

# Somatic embryogenesis in guava (*Psidium guajava* L.): current status and future perspectives

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**Abstract** Guava (*Psidium guajava* L.) is a highly perishable fruit crop comparable to mango owing to its high medicinal value and intense aroma. The presence of high genetic variability limits the chances of further expansion of guava improvement using biotechnological interventions. Conventional methods of guava improvement encountered with restricted achievement in progress of disease resistant varieties because of existing high genetic variability in the germplasm. There is a considerable demand for the establishment of successful and efficient regeneration protocols via somatic embryogenesis. Plants regenerated through somatic embryogenesis could be more useful than plants obtained through organogenesis because, in most cases, somatic embryos are of single-cell origin, and have a low frequency of chimeras and a high number of regenerations. This review is a snapshot of the recent status of somatic embryogenesis as a basis for expanding genetic improvement in guava for quality traits and future perspectives using advanced technologies.

**Keywords** Somatic embryogenesis · Regeneration · Maturation · Conversion · Acclimatization · In vitro selection · Genetic transformation

## Introduction

*Psidium guajava* L. is a fruit of high nutraceutical value, which belongs to the family Myrtaceae, and is commonly referred to as 'guava' (Chandra et al. 2010a). Guava is native to tropical America but is widely distributed all over the equatorial regions of tropical and sub-tropical countries. It is the fourth most important fruit crop in terms of area and production following mango, banana, and citrus. World production statistics for guava are not yet available, but its production has major commercial importance in India, Egypt, South Africa, Brazil, Colombia, and the Caribbean region (Chandra et al. 2010a). Guava has a vast gene pool, genetically diverse in nature, and considered economically valuable crop around the globe. Guava fruit is highly palatable due to its intense fruity aroma and has high medicinal value (Barbalho et al. 2012). The fruit contains vitamin C, lycopene, including calcium, phosphorus, and iron (Singh et al. 2005), making the exotic fruit more valuable (Barbalho et al. 2012). In traditional medicine, different parts of the plant, i.e., fruits, leaves, roots, and bark with high therapeutic values, are utilized for the treatment of gastroenteritis, diarrhea, and dysentery (Barbalho et al. 2012). The high pectin content of guava helps to significantly reduce the accumulation of cholesterol and, thus, limits the risk of developing cardiovascular diseases (Singh et al. 2005).

The genus *Psidium* ( $2n = 2x = 22$ ) comprises approximately 150 species that are predominantly fruit-bearing trees or shrubs. Guava is diploid in nature, while artificially produced triploid cultivars ( $2n = 3x = 33$ ) also exist mostly as seedless varieties (Jaiswal and Amin 1992). The guava flower is epigynous with profuse stamens of various sizes and existence of extended juvenile period restricts the genetic improvement in guava using conventional breeding

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programmes. Genomic incompatibility is found dominant in guava being self-pollinated and, thus, restricting combining traits in single genotype by interspecific crosses (Jaiswal and Amin 1992). Due to these complications, only some successful research papers are available in breeding (Ribeiro and Pommer 2004; Sharma et al. 2013). However, widespread genetic diversity exists in guava, and the genotypes are adapted to a wide range of soil and environmental conditions.

Generally, guava propagation is performed by seeds whereas seedling trees carried genetic variability in characteristic features in crop and late-flowering (3–4 years after plantation). However, plants propagated through somatic embryogenesis start flowering in 2–3 years (Yadava 1996). Conventional breeding is a tiresome, labor-intensive and tedious approach for developing plants with additional and improved quality traits (Chandra et al. 2004). Moreover, the commencement of a guava expansion program must focus on traits for fruit quality, high yield, disease resistance, long shelf-life, intense aroma, attractive skin, flesh color, and seedlessness (Dinesh and Iyer 2005). However, such an ideal guava phenotype is not conceivable using conventional methodologies. Planting of extensive new guava orchards using vegetative propagated clones is sometimes limited by pathogens. Therefore, the best alternative is to resort toward somatic embryogenesis, which allows for the mass multiplication of plants with superior quality traits in elite cultivars in a short span of time by employing advanced technologies.

### Advancement in SE in guava

SE is the perfect model of totipotency where a single somatic cell progress through the embryogenic stages and ultimately outcomes forms a plant (Zimmerman 1993). In comparison with organogenesis, SE offers a model scheme aimed at exploring plant differentiation, along with the mechanism for the expression of totipotency in plant cells (Litz and Gray 1992). Conventional breeding approaches integrated with cellular and molecular biology can be a suitable way to augment the genetic improvement of plants with marketable value (Stasolla et al. 2003). Hence, SE is a preferred method for genetic improvement and the exponentiation of valuable germplasm for perennial fruit trees (Raj-Bhansali 1990; Litz and Gray 1992). Furthermore, the development of competent somatic embryogenesis protocols paves the way for future genetic modification or gene-editing in guava with improved yield and quality traits. Extensive efforts have been performed for the establishment of SE in guava (Table 1). In Fig. 1, an orderly series of characteristic SE stages and the factors influencing the establishment of successful SE in guava are

described. As in plant genetic modification, plants regenerated via SE are more useful than plants obtained through organogenesis since SE plants are derived from a single celled origin; therefore, there is a low frequency of chimeras and the production of an increased number of regenerations. The exploitation of in vitro regeneration protocols is limited to a few fruit-bearing species of Myrtaceae. The short juvenile period in guava provides a unique model for the application of SE and genetic transformation approaches for long shelf-life, seedlessness, and resistance to root and fruit diseases.

## Factors affecting SE in guava

### Induction factors

#### *Explants*

Guava is a perennial fruit tree in which tissue culture is difficult owing to its recalcitrant nature (Thorpe et al. 1991). Selection of potential explant is a critical factor in the establishment of a successful in vitro regeneration system. Fruit trees have marked phase changes in their life cycle, which result in a decline in their potential for inducing SE (Von Aderkas and Bonga 2000). SE is generally initiated through the juvenile tissues, which is a major constraint for the propagation of woody perennials. Juvenile-mature phase changes can be reformed in some trees ex vivo or in vitro. Among fruit trees, the preferred explants used to induce somatic embryos are immature or mature zygotic embryos (ZEs), cotyledons, hypocotyls, ovaries, ovules, inner integuments, nucellar, endosperms, anthers, filaments, leaves, axillary buds, and protoplasts (Dunstan et al. 1995). Phenol exudation and inborn contamination are the two major bottlenecks faced in guava tissue culture. The importance of embryonic tissue as explant source for inducing SE has been well documented (Thorpe et al. 1991; Dunstan et al. 1995) in several woody perennials including guava (Jaiswal and Jaiswal 2005; Rai et al. 2007; Akhtar 2010, 2013; Kamle et al. 2013). Therefore, an immature zygotic embryo (IZE) considered the most juvenile explant, especially effective for the induction of embryogenic competency when compared with the mesocarp and an in vitro leaf. Researchers revealed that IZE of guava undergo maximum SE and results contrast in immature mesocarp (Akhtar 2010; Rai et al. 2010; Kamle et al. 2013, 2016).

ZEs are made up of pre-embryogenic determined cells (PEDCs), which have embryogenic competency and can be effortlessly tempted to track the embryogenic pathways (Kamle et al. 2016). The first report of introducing SE in guava from zygotic embryo cultures appeared (Jaiswal and

**Table 1** Innovations in guava through somatic embryogenesis

No	Explant	Growth media	Response	References
1.	ZE	MS + NAA + BAP	Induction	Gaffoor and Alderson (1994)
2.	Immature ZE	MS + zeatin	Callusing and somatic embryogenesis	Ramirez-Salazar (1998)
3.	Immature ZE	MS + 2,4-D	Somatic embryogenesis	Vilchez et al. (2002)
4.	Immature mesocarp	MS + 2,4-D + L-glutamine + sucrose	Induction, maturation and plantlet formation	Chandra et al. (2004)
5.	Immature ZE	½ MS + 2,4-D + ascorbic acid + sucrose + L-glutamine	Somatic embryogenesis and germination	Kosky et al. (2005)
6.	Immature ZE	MS + 2,4-D	Induction, maturation and plantlet formation	Rai et al. (2007)
7.	Immature ZE	GSEM + IAA + L-glutamine	Induction, maturation	Biswas et al. (2007)
8.	Immature mesocarp	MS + 2,4-D + L-glutamine + malt extract	Direct embryogenesis	Bajpai et al. (2008)
9.	Immature seeds	MS + 2,4-D + CPA	Callusing followed by embryogenesis	Moura and Motoike (2009)
10.	Immature ZE	MS + 2,4-D + putrescine + sucrose	Induction, maturation and plantlet formation	Kamle et al. (2009)
11.	Immature ZE	MS + 2,4-D + putrescine	Induction, maturation and plantlet formation	Kamle et al. (2009)
12.	Immature ZE	MS + 2,4-D + spermidine	Induction, maturation and plantlet formation	Kamle et al. (2010)
13.	Immature ZE	MS + 2,4-D + sucrose	Induction, maturation	Akhtar (2010)
14.	Immature ZE	MS + 2,4-D + NaCl	Induction	Rai et al. (2010)
15.	Immature ZE	MS + 2,4-D + sucrose	Induction, maturation and plantlet formation	Kamle et al. (2013)
16.	Immature ZE	MS + 2,4-D + sucrose	Induction	Rai et al. (2012)
17.	Immature ZE	MS + 2,4-D	Induction, maturation	Akhtar (2013)
18.	Immature ZE	MS + 2,4-D + L-glutamine + sucrose	Induction, maturation and plantlet formation	Kamle et al. (2016)

ZE zygotic embryo, GSEM guava somatic embryogenesis medium, MS Murashige and Skoog medium, CPA *p*-chloro phenoxy-acetic acid

Amin 1992; Akhtar 1997, 2013). Following similar protocols, the induction and development of somatic embryogenesis from ZEs of cultivar Paluma in Brazil were performed (Vilchez et al. 2002). Later, applications in the improvement of guava and other tropical and sub-tropical fruit species were reviewed (Akhtar et al. 2000; Jaiswal and Jaiswal 2005). Advancement of SE in guava has been reported by several researchers (summarized in Table 1). Numerous problems were encountered during the optimization of a high-efficiency protocol concerning the induction of SE from ZEs in a few genotypes of guava (Kamle et al. 2016). IZEs were utilized as the primary explants for the induction of SE by a group of researchers (Akhtar et al. 2000; Rai et al. 2007; Akhtar 2013; Kamle et al. 2013, 2016).

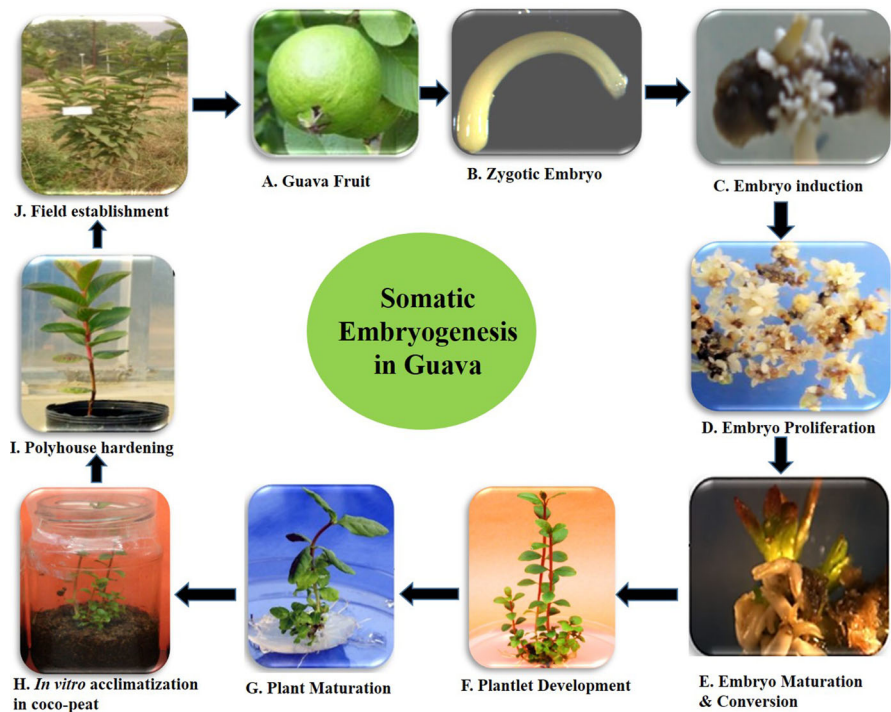
Various other explants were tried but proved unsuccessful except the immature mesocarp tissue with limited success (Biswas et al. 2005; Chandra et al. 2004, 2005). It is presumed that less differentiated cells (immature

embryos) are more receptive to the induction of SE as genes responsible for the embryogenic developmental program are released by chromatin mediated gene silencing in vegetative cells and are activated due to a strong signal such as high doses of auxin (Feher 2005, 2008).

### Developmental age of the explant and embryogenic potential of genotype

The developmental age of explant to be cultured is of prime importance in acquiring embryogenic competence. IZEs are preferred explants in embryogenesis as the developmental stage of explants plays a very critical role in growth of in vitro cultures (Yadava 1996). Numerous research reports are available on the induction of embryogenesis using IZEs (Vilchez et al. 2002; Rai et al. 2007; Moura and Motoike 2009; Akhtar 2010). According to the recent research findings, 10-week-old embryos of genotype Allahabad Safeda (Kamle et al. 2016) proved to

**Fig. 1** Schematic representation of the somatic embryogenesis process for guava



be the best responsive explant source encouraging SE in accordance with other researchers (Kosky et al. 2005; Rai et al. 2007; Akhtar 2010; 2013) in cv. Banarasi local, while, in cv. Paluma 7-week-old IMZE found potent to induce SE (Moura and Motoike 2009). Occurrence of variability in regeneration ability is probably due to their physiological stage of development. Similarly, mesocarp tissue obtained from IZE has the better potentiality to induce SE than the MZE (Chandra et al. 2004).

### Genotype

Genotype is also considered an important factor influencing embryogenic response in plant tissue culture, and SE is a highly genotype-dependent regeneration process. Selection of promising genotype to initiate the SE under in vitro conditions is of prime importance to avoid recalcitrant genotypes. However, variability exists in between the genotypes and within the cultivars resulting into the embryogenic potency of the species (Canhoto et al. 1999). Genotype-dependent embryogenic capability was widely reported and if the aim is for the genetic improvement of plants, then selection of the potential genotype is extremely important. Somatic embryo development in guava was asynchronous with enormous variation in somatic embryo number at different developmental stages. Allahabad Safeda was reported to be the most responsive genotype in terms of embryogenic behavior. Regeneration potential varies among genotypes in each plant species and this is the

most influential factor affecting the establishment of SE, especially in the case of perennial fruit trees (Canhoto et al. 1999).

### Auxin

Auxin is the main driving regulator of SE induction, and gives rise to different effects in different phases of embryogenesis (Feher 2005). 2,4-D is a prerequisite for inducing embryogenesis in an array of crops widely used in developing embryogenic competence in several explants leading to SE (Arnold et al. 2002). It is also considered as one of the main inducing factors influencing the SE (Amirato 1993) and established well in numerous tree species (Dunstan et al. 1995; Canhoto et al. 1999) including Myrtaceae. Exogenous addition of 2,4-D in the medium for the induction of SE in recalcitrant species is a key factor in deciding the establishment of successful protocols and for the commercial viability of micropropagation systems.

Different concentrations of 2,4-D were utilized in the induction of SE in guava (Kamle et al. 2016). 2,4-D (2.0 mg/l) remained ideal condition for evoking SE in four elite genotypes, viz. Allahabad Safeda, Sardar (L-49), Lalit, and Shweta and verified as per embryogenic frequency and intensity. Previously, 2,4-D (1.0 mg/l) was also found operative in inducing SE in the cultivar Banarasi local (Akhtar 2010; Rai et al. 2007; Akhtar 2013). Lower concentration of 2,4-D (1.0 mg/l) was recommended in few cultivars for embryogenesis, while increased concentrations

improved somatic embryo diminution. In some guava genotypes, 2,4-D concentration in media up to 45–60 days led to satisfactory SE with fewer morphological abnormalities. Direct SE induction by employing 2,4-D at the adaxial surface of zygotic embryos of *M. communis* has been reported (Canhoto et al. 1999).

In general, complete removal of auxin or decreased concentration in the culture medium serves as a trigger for somatic embryo conversion and development (Zimmerman 1993). Auxin encouraged embryogenic determination proportionately in cells and suspension cultures and, simultaneously, ceased further cell division (Sharp et al. 1980). On the contrary, some researchers claim that 2,4-D alone is not sufficient for the induction of SE. However, a combination of auxin and cytokinins or amino acids has been proved effective in SE in guava cultivars Paluma and Allahabad safeda (Moura and Motoike 2009; Kamle et al. 2016).

### Carbohydrates

Carbohydrates play a vital role in plant life and are substrates for respiration, perform functions in the synthetic pathways of many compounds, and are the building blocks of macromolecules (Gibson 2000). During heterotrophic mode, the nutrition uptake by tissue, carbohydrate in the form of sucrose is the key source found abundantly in phloem (Zimmermann and Ziegler 1975). It is proposed that carbohydrate content in the medium enhances the development of somatic embryo induction (Gray et al. 1993; Lou and Kako 1995) as in cucumber (Luo et al. 1996) and iris (Jehan et al. 1994). However, we reported 6% sucrose proved optimum quantity for evoking SE among four potential genotypes of guava (Allahabad Safeda, Lalit, Sardar (L-49), and Shweta) in India (Kamle et al. 2013). 5% sucrose is reported to have similar effects (Kosky et al. 2005; Rai et al. 2007). The availability of high carbohydrate content in culture medium was found to be effective in inducing SE in many plant species (Gray et al. 1993; Luo et al. 1996). Several other forms of carbohydrate source are attempted for zygotic embryo explants for SE induction (Akhtar et al. 2000; Akhtar 2013). The basis of these findings revealed that sucrose is the main carbon source for encouraging SE process and all other carbon sources are not metabolized easily in guava.

Furthermore, increasing sucrose concentration improved the initiation of SE in guava and similar findings have been reported for another plant species (Jehan et al. 1994; Luo et al. 1996). However, several researchers reported that high carbohydrate percentages in a medium lead to cell osmolarity (Litz 1986; May and Trigiano 1991). Hence, it concluded that sucrose serves as both nutritional carbon source for SE initiation and as osmoticum for conversion

and maturation of SEs. The positive effect of high osmolarity might mimic the osmolarity alterations that occur surrounding the embryo in nature (Merkle 1995).

### Molecular aspects of SE

Significant progress has been made over the last decade in elucidating the genes that play critical roles in the process of SE (Namasivayam 2007). The molecular mechanisms of SE are complex; several other factors are also responsible, and many genes have been reported in different crops like the *SE receptor kinase (SERK)* gene in *Daucus carota* (Schmidt et al. 1997) and banana (Huang et al. 2007). *Arabidopsis Leafy cotyledon (LEC)* genes are essential for the accomplishment of embryo maturation in *Arabidopsis* (West et al. 1994) and *LEC1* is overexpressed during early embryogenesis and can be utilized as efficient marker for early embryogenesis. Another gene *LEC2* encodes a transcription factor with a B3 domain and can regulate the aspects of SE in *Arabidopsis* (Stone et al. 2008). *LEC2* gene upregulation is auxin dependent, and may compensate for the loss of auxin when transgenic explant cultured on auxin containing medium displays a significantly reduced level of embryogenic potential. Genes *PICKLE (PKL)* and *WUSCHEL (WUS)* that encode for stem cell populations in the shoot meristem regulated by *CLAVATA (CLV)* genes (Bhalla and Singh 2006; Busch and Benfey 2010) are also related to embryogenesis and formation of seeds. Numerous changes occur during the process of SE, and several genes are involved; although many of these genes have been explored, there are presently no reports of SE-responsive genes in guava.

### Conversion and maturation

Maturation is a key phase between embryo development and germination (Quatrano 1987) and the culture conditions must be changed in a sequential manner during somatic embryo development and germination (Ammirato 1993). Continuous treatment up to 60 days provokes recurrent somatic embryogenesis in guava (Kamle et al. 2016) along with polyethylene glycol (50 mg/l) and 3% sucrose for achieving maximum embryo maturation. Augmentation of PEG in media is preferred for normal somatic embryo development and differentiation (Stasolla et al. 2003) because it does not cause plasmolysis of cells and proved efficient for embryo maturation (Capuana and Debergh 1997; Kamle et al. 2016). Similarly, MS medium in addition to PEG (45 mg/l) in *Eruca sativa* produced enhanced mature embryos. After desiccation, somatic embryos were converted into plantlets by culturing the mature embryos on ½ MS medium containing 0.24 μM indole-3-butyric acid. Partial desiccation improves

conversion frequencies but mimics the environment of zygotic embryos, which further improves the maturation of somatic embryos (Finkelstein and Crouch 1986). Factors that determine the ability of embryos to convert into plants include the synthesis and accumulation of storage compounds, especially storage proteins, and the acquisition of desiccation tolerance (Kermode 1990). The importance of water in relation to controlling embryo maturation was proposed and supported by evidence from both embryo culture (Xu et al. 1997) and in situ studies. Efforts to simulate the in vivo environment through modification in the composition of maturation media result in increased storage compounds and tolerance to the desiccation (Finkelstein and Crouch 1986; Xu et al. 1997).

The requirement for high osmolarity may reflect changes in osmolarity in the environment surrounding the zygotic embryo (Merkle 1995). ABA and polyethylene glycol (PEG) provoke somatic embryo maturation in *Aesculus hippocastanum* (Capuana and Debergh 1997). A stimulatory effect of 7.5% PEG on somatic embryo maturation was also recorded for *Picea abies* (Bozhkov and Arnold 1998).

### Plantlet regeneration

Regeneration is used to promote vigorous growth and development of fully developed and matured somatic embryos (cotyledonary stage) into plantlets that are ready for transition to autotrophic growth. The regeneration process is improved for the developmental stages of SEs (globular, heart-shaped, torpedo and cotyledonary) and in some cases, precocious germination is advanced (Arnold et al. 2002; Gaj 2004; Jimenez 2005; Kamle et al. 2016). Subsequently, to develop healthy plants through somatic embryogenesis, a critical review of factors for the regeneration of somatic embryos is required (Kamle et al. 2016).

Additionally, in few reports, medium devoid of any plant growth regulators/hormones is capable of conversion of embryos into maturation stage, while some plants needed very less concentration of auxin/cytokinins for enhanced conversion. While, reducing the medium and sucrose concentration proved efficient during the conversion of somatic embryos into plantlets (Rai et al. 2010; Kamle et al. 2011).

Different genotypes of guava show variation in frequency, intensity, and efficiency of somatic embryo maturation and germination. Somatic embryos greater than 8 weeks old were not converted well, while plantlets were normal (Kamle et al. 2016). Maximum conversion of plantlets is achieved 8 weeks after somatic embryo induction. Elongated torpedo stage embryos appear to convert into normal plantlets at a high frequency and intensity. The augmentation of a liquid basal medium

fortified with 6-benzyl amino-purine, Biobras-6 (brassinosteroid analog) along with less (2%) sucrose gives rise to improved germination (Kosky et al. 2005). Furthermore, BAP (0.5 mg/l), NAA (0.1 mg/l) and sucrose (3%) resulted in maximum conversion of SE into 42.5% of plantlets (Kamle et al. 2013). In addition to this, combination of abscisic acid and sucrose also proved effective in maturation and germination of somatic embryos in guava cv. Banarasi local (Rai et al. 2008, 2010).

### Acclimatization

The establishment of SE-regenerated plants into the field is one of the most critical factors in the establishment of a successful SE system and causes higher production costs (Kamle et al. 2016). However, a high mortality rate is often observed while transferring plants to ex vivo conditions as the micropropagated plantlets have non-functional stomata, weak root systems, and a less developed cuticle. For improved growth and reduced mortality of regenerated plants during the acclimatization is mainly emphasized on the controlled environmental condition for successful plantation. A rapid multiplication rate, successful acclimatization of plantlets in soil, uniform genetic fidelity of progeny, and cost effectiveness are the key considerations for developing a successful SE protocol (Mathur and Mathur 2003; Debnath et al. 2006; Kamle et al. 2013). The ultimate success of such efforts on a commercial scale depends upon a high frequency of acclimatization of the regenerated plants utilized for the field transplantation. The highly protective environment of the SE-derived plants coupled with the lack of exposure to light, temperature, humidity, and interaction with microorganisms normally found in nature makes it difficult for the plants to sustain growth and survive.

The SE-derived regenerates are first grown in a coco-peat substrate moistened with ½ MS plant salt mixture for about 30 days and then transferred to soil for field establishment. The acclimatization of plants resulting from SE by utilizing an inorganic salt solution before transplantation to soil was previously reported as an essential step for both herbaceous and woody perennials and a deciding factor for the survival of the plants in soil. The augmentation of plant salt solution for a short duration permits the rebuilding of photosynthesis, and giving plants access to essential mineral salts due to the liquid state of the nutrient medium. Carrier substrates are important source for successful acclimatization of plants and further result in increased plant growth and canopy including the percent survival. Coco-peat is preferred as the most successful and efficient substrate for the gradual absorption and release of water, providing adequate ventilation intended for the growth of strong roots. In guava, successful acclimatization

of SE regenerates was observed in peat-based compost and sterilized farm yard manure (FYM) + sand (1:1) (Prakash and Tiwari 1993, 1996).

Hardened plants were shifted to the field where they grew vigorously, and the survival of the rooted plants of guava was high in the coco-peat substrate (Kamle et al. 2016). Maximum survival of SE plants (84%) was observed on autoclaved coconut husk fortified with ½ MS plant salt mixture (Kamle et al. 2016). The application of useful microorganisms like *Rhizophagus* and *Trichoderma* as bioagents benefits the plants and encourages a mutual association, through which the plants get protected from harmful pathogens and successfully acclimatized in the field (Varma and Schuepp 1996; Sahay and Varma 1999; Kamle et al. 2016).

### In vitro selection

The in vitro selection (IVS) approach appears to be a promising tool for rectifying specific defects to develop a plant with a desirable trait of highly adapted cultivars without involving a sexual cycle. In vitro selection technology has potential applications in the development of disease-resistant plants in various crops (Hammerschlag 1988; Chandra et al. 2010b). The establishment of a host–pathogen interaction in vitro warrants screening and the selection of plantlets for disease tolerance at the cellular level (Sengar et al. 2009). The availability of a defined toxin is advantageous for mounting IVS protocols. Culture filtrates or partially purified toxins obtained from fungal pathogens are extensively used as selection agents for resistant host species (Buiatti and Ingram 1991). The basic prerequisite for the selection of disease-tolerant lines at the cellular level is to generate genetic variability in the cells. The induction of high genetic variability and the correlation observed between the response of a host to a challenging pathogen in vitro and in vivo are the foundations for the use of the IVS technique in resistance breeding (Van den Bulk 1991).

The exploitation of IVS method for traits of economic importance in trees breeding is advantageous over the conventional breeding approach (Chandra et al. 2010b). Genus *Fusarium* is broadly known for production of many biologically active metabolites and mycotoxins and effectively characterized (Kosiak et al. 2003; Uhlig et al. 2007). However, some biologically active metabolites (culture filtrate) produced by the fungus spp. are utilized for IVS to develop disease-tolerant plants. Several plant pathogenic fungi produce toxins that are associated with several different diseases in many fruit trees, e.g.: in mango (Jayasankar and Litz 1998), banana (Matsumoto et al. 1995), apple (Utkhede 1986), guava (Vos et al. 1998; Bajpai et al. 2007; Kamle et al. 2012). Moreover, Chinese guava (*P.*

*friedrichsthalianum*) and Philippine guava (*P. molle*, and *P. guinense*) were wilt resistant (Edward and Shankar 1964), though a wilt-resistant rootstock has been developed using IVS in guava from South Africa (Vos et al. 1998). Culture filtrate from *Penicillium vermoeseni* was used for screening of shoots and recovered 100% resistance to wilt disease successfully. Furthermore, IVS for the screening of mesocarp-derived calluses of *P. guajava* against *Fusarium* spp. and *Gliocladium* spp. culture filtrates (Bajpai et al. 2007) has been performed with limited success. Later, IVS reported using *F. oxysporum* and *F. solani* culture filtrates for attaining resistance/tolerance to wilt disease in guava. *F. solani* (isolate F2 and F15) culture filtrate provided a positive selection agent in which susceptible shoots presumably grow slowly, and resistant shoots proliferate (Kamle et al. 2009, 2012). A second selection cycle allowed for the recovery of a higher percentage of resistant/tolerant plantlets. The source of variation, when linked to cell-based selection, can be used most effectively.

### Genetic transformation

Guava is an economically important fruit tree and small stakeholders favored for its high yield, consumption and marketable value. However, its production is affected by several pathogens causing major production losses as a result of wilt disease. Various management practices have been deployed to control wilt disease; however, a solution has yet to be achieved successfully. In the improvement of guava using advanced technologies, genetic transformation is one of the alternative approaches to controlling diseases. Numerous efforts have been made over the decades for the genetic expansion of guava for quality traits but, to this day, no successful genetically modified guava plant has been reported. The first report of preliminary genetic transformation was made feasible for cold hardness (Biswas et al. 2005, 2007; Rai et al. 2010). Cloning and sequencing of the hydroperoxide lyase gene (*hpl*) for enhanced flavor were performed for analysis of *hpl* gene silencing in transformed guava plants (Valecillos and Fermin 2010). Moreover, Mishra et al. (2014) claimed genetic transformation in guava against wilt disease using endochitinase gene.

Wilt is a soil born disease reported to be caused by *Fusarium* spp. which greatly damages the vascular system via roots. Subsequently, employing *rolD* (a gene with a pleiotropic effect and disease resistance) and *rolC* (better rooting ability and reduced flowering time) genes from *A. rhizogenes* for the introduction of disease resistance could be an interesting approach toward producing transgenic guava and has been successfully employed in tomato (Bettini et al. 2003) and strawberry (Landi et al. 2009). Consequently, variable responses found over the transgenic

genotypes or cultivars intended for resistance by the pathogens. Similarly, different *rol* genes produce different responses for the growth and metabolism of plants (Bulgakov et al. 2013). The defense response in plants transformed with *rol* genes is activated by a protein kinase/phosphatase cascade through signal transduction (Shirasu et al. 1997) and phytoalexin synthesis by phosphatases (Zhao et al. 2005; Bulgakov et al. 2003). The expression of *rol* genes under the CaMV35S promoter modifies the calcium-dependent protein kinase (CDPK) genes in the transgenic cells, leading to an increased level of resistance/tolerance of transformed plants against multiple pathogens (Landi et al. 2009). However, the complete regeneration of transformed guava plants in field trials is still currently unachievable.

### Future perspectives

Establishment of successful somatic embryogenesis protocol for mass production of plants depends upon the various factors like genotype, auxin, carbohydrate source, etc; the current review describes the recent updates on guava SE. Researchers may concentrate further on the genetic improvement of guava as there are many gaps in research which need to be filled with advanced technologies. Whole-Genome sequencing of guava is required in order to proceed towards functional genomic and transcriptome studies. This is necessary to unravel the mechanisms of fungus–plant interactions and the genes involved in disease progression. The recent release of the genomes of many fruit trees paves the way to a better understanding of quality traits and results in the consideration of genetic enhancement with the implication of genome editing technology. Many countries around the world are considering the use of genome editing tools. Up to present, no further studies have reported genetic modification through genome editing in fruit crops. Most transgenic fruit crops are developed with *Agrobacterium tumefaciens*-mediated genetic transformation; therefore, genome edited guava fruit would be an asset for the coming millennium as this approach is easy, eco-friendly, and the edited fruit is not considered transgenic. This adds significance to genome editing tools for guava improvement and welfare to ease any regulatory procedures for further commercialization.

Overall, this tool opens the door for the development of genetically altered, non-transgenic guava with superior quality traits like enhanced aroma, seedlessness, disease resistance, cold hardiness, greater shelf-life, and ease of acceptance. Genome editing technology in guava holds the potential to offer the consumer products which would be difficult or impossible through traditional breeding approaches. Combined knowledge of the genome of fruits

with novel DNA-editing technologies produces guava with novel quality traits. Hence, genetically edited guava will explore the market in the future, as guava is a highly perishable fruit with great medicinal value.

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### Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

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