the variety, while 3500 ppm IBA decreased the rooting parameters (Debnath and Maiti 1990). Several workers had conducted experiments on the effects of different concentrations of IBA, NAA treated with different combinations in percentages of rooting percentage, number of roots, promoting the best characters observed with different media and better performance observed at the root and shooting characteristics (Pandey and Bisen, 2010; Kareem *et al.* 2013; Gautam *et al.* 2010, Rahman *et al.* 2004; Vale *et al.* 2008; Wahab 2001; Noor *et al.*, 2004).

The mixture of vermiculite, perlite, sand and peat in the rooting medium, increases rooting with or without using auxins such as IBA and NAA (Gautam *et al.*, 2010, Cheng *et al.*, 2011). But, the main reasons for rooting in cuttings are plant age, harvest time, temperature and humidity.

Semi-hard cuttings are not normally used commercially. Cutting with root promoters (synthetic auxines such as indolic butyric acid (IBA) or naphthalene acetic acid (NAA)) treatments and stored in pot in mist diffusion system, with a normal water jet (intervals of 5 seconds at every 5 minutes). After about three months, the cutting can induce roots in the field. The cuttings prepared

by the young and juvenile branches offers a better rooting than the mature branches.

Wahab *et al.* (2001) revealed that “cuttings of guava stem (*Psidium guajava* L.) cv Safeda discovered that the auxin had no effect on the number of days for outbreaks of buds, while sprouting in itself increased significantly with IBA at 1000 and 3000 ppm and NAA at 2000 ppm are 79.84, 75.96 and 76.59% respectively. Maximum survival (12.50%) was observed in cuttings treated with IAA at 3000 and 6000 ppm, IBA and NAA, both at 6000 ppm. IAA at 3000 ppm significantly increased the number of leaves (16) per cutting. The highest number of roots (23.75) per cutting was recorded in the cutting treated with IBA at 4000 ppm. The significantly longer root length (4.13 cm) was observed in the stem treated with IAA 3000 ppm. The cutting treated with IAA and IBA at 5000 ppm at 4000 ppm showed significantly higher roots weight and 16.62g and 16.25g respectively.”

While Zamir *et al.* (2017) found that “As compared with the control, auxin treatment favored rooting in both cuttings. The maximum number of roots per cutting was between 11 and 17.3 for semi-hardwoods, while that for softwood was between 15.6-27.6. Whatever the nature of cuttings the IAA and NAA seems to be the most effective at promoting roots. In contrast, IBA was more effective on softwood cuttings only. However, the ultimate survival of plants is the key to such studies. Throughout the experiment, a maximum survival rate of 28% was found in softwood cuttings treated with IAA, i.e. 100 mg per 100 g of talc.”

Kareem *et al.* (2016) observed that the IBA (4000ppm) showed maximum results in terms of shooting percent, roots number per cutting, normal root length respectively.

Root Cutting

Webber (1944) pointed out that “adopting root cutting in colder regions such as California was the main advantage, as well as the prospect of propagating guava plants by cuttings. Because of the intense cold that caused the freezing and death of trees in orchards, it was likely to use new shoots from the root system to propagate new plant without a graft because the root system is genetically identical to the stem. It has been used effectively in the past. The roots are cut about 0.5-1 m from the trunk of a mature tree.” The shoots that grow during root cutting are removed with their

roots and planted in 5-liter plastic bags. With this method, however, it is possible to induce guava wilt disease through the root wounds, which is why it is not recommended. The results showed that the plant did not have a root absorption system with this method. Fracaro, 2004 found, the primary roots had sufficient numbers, a distributed around the plant, suitable growth and high branching, providing sufficient anchorage and simple conditions for the exploration of large volumes of soil in search of water and nutrients.

Layering

In layering, success depends on the early beginning of the root and on the formation of sufficient fibrous roots. In guava, two types of layering is commonly adopted viz Air layering and Mound/Stool layering.

Air layering

Air layering was the main method for the spread of guava in the old days. “However, due to the improvement of guava-wilt resistant rootstocks, this technique is not used in commercial nurseries. Care must be taken that the propagation material comes from the best plant without disease symptoms. The most suitable period for conducting air-layering is from August to February (southern hemisphere). A strong and direct shoot on from girdled area must be selected. The selected shoots must be 500 mm from the tip to the point where the bark ring is made. A piece of bark, usually 25 to 40 mm wide, is peeled off around the chosen branch. The cambium layer between bark and wood must be scraped or opened for drying for 2 days. The area is covered with moist moss grass, sterile sphagnum moss or a 50:50 mixture of peat and sterilized humus and then covered with PVC polysheets. Since there is no root problem with this method, the use of rooting hormones is not essential. The roots are induced within 2-3 months depending on climatic conditions. Once 50% of the roots are induced, the layers are removed from the parent plant and stored in a 5 liter polyethylene bag until they become strong enough to be transplanted into the garden.” (Hartman and Kester, 2002).

The regeneration of the roots in the air layering is largely controlled by a series of external and internal factors. The etiolation stimulates rooting in the etiolated portion and has led to better rooting in different fruit plants. It is now known that treatment with etiolation increases the temporary

accumulation of endogenous growth substances in the etiolate portion due to some anatomical abnormalities, which promote better root rooting and quality (Dhua and Sen, 1984). This is a quick, efficient & simple, way to clone guava plants and could be the most inexpensive technique. Singh *et al.* (2007) observed that the combination of IBA with rooting media helped produce the maximum number of primary roots, secondary roots, leaves in 60 days and length of shoots in 60 days. IBA 5000 ppm and the combination of poultry manure were the second best for the survival of air layers.

The physiological state of branches, type of application, concentration of PGRs, the kind of auxin & media applied are significant aspects to consider when guava plants are propagated by air layering (Urdaneta *et al.*, 2009). But, major disadvantage, the air layerings have a less output per mother plant, compared to cuttings budding or grafting.

In another experiment, a year of shooting guava cv. "Lucknow-49" has been treated in surface area ringed with various concentrations of IBA with organic media, ie, poultry manure, vermin-compost and FYM. The air layers guava with a concentration of 6000 ppm IBA with soil: sand: poultry manure rooting media produced the highest percentage (76.75%) of survival of the plants after 60 days grown in polyethylene bags (Singh *et al.* 2007). Kakon *et al.* (2005) reported that, "the combination of medium rooted IBA helped to produce the maximum number of primary roots (18.57), secondary roots (23.91), leaves at 60 days (14.36) and shoot length at 60 days (5.31 cm) (Singh *et al.*, 2007)." Maurya *et al.* (2012) reported that "the air layering are made with soil and dung + sphagnum + 6000 mg IBA / L showed early emergence of the roots (16.33 days), increase in the number of primary roots (17.49), secondary roots (47.73), the maximum root length (10.20 cm), fresh root (3.31g) and dry root weight (0.68g) compared to control. He also reported the maximum survival rate (90.67), the length shoots (7.93 cm) and the number of shoots (18.33) at 60 days of air layers in the polyethylene bag after transferring with the maximum cost benefit ratio (CBR 1: 3.59)."

Manga and Jholgiker (2017) reported that the minimum days for the root beginning (84 days), the maximum number of roots, the highest rooting and the survival rate were found in layers

prepared in August were applied with IBA-4000 ppm. The growth of these layers was vigorous, as demonstrated by the maximum number of shoots, the number of leaves and the length of the shoots to 90 days from the separation of the layers of mother plants placed in the shade of the house for hardening.

Mound Layering/Stooling

In this method, a plant is cut into the ground during the dormant season and covered with soil at the base of the newly developed shoots. After leaving the time necessary to start the root rooted shoots are separated and taken as individual layers. In live propagation through layers of heap kept in guava cv. L-49 (cv. Sardar) in the range of mid-June to September. Early sprouting (7.90 days), maximum rooting rate (90.73%), maximum number of primary roots (15.72), maximum number of secondary roots (30.82), more leaves 45 days after transferring into bags of polyethylene (8.94) were recorded (Patil *et al.*, 2016).

In one of the studies conducted by Lal *et al.* (2007) that, "on the potential of stooled shoots of guava cv. Sardar (Lucknow-49), the results revealed that different concentrations of IBA, NAA and their combination significantly increased the rooting rate, mean number of roots per shoot, the mean root length (cm), sprouting rate and the survival of the stooled shoots rooted in the control field. IBA (7500 ppm) of treatment has a maximum rooting rate (96.67%), average number of roots per shoot (46.93) average root length (8.45 cm) and survival (75%) after transplantation in the field." Rymbai and Reddy 2010, found that "the application of IBA was found to be significantly effective in inducing rooting, promoting root characters, facilitating the initial sprouting and the maximum number of leaves in stooled shoots." IBA-7000ppm proved to be the most effective for rooting, the following characters, the creation and growth of stooled plantlets. Maximum survival, minimum sprouting days and the maximum number of shoots in polyhouse, regardless of the possible IBA concentrations that provides good environmental conditions seedlings compared to uncontrolled environmental conditions open nurseries.

Budding

Various budding techniques were developed from the buds of green shoots in guava

seedlings (5 mm in diameter) have been tried, such as Forkert, shield, patch, chip, etc. in guava with different degrees of success (Jaffco, 1970). Sohnika *et al.* (2015) reported that the patch budding from August 15th to 21st showed the highest success rate (92.07%) observed after 90 days of guava propagation. Bhatt *et al.* (2013) reported that the patch budding mid-June showed a better response to the number of sprouting (7.49), percentage of survival (73.33%), average length of the shoot (50.27 cm), average leaf length in new growth (6.67 cm), average leaf width (3.71 cm) and leaf area (53.86 cm²). T-budding is carried out instantaneously prior to or during the growth period. Nevertheless, a few species may sprout throughout winter when they are inactive. It is essential to ensure that the rootstock and scion are compatible. "T" budding or shield budding is the most common budding method in guava. Patch budding was more popular method to propagate for Allahabad Safeda among budding (Bhatt *et al.*, 2013).

Grafting

Webber (1944) explained the success of guava transplantation. Apical stem branches is used to perform the grafting, this should be 3 or 4 months old. "The shoot should be made with 3-4 buds, 15-18cm long and 8mm in diameter. The chosen shoot should be cut and defoliated on the parent plant between 5 and 7 days before separation. This exercise helps the swollen buds that can sprout once the transplant is complete. This is considered necessary for successful grafting" (Singh, 2007). The rootstock stem is separated between 15 to 18 cm from the soil surface and grafting is done. The graft should be wrapped in clear polyfilm after union, to avoid dehydration and to boost the success rate. "Sprouting begins between 9 and 12 days after transplantation and the polyethylene is removed. In the greenhouse, the success rate can vary between 70 and 92%." (Singh, 2007). When rootstocks attain a height of 200mm, the rootstock may be grafted and diameter of about 5-7mm. Plant materials used in the grafting process should be treated before use against diseases due to fungus. For grafting, scion shoot should be exactly of same thickness as the shoot of rootstock or should be thinner than the rootstock to graft. Cut is completely covered with a biodegradable material: Parafilm® film. After about 2-3 weeks, the buds

begin to swell. If the shoots break the Parafilm® leaf and have about 6 to 8 hard leaf buds, the leaf can be removed. All shoots growing under the graft should be removed regularly to avoid competition for growth of rootstocks.

Grafting is practiced to exploit the well-developed root system of the rootstock. It can be done by inarching in guava. This technique can produce up to 95% of success, but more laborious as compare to cutting or layering. The maximum rate of graft sprouting, the number of sprouted shoots occurred in grafted plants under shade net condition as compared to grafted plants under polyhouse condition and also the maximum percentage of graft sprouting, fewer days taken by 50% of the sprouting of grafts and the number of days taken for sprouting was also recorded in grafted plants in January (Vanaja *et al.*, 2017). Sweeti *et al.* (2016) reported that the maximum percentage of sprouting (44.76%) was recorded on March 5 and the success rate of the transplant (69.08%) and the maximum length of sprouts (14.00cm) was observed in grafted plants on 20 February after 90 days of implantation of the first draft of the draft fabric between the warehouse and the stem cells increased the percentage of transplantation and the development of new buds in the bud (Taiz and Zeiger, 2012). Gotur *et al.* (2017) reported that the wedge grafting in August gave better results in the poly (69.88%) and open field (67.12%) households. The most successful grafts in August could be due to the optimal temperature and high humidity that prevails during this period, which resulted in the successful bonding of the layers of cambium of stocks and scion, the first formation of calluses and the beginning of the subsequent growth. A high success rate and graft survival was observed in 35% of shaded houses (68.80 and 87.19% respectively), followed by a 50% shaded case (58.00 and 79.13% respectively) (Manga and Jholgiker, 2017). Several *Psidium guajava* workers have carried out similar experiments in various grafting parameters and larger number of sheets in a minimum time compared to different sowing periods (Mahendra *et al.* 2015; Syamal *et al.* 2012; Rani *et al.* 2015; Beera *et al.* 2013; Nanditha *et al.* 2017; Singh *et al.*, 2018 Shashi *et al.* 2012; Gurjar *et al.* 2012).

Micropropagation

The expansion of clonal propagation

in vitro can facilitate rapid clonal propagation. However, its profitability must be addressed in time of energy crisis. Other aspects of guava cultivation, such as wilt disease, fruit flies, shorter shelf life and increased sensitivity to abiotic stress such as frost, must be taken into account. Tools for molecular biology such as marker-based selection, genetic alteration for stress tolerance, genomics use, molecular physiology and bioinformatics can be used to understand plant physiology in plants better with changing weather conditions and development of resistant varieties. (Ali *et al.*, 2007)

Organogenesis and embryogenesis are two known models of *in vitro* regeneration using *in vitro* explants of plant or elite seedlings. Though, the regeneration of tissues from *in vivo* sources has been hampered by higher microbial contamination and exudation of the phenols at higher level from plant tissues will resulting in the removal of the darkness and recalcitrant nature of plant tissues. Soaking & agitation of plant tissue in antioxidant, such as citric acid, ascorbic acid and polyvinylpyrrolidone (PVP) solution and PVP use, in nutritive medium drastically reduced the phenolic exudation in the medium and contributed to the plant tissue formation from explants from *in vivo* sources (Liu and Yang, 2011). Tall explant seedlings or greenhouse grown plants (low light and low temperatures) produced a smaller amount of contamination and less phenol due to the reduced synthesis of phenolic compounds in seedlings (Chandra and Mishra 2005, Ali *et al.*, 2007, Usman *et al.*, 2012). "Microbial contamination was also reduced when explants were collected from plants grown in greenhouses compared to plants grown in open environments. Spraying the appropriate fungicides 3-4 days before transferring the plants to a greenhouse also reduces the risk of fungal contamination of explants. The use of 5-10% sodium hypochlorite, followed by 0.5% to 1.0% mercury chloride, reduced the microbial contamination of explants from mature woody plants" (Usman *et al.*, 2012). Young explants plant respond better to the plant than mature trees (Shah *et al.*, 2008). The latest information has revealed that *in vitro* regeneration reactions to organogenesis and embryogenesis are reviewed here.

Organogenesis

The adventitious shoots regeneration from differentiated tissues or callus or is called

organogenesis. In guavas, it has been found in two young plants grown *in vitro* (Mishra *et al.*, 2014, Usman *et al.*, 2012). *In vitro* organogenetic reactions are largely regulated by the type of explants and plant growth regulators (PGR), as well as by inorganic inorganic additives and growth and medium conditions. Singh *et al.* (2007) investigated the effect of explants and media compositions to callus induction from guava seedlings Cv. Pant Prabhat, which were grown *in-vitro* and *ex-vitro*. In MS media having NAA (1 mg/L) + 2,4-D (2 mg/L) + Kinetin (1 mg/L), the maximal callus induced by hypocotyl explants in both explant species independently of hormonal combinations. In very difficult species, such as guava, the efficacy of clonal multiplication *in vitro* depends on the quality of the genotype, extraction, the physical state of the tissue and the duration of the experiment. Organogenesis was found in Banarasi, Safeda, Allahabad Safeda and Beaumont varieties in Pakistan, India, Iran and Malaysia. Regeneration was more effective than explants of mature woody plants and explants of young greenhouse-grown plants grown *in vitro* (Usman *et al.* 2012). Meghwal *et al.*, (2010) and Liu and Yang, 2011 also found that though, in the cultivars Beaumont and Allahabad Safeda shoots regeneration was established by explants from nodes of mature plants. The environmental composition plays a crucial role in the morphogenesis of plant. An *in-vitro* regeneration guava cultivar was first reported in a MS medium (Murashige Skoog, 1962) plus 6-benzylaminopurine (BAP) in explants collected from mature plants and plants *in vitro* (Usman *et al.*, 2012; Mishra *et al.*, 2014), which indicates a high rate of metabolism of guava tissue due to BAP as compare to other cytokinins (Malik *et al.*, 2005; Rai *et al.*, 2010). Shah *et al.*, 2008; Liu and Yang, 2011 also found Kinétine & Zeatin were sometimes used in combination with BAP. Thomas (2008) and Usman *et al.*, (2012) also revealed that adenine sulfate & sucrose are other additives to improve rooting induction and growth rate, while activated carbon improves frequency of rooting. Rai *et al.*, 2010 & Usman *et al.*, (2012) reported that the roots development in shoots extracted from an mature plant requires IBA and NAA supplements for the induction medium. In the case of shoots removed seedlings established *in vitro*, the roots were grown in MSO medium without PGRs. Among various

gel substrates, agar has been widely used during organogenesis in various cultivars and there is no report of using liquid medium.

Embryogenesis

“Somatic embryogenesis is a helpful means for plant biotechnology and is broadly used for mass propagation of best cultivars. Somatic cells produce masses that develop into mature embryos under favorable growth conditions in embryogenesis” (Nic-Can *et al.*, 2015). Rai *et al.*, 2012 & Kamle *et al.*, 2014 found that somatic embryogenesis helps to study plant differentiation, totipotent cell expression level also and has been extensively used for the modification at genetic level for woody plants. Among the Allahabad Safeda and Banarasi cultivars, using the zygotic embryos as explants. Explants from other sources were also explored, such as leaf disks, internodes, mesocarp and petals for embryogenesis; however, only embryos from induced mesocarp were developed (Chandra *et al.*, 2007, Butt *et al.*, 2013). Embryos from immature zygote attained after 8-10 weeks of embryogenesis induced in cv. Banarasi (Rai *et al.*, 2010). In MS media embryogenesis was induced (Rai *et al.*, 2012; Kamle *et al.*, 2014) with mixture of 2,4-D and other auxin or cytokinins. Ascorbic acid and L-glutamine which are sources of nitrogen actually induced somatic embryogenesis, while PEG and L-proline accelerated the maturity of somatic embryos (Rai *et al.*, 2012). Among the different sources of carbon, 5-6% sucrose was better for inducing and maturing of somatic embryos, while the addition of glucose, fructose, maltose, sorbitol and mannitol in the medium had reducing effects (Rai *et al.*, 2008). The agar (0.7-0.8%w/v) mixed solidified medium showed a better initiation of embryos as compared to the liquid medium. The embryo germination can be enhanced by sucrose (3%) and reducing salts (half) in the medium (Rai *et al.*, 2007). In conclusion, development in the clonal propagation of guava was strongly promoted by the organogenesis and somatic embryogenesis usage in elite cultivars; However, its commercialization is still in its initial stages.

Challenges and perspective of Guava Propagation

There are three major factors in production, which can cause to a drastic drop in production. First, the soil fungus caused guava wilt disease to

a damaging effect. By using chemicals, presently, there are no control measures. The only way out is the varietal development those are resistant to diseases. The lack of varieties resistant to GWD is a major concern. Cultivation of young guava plants in the field is difficult (Amin and Jaiswal 1987). Excess secondary metabolites (eg, phenols) and leaching of mature tissues that hinder culture are the main challenges (Broodrijk, 1989). In addition, the difficulty of removing contaminating microorganisms from the surface and from the interior, still when attenuation methods are used (Zamir *et al.*, 2004). Genotypic variants also play a role in the development of varieties (Joshee *et al.*, 2004). Somatic embryogenesis methods like indirect propagation methods (Jaiswal, 1994) and / or organogenesis of plant organs (eg, leaf and stem sections) are recalcitrant. However, these methods have also been analyzed, as well as of an entire plant development by embryogenesis and / or organogenesis. An effective culture method facilitates the replication of elite genotypic plants for later evaluation and also provides orchard grower with disease-free vegetative propagation material.

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