

Review Article

Genetic Transformation in *Citrus*

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Citrus is one of the world's important fruit crops. Recently, citrus molecular genetics and biotechnology work have been accelerated in the world. Genetic transformation, a biotechnological tool, allows the release of improved cultivars with desirable characteristics in a shorter period of time and therefore may be useful in citrus breeding programs. *Citrus* transformation has now been achieved in a number of laboratories by various methods. *Agrobacterium tumefaciens* is used mainly in citrus transformation studies. Particle bombardment, electroporation, *A. rhizogenes*, and a new method called RNA interference are used in citrus transformation studies in addition to *A. tumefaciens*. In this review, we illustrate how different gene transformation methods can be employed in different citrus species.

1. Introduction

Citrus species are the most widely grown fruit crops. Despite substantial genetic diversity and interspecific fertility, the genus *Citrus* includes some of the most difficult species to breed [1, 2]. This is due to several obstacles for conventional breeding. For example, most species are highly heterozygous and produce progeny that segregate widely for many characters when crosses are made. The juvenile periods are often very long, self- and cross-incompatibility and pollen and/or ovule sterility are relatively common, and the presence of adventitious somatic embryos in the nucellus of developing ovules of the most of *Citrus* greatly limits hybrid production [2, 3].

The genus *Citrus* possesses several undesirable characteristics including salt and cold sensitivity [4, 5]; they are also susceptible to diseases caused by fungi, bacteria and viruses, such as *Citrus* exocortis viroid (CEV), *Citrus* infectious variegation virus (CIVV), *Citrus* cachexia viroid (CCaV) and *Citrus* tristeza closterovirus (CTV) [5, 6]. Classical genetic selection, gene transfer, grafting, and micrografting techniques can contribute to the improvement of *Citrus* and propagation of selected species. Therefore, *in vitro* manipulation procedures leading to a rapid, direct bud regeneration for efficient micropropagation as well as genetic transformation

are needed as a first step towards *Citrus* improvement. Practical benefits resulting from *in vitro* culture methods have already been reported in *Citrus* [5, 7, 8]. Recent developments in gene transfer techniques via the classical regeneration method have been applied to this genus and have opened the way to induce a specific genetic change within a period of time shorter than using the classical genetic selection method [5, 9, 10].

Conventional breeding methods have demonstrated limitations with respect to citrus improvement due to some of the biological characteristics of woody plants such as nucellar polyembryony, high heterozygosity, long juvenile period, and autoincompatibility [11, 12]. The development of biotechnological tools has made it possible to overcome some of these problems. In the specific case of citrus breeding programs, somatic hybridization [12–14] and genetic transformation [12, 15, 16] have been applied in many countries [10, 12, 17, 18].

In recent years, there has been a major thrust in citrus improvement as competition from international citrus markets, disease, and pest pressure and other abiotic and biotic stress conditions stimulate worldwide interest [19, 20]. Several strategies exist for the genetic improvement of citrus including conventional breeding and genetic transformation [20, 21]. Currently, genetic transformation of citrus as a tool

for citrus improvement is gaining popularity. This method is especially useful in cases where it is not possible to introduce a particular trait of interest to another elite cultivar using conventional breeding. *Citrus* cultivars vary in their response to *in vitro* organogenesis and genetic transformation. This results in the need for cultivar-specific optimization of *in vitro* protocols [20, 22].

Among the several methods available for the genetic transformation of citrus, the most popular method to transform a wide range of citrus cultivars is *Agrobacterium*-mediated transformation using epicotyl explants as target cells for incorporation of the T-DNA [20, 23]. However, this method is not suitable for the transformation of any seedless cultivar. Also, special cultivars in the mandarin group remain robust to transform using this method [20, 22, 24].

2. Transformation Studies in Citrus

Genetic transformation and somatic hybridization studies are already integrated in *Citrus* breeding programs in several countries. Genetic transformation of *Citrus* is a promising tool that enables the introduction of desirable traits without altering the genetic background [25]. Genetic transformation of citrus has been reported, by using several methods (Table 1).

Agrobacterium has been the most frequently used genetic transformation method in *Citrus* with explants collected from seedlings germinated *in vitro* or under greenhouse conditions [68].

Transformation studies have been done for two decades in citrus. In the last few years, different transformation methods such as RNA silencing are used. In order to carry out successful gene transformation studies in citrus, optimized *in vitro* regeneration protocol is needed. Researchers should optimize efficient regeneration protocol before starting transformation studies. There are also many efficient regeneration protocols published in different citrus species.

Orbović et al. [36] investigated the effects of seed age on shoot regeneration potential and transformation rate of “Duncan” and “Flame” grapefruit cultivars, along with “Hamlin” sweet orange cultivar. Genetic transformation of citrus explants was carried out as previously described [93] using *A. tumefaciens* strain EHA105 [94] containing a binary vectors derived from pD35s [22]. In conclusion, the regeneration potential and transformability of citrus juvenile explants are different among cultivars and also change within the fruit harvest season. Because of these findings, especially the latter one, it will be extremely difficult to develop a universal protocol for genetic transformation of citrus. Optimal transformation efficiency will require flexible procedures that account for cultivar variability and timing of seed collection. In another study, a protocol was developed for regeneration of transgenic plants via *A. tumefaciens*-mediated transformation of leaf segments from “Valencia” sweet orange (*C. sinensis* L. Osbeck) using *gfp* (green fluorescence protein) as a vital marker [27]. The transformation methodology described by Khan et al. [27] was an important finding for generating transgenic plants using leaf segments as explants.

In addition to transformation studies via *A. tumefaciens*, recently, *A. rhizogenes* has been used. Many reports suggest the use of *A. rhizogenes* for expression of the *rol* genes and also to deliver foreign genes to susceptible plants [95]. The hairy root harbours the T-DNA segment of Ri-plasmid within its nuclear genomes [96]. *A. rhizogenes* are also capable of transferring the T-DNA of binary vectors *in trans*, thereby facilitating the selection of transgenic plants from screened hairy roots [95]. *A. rhizogenes*-mediated transformation system was found to be very useful in genetic manipulation of plants for the production of phytochemicals [97], large scale secondary metabolite production [98], monoclonal antibody production [99], and phytoremediation [100]. There are many reports that suggest the successful use of *A. rhizogenes* harbouring binary vectors with desired gene constructs [95] for plant genetic transformation [101]. Due to low transformation efficiency of *A. rhizogenes*, many researchers have worked to optimize transformation methods.

Chávez-Vela et al. [72] used *A. rhizogenes* A4 agropine-type strain to develop the transformation system. A4 contains wild-type plasmid pRi A4 which confers hairy-root genotype and binary vector pESC4. In the study seventy-five-day-old sour orange seedlings were used and transgenic sour orange (*C. aurantium* L.) plants were regenerated from *A. rhizogenes* transformed roots. 91% of explants produced transformed roots with an average of 3.6 roots per explant.

In another study transgenic Mexican lime (*C. aurantifolia* (Christm.) Swing) plants were regenerated from tissues transformed by *A. rhizogenes* strain A4, containing the wild-type plasmid pRiA4 and the binary vector pESC4 with *nos-npt II* and *cab-gus* genes. More than 300 Mexican lime transgenic plants were obtained, 60 of which were adapted to growing in soil [2].

In addition to the indirect gene transfer methods, there are studies performed by direct gene transfer methods in citrus. Besspalhok Filho et al. [69] carried out to optimize the conditions for transient gene expression through particle bombardment on Carrizo citrange (*C. sinensis* × *Poncirus trifoliata*) thin epicotyl sections. The best conditions for transient GUS expression were M-25 tungsten particles, 1550 psi helium pressure, 9 cm distance between specimen, and DNA/particle holder and culture of explants in a high osmolarity medium (0.2 M mannitol + 0.2 M sorbitol) 4 h prior and 20 h after bombardment. Under these conditions, an average of 102 blue spots per bombardment (20 explants/plate) were achieved. It is stated that protocol is currently being used for transformation of Carrizo citrange and sweet orange (*C. sinensis*).

Electroporation is an effective direct gene transfer system used for citrus transformation. Hidaka and Omura [90] used electroporation methods for gene transformation in citrus. Protoplasts were prepared from embryogenic callus of “Ohta” ponkan (*C. reticulata* Blanco) and electroporation with exponential decay pulses was carried out in the solution containing the β -glucuronidase (GUS) chimeric gene coupled to the CaMV 35S promoter (pBI221). At 24 hr after incubation, significant GUS activity was detected in the cells by fluorometric assay. Another alternative method for direct gene transformation had been developed in sweet

TABLE I: Transformation researches in citrus.

Species	Transferred genes	Transformation method	References
<i>C. sinensis</i> L. Osb.	<i>GUS</i> and <i>nptII</i>	<i>A. tumefaciens</i>	[26]
<i>C. sinensis</i> L. Osb.	<i>gfp</i>	<i>A. tumefaciens</i>	[27]
<i>C. sinensis</i> L. Osb.	<i>GUS</i>	<i>A. tumefaciens</i>	[28]
<i>C. sinensis</i> L. Osb. and Carrizo citrange	<i>uidA</i> , <i>nptII</i>	<i>A. tumefaciens</i>	[29]
<i>C. paradisi</i> Macf.	<i>RdRp</i> , <i>Gfp</i> , and <i>Gus</i>	<i>A. tumefaciens</i>	[30]
<i>C. sinensis</i> L. Osb.	<i>CTV-CP</i>	<i>A. tumefaciens</i>	[31]
<i>C. aurantifolia</i>	<i>p25</i> , <i>p20</i> , and <i>p23</i>	RNA interference	[32]
<i>C. aurantifolia</i> Swingle	<i>AtSUC2</i> , <i>RSsl</i> , <i>RTBV</i> , <i>GUS</i> , <i>rolC</i>	<i>A. tumefaciens</i>	[33]
<i>C. paradisi</i>	<i>attE</i>	<i>A. tumefaciens</i>	[34]
<i>C. unshiu</i> Marc	<i>miraculin</i>	<i>A. tumefaciens</i>	[35]
<i>C. sinensis</i> L. Osbeck and <i>C. paradisi</i> Macf.	<i>GFP</i>	<i>A. tumefaciens</i>	[36]
<i>C. sinensis</i> L.	<i>CTV-GFP</i>	<i>A. tumefaciens</i>	[37]
<i>C. sinensis</i> Osb.	<i>Shiva A</i> and <i>Cecropin B</i>	<i>A. tumefaciens</i>	[38]
<i>C. sinensis</i>	CPsV <i>cp</i> (ihpCP), <i>54K</i> (ihp54K), and <i>24K</i> (ihp24K)	<i>A. tumefaciens</i> RNA silencing	[39]
<i>C. sinensis</i> L. Osb.	<i>GFP</i> and <i>nptII</i>	<i>A. tumefaciens</i>	[40]
<i>C. sinensis</i> L. Osb.	<i>pthA-nls</i>	<i>A. tumefaciens</i>	[41]
<i>Poncirus trifoliata</i> L. Raf.	<i>AhBADH</i>	<i>A. tumefaciens</i>	[42]
<i>C. sinensis</i> L. Osb.	<i>Cy-GFP</i> and <i>Er-GFP</i>	<i>A. tumefaciens</i>	[43]
<i>C. aurantifolia</i> Swingle	<i>gus-egfp</i>	<i>A. tumefaciens</i>	[44]
Tetraploid citrus rootstock selection "Orange #16"	<i>egfp-nptII</i>	<i>A. tumefaciens</i>	[45]
Carrizo citrange	<i>manA</i> and <i>egfp</i>	<i>A. tumefaciens</i>	[22]
<i>C. sinensis</i> , <i>C. reticulata</i> <i>C. amblycarpa</i> and <i>C. depressa</i>	<i>nptII</i> , <i>hptII</i> , and <i>GFP</i>	<i>A. tumefaciens</i>	[20]
Carrizo citrange and <i>C. sinensis</i> L. Osb.	<i>Gfp</i>	<i>A. tumefaciens</i>	[46]
Carrizo citrange, <i>C. paradisi</i> Macf., <i>C. aurantifolia</i> Swingle	<i>EGFP</i>	<i>A. tumefaciens</i>	[23]
<i>C. sinensis</i> L. Osb. "Swingle" citrumelo and <i>C. sinensis</i> L. Osb.	<i>GUS</i> and <i>nptII</i>	Sonication-assisted <i>A. tumefaciens</i> (SAAT)	[47]
<i>C. sinensis</i> cv. Hamlin	<i>hrpN</i>	<i>A. tumefaciens</i>	[48]
<i>Poncirus trifoliata</i> [L.] Raf.	<i>uidA</i> and <i>nptII</i>	<i>A. tumefaciens</i>	[49]
<i>Poncirus trifoliata</i> [L.] Raf	<i>GFP</i> and <i>MAC12.2</i>	<i>A. tumefaciens</i>	[50]
Carrizo citrange and <i>C. sinensis</i> L. Osb.	<i>uidA</i> and <i>iaaM/H</i> marker genes	<i>A. tumefaciens</i>	[51]
<i>C. sinensis</i> L. Osb.	<i>cp</i> and <i>nos</i> genes	<i>A. tumefaciens</i>	[52]
<i>C. sinensis</i> L. Osb.	Nospro- <i>nptII</i> -Noster	<i>A. tumefaciens</i>	[53]
Carrizo citrange and <i>C. sinensis</i> L. Osb.	<i>ipt</i> gene	<i>A. tumefaciens</i>	[54]
<i>C. paradise</i> Macf.	<i>CTV</i> -derived candidate resistance	<i>A. tumefaciens</i> RNA-mediated resistance	[55]
<i>Poncirus trifoliata</i> L. Raf.	<i>gfp</i>	<i>A. tumefaciens</i>	[56]
Carrizo citrange	<i>nptII</i>	<i>A. tumefaciens</i>	[57]
<i>C. aurantium</i> , <i>C. macrophylla</i> , <i>C. limon</i> and Troyer citrange	<i>CTV-p61</i> and <i>p23U</i>	<i>A. tumefaciens</i>	[58]
<i>C. sinensis</i> L. Osb.	<i>attA</i>	<i>A. tumefaciens</i>	[59]
<i>C. limonia</i> Osb.	<i>bO</i>	<i>A. tumefaciens</i>	[60]
<i>C. paradisi</i>	<i>RdRp</i>	<i>A. tumefaciens</i>	[61]

TABLE I: Continued.

Species	Transferred genes	Transformation method	References
<i>C. jambhiri</i> Lush	<i>GUS</i> and <i>nptII</i>	<i>A. tumefaciens</i>	[62]
<i>C. sinensis</i> L. Osb.	<i>gfp</i> and <i>pme</i>	PEG	[63]
Swingle citrumelo	<i>uidA</i> , <i>nptII</i> , and <i>GUS</i>	<i>A. tumefaciens</i>	[64]
Carrizo citrange	<i>GUS</i> and <i>nptII</i>	<i>A. tumefaciens</i>	[65]
Carrizo citrange and <i>C. aurantifolia</i>	<i>GUS</i> , <i>GFP</i> , and <i>nptII</i>	<i>A. tumefaciens</i>	[66]
Carrizo citrange	Citrus blight-associated	<i>A. tumefaciens</i>	[67]
<i>C. sinensis</i> and <i>C. limonia</i>	<i>GUS</i>	<i>A. tumefaciens</i>	[68]
Carrizo citrange	<i>uidA</i> and <i>nptII</i>	Particle bombardment	[69]
<i>C. sinensis</i>	<i>pTA29-barnase</i>	<i>A. tumefaciens</i>	[70]
<i>Citrus sinensis</i>	<i>PMI</i>	<i>A. tumefaciens</i>	[12]
<i>Citrus sinensis</i>	<i>GUS</i> and <i>nptII</i>	<i>A. tumefaciens</i>	[71]
<i>Citrus aurantium</i> L.	<i>GUS</i> and <i>nptII</i>	<i>A. rhizogenes</i>	[72]
<i>Citrus paradisi</i> Macf.	<i>cp</i> and <i>GUS</i>	<i>A. tumefaciens</i>	[73]
<i>C. sinensis</i> L. Osb.	<i>GUS</i>	Electroporation	[74]
Carrizo citrange and <i>C. sinensis</i> L. Osb.	<i>GUS</i>	<i>A. tumefaciens</i>	[75]
<i>Citrus sinensis</i> L. Osbeck	<i>GUS</i> and <i>nptII</i>	<i>A. tumefaciens</i>	[18]
<i>C. reticulata</i> Blanco	<i>pTA29-barnase</i>	<i>A. tumefaciens</i>	[76]
<i>C. paradisi</i> Macf.	Carotenoid biosynthetic genes	<i>A. tumefaciens</i>	[16]
Carrizo citrange	<i>LFY</i> and <i>API</i>	<i>A. tumefaciens</i>	[77]
<i>C. aurantium</i> L.	<i>cp</i>	<i>A. tumefaciens</i>	[78]
<i>C. paradisi</i> Macf.	<i>CP</i> and <i>T36</i>	<i>A. tumefaciens</i>	[79]
Troyer citrange	<i>Bar</i> and <i>uidA</i>	<i>A. tumefaciens</i>	[80]
<i>C. aurantifolia</i> Swing.	<i>cp</i>	<i>A. tumefaciens</i>	[81]
<i>C. sinensis</i> (L.) Osb.	<i>Gfp</i>	PEG	[82]
<i>C. aurantifolia</i> Swing.	<i>GUS</i>	<i>A. tumefaciens</i>	[83]
<i>C. paradisi</i> Macf.	<i>GUS</i> , <i>uncp</i> , <i>gna</i>	<i>A. tumefaciens</i>	[84]
Carrizo citrange	<i>uidA</i> and <i>nptII</i>	<i>A. tumefaciens</i>	[85]
<i>C. sinensis</i> L. Osb.	<i>GUS</i>	<i>A. tumefaciens</i>	[86]
<i>C. aurantifolia</i> (Christm.) Swing.	<i>GUS</i> and <i>nptII</i>	<i>A. rhizogenes</i>	[2]
<i>C. aurantium</i> L.	<i>cp</i>	<i>A. tumefaciens</i>	[10]
Tangelo	<i>GUS</i> and <i>nptII</i>	Particle bombardment	[87]
Carrizo citrange	<i>GUS</i> and <i>nptII</i>	<i>A. tumefaciens</i>	[88]
<i>C. sinensis</i> L. Osb.	<i>GUS</i> and <i>nptII</i>	<i>A. tumefaciens</i>	[89]
<i>C. reticulata</i> Blanco	<i>GUS</i>	Electroporation	[90]
<i>Citrus</i> spp.	<i>GUS</i> and <i>nptII</i>	<i>A. tumefaciens</i>	[91]
<i>Citrus</i> spp.	<i>cat</i> and <i>nptII</i>	PEG	[92]

orange (*C. sinensis* (L.) Osbeck). Plasmid DNA encoding the nondestructive selectable marker enhanced green fluorescent protein gene was introduced using polyethylene glycol into protoplasts of "Itaborai" sweet orange isolated from an embryogenic nucellar-derived suspension culture. Following protoplast culture in liquid medium and transfer to solid medium, transformed calluses were identified via expression of the green fluorescent protein, physically separated from nontransformed tissue and cultured on somatic embryogenesis induction medium. Transgenic plantlets were recovered from germinating somatic embryos and by *in vitro* rooting of shoots [82].

As well as the transformation studies conducted for gene expression, several studies conducted for gene silencing. RNA interference (RNAi) are a posttranscriptional gene-silencing phenomenon induced by double-stranded RNA. It has been widely used as a knockdown technology to analyze gene function in various organisms. Although RNAi was first discovered in worms, related phenomena such as posttranscriptional gene silencing and coat protein-mediated protection from viral infection had been observed in plants prior to this. In plants, RNAi is often achieved through transgenes that produce hairpin RNA. For genetic improvement of crop plants, RNAi has advantages over antisense-mediated

gene silencing and cosuppression, in terms of its efficiency and stability [102]. Soler et al. [32] stated Citrus tristeza virus (CTV), the causal agent of the most devastating viral disease of citrus, has evolved three silencing suppressor proteins acting at intra- (p23 and p20) and/or intercellular level (p20 and p25) to overcome host antiviral defence. Mexican lime was transformed with an intron-hairpin vector carrying full-length, untranslatable versions of the genes p25, p20, and p23 from CTV strain T36 to silence the expression of these critical genes in CTV-infected cells. Three transgenic lines presented complete resistance to viral infection, with all their propagations remaining symptomless and virus-free after graft inoculation with CTV-T36, either in the nontransgenic rootstock or in the transgenic scion. Accumulation of transgene-derived siRNAs was necessary but not sufficient for CTV resistance. Inoculation with a divergent CTV strain led to partially breaking the resistance, thus showing the role of sequence identity in the underlying mechanism. Results are a step forward to developing transgenic resistance to CTV and also show that targeting simultaneously by RNA interference (RNAi) the three viral silencing suppressors appear critical for this purpose, although the involvement of concurrent RNAi mechanisms cannot be excluded.

3. Conclusion

Genetic transformation is an attractive alternative technique for citrus genetic improvement. However, transformation efficiencies are generally low, and protocols are dependent on species, or even cultivar dependent. One of the limitations within this technology is low plant regeneration frequencies especially for many of the economically important citrus species [65]. In addition, difficulty in rooting transgenic shoots for some citrus cultivars has been reported [10, 89, 91]. Development of effective genetic transformants therefore requires specific studies on *in vitro* regeneration conditions for each genotype.

The development of direct genetic manipulation techniques has provided new opportunities for plant improvement. Plant transformation has made it possible to modify just one or two traits, while retaining the unique characteristics of the original cultivar. The characters that could potentially be manipulated by genetic transformation of *Citrus* include pest and disease resistance, growth habit, and fruit quality. In order to use this technology, it is essential to develop efficient genetic transformation systems for *Citrus*. [2].

References

- [1] F. G. Gmitter, J. W. Grosser, and G. A. Moore, "Citrus," in *Biotechnology of Perennial Fruit Crops*, F. A. Hammerschlag and R. E. Litz, Eds., pp. 335–369, CAB International, Wallingford, UK, 1992.
- [2] E. Pérez-Molphe-Balch and N. Ochoa-Alejo, "Regeneration of transgenic plants of Mexican lime from *Agrobacterium rhizogenes*-transformed tissues," *Plant Cell Reports*, vol. 17, no. 8, pp. 591–596, 1998.
- [3] G. A. Moore, C. C. Jacono, J. L. Neidigh, S. D. Lawrence, and K. Cline, "Transformation in Citrus," in *Plant Protoplasts and Genetic Engineering IV*, Y. P. S. Bajaj, Ed., vol. 23 of *Biotechnology in Agriculture and Forestry*, pp. 194–208, Springer, Berlin, Germany, 1993.
- [4] P. Garcia-Agustin and E. Primo-Millo, "Selection of a NaCl-tolerant Citrus plant," *Plant Cell Reports*, vol. 14, no. 5, pp. 314–318, 1995.
- [5] B. Van Le, N. Thanh Ha, L. T. Anh Hong, and K. Trần Thanh Vân, "High frequency shoot regeneration from trifoliolate orange (*Poncirus trifoliata* L. Raf.) using the thin cell layer method," *Comptes Rendus de l'Academie des Sciences*, vol. 322, no. 12, pp. 1105–1111, 1999.
- [6] V. Greño, L. Navarro, and N. Duran-Vila, "Influence of virus and virus-like agents on the development of citrus buds cultured *in vitro*," *Plant Cell, Tissue and Organ Culture*, vol. 15, no. 2, pp. 113–124, 1988.
- [7] J. W. Grosser, F. G. Gmitter Jr., and J. L. Chandler, "Intergeneric somatic hybrid plants of *Citrus sinensis* cv. Hamlin and *Poncirus trifoliata* cv. Flying Dragon," *Plant Cell Reports*, vol. 7, no. 1, pp. 5–8, 1988.
- [8] R. Ghorbel, L. Navarro, and N. Duran-Vila, "Morphogenesis and regeneration of whole plants of grapefruit (*Citrus paradisi*), sour orange (*C. aurantium*) and alemow (*C. macrophylla*)," *Journal of Horticultural Science and Biotechnology*, vol. 73, no. 3, pp. 323–327, 1998.
- [9] J. Kaneyoshi, S. Kobayashi, Y. Nakamura, N. Shigemoto, and Y. Doi, "A simple and efficient gene transfer system of trifoliolate orange (*Poncirus trifoliata* Raf.)," *Plant Cell Reports*, vol. 13, no. 10, pp. 541–545, 1994.
- [10] M. A. Gutiérrez-E, D. Luth, and G. A. Moore, "Factors affecting *Agrobacterium*-mediated transformation in *Citrus* and production of sour orange (*Citrus aurantium* L.) plants expressing the coat protein gene of *Citrus tristeza virus*," *Plant Cell Reports*, vol. 16, no. 11, pp. 745–753, 1997.
- [11] R. Ghorbel, J. Juárez, L. Navarro, and L. Peña, "Green fluorescent protein as a screenable marker to increase the efficiency of generating transgenic woody fruit plants," *Theoretical and Applied Genetics*, vol. 99, no. 1-2, pp. 350–358, 1999.
- [12] R. L. Boscarol, W. A. B. Almeida, M. T. V. C. Derbyshire, F. A. A. Mourão Filho, and B. M. J. Mendes, "The use of the PMI/mannose selection system to recover transgenic sweet orange plants (*Citrus sinensis* L. Osbeck)," *Plant Cell Reports*, vol. 22, no. 2, pp. 122–128, 2003.
- [13] J. W. Grosser and F. G. Gmitter, "Protoplast fusion and citrus improvement," *Plant Breeding Reviews*, vol. 8, pp. 339–374, 1990.
- [14] B. M. J. Mendes, F. D. A. A. Mourão Filho, P. C. D. M. Farias, and V. A. Bedito, "Citrus somatic hybridization with potential for improved blight and CTV resistance," *In Vitro Cellular and Developmental Biology—Plant*, vol. 37, no. 4, pp. 490–495, 2001.
- [15] L. Peña and L. Navarro, "Transgenic citrus," in *Transgenic Trees*, Y. P. S. Bajaj, Ed., pp. 39–54, Springer, Berlin, Germany, 1999.
- [16] M. G. C. Costa, W. C. Otoni, and G. A. Moore, "An evaluation of factors affecting the efficiency of *Agrobacterium*-mediated transformation of *Citrus paradisi* (Macf.) and production of transgenic plants containing carotenoid biosynthetic genes," *Plant Cell Reports*, vol. 21, no. 4, pp. 365–373, 2002.
- [17] M. Cervera, C. Ortega, A. Navarro, L. Navarro, and L. Peña, "Generation of transgenic citrus plants with the tolerance-to-salinity gene HAL2 from yeast," *Journal of Horticultural Science and Biotechnology*, vol. 75, no. 1, pp. 26–30, 2000.

- [18] B. M. Januzzi Mendes, R. Luciana Boscaroli, F. D. A. A. Mourão Filho, and W. A. Bastos de Almeida, "Agrobacterium-mediated genetic transformation of "Hamlin" sweet orange," *Pesquisa Agropecuaria Brasileira*, vol. 37, no. 7, pp. 955–961, 2002.
- [19] J. W. Grosser, P. Ollitrault, and O. Olivares-Fuster, "Somatic hybridization in citrus: an effective tool to facilitate variety improvement," *In Vitro Cellular and Developmental Biology—Plant*, vol. 36, no. 6, pp. 434–449, 2000.
- [20] M. Dutt and J. W. Grosser, "An embryogenic suspension cell culture system for *Agrobacterium*-mediated transformation of citrus," *Plant Cell Reports*, vol. 29, no. 11, pp. 1251–1260, 2010.
- [21] L. Peña, M. Cervera, R. Ghorbel et al., "Genetic transformation," in *Citrus Genetics, Breeding, and Biotechnology*, I. A. Khan, Ed., pp. 329–344, CABI International, Wallingford, UK, 2007.
- [22] M. Dutt, D. H. Lee, and J. W. Grosser, "Bifunctional selection-reporter systems for genetic transformation of citrus: mannose- and kanamycin-based systems," *In Vitro Cellular and Developmental Biology—Plant*, vol. 46, no. 6, pp. 467–476, 2010.
- [23] M. Dutt and J. W. Grosser, "Evaluation of parameters affecting *Agrobacterium*-mediated transformation of citrus," *Plant Cell, Tissue and Organ Culture*, vol. 98, no. 3, pp. 331–340, 2009.
- [24] R. N. Khawale, S. K. Singh, G. Garg, V. K. Baranwal, and S. A. Ajirlo, "Agrobacterium-mediated genetic transformation of Nagpur mandarin (*Citrus reticulata* Blanco)," *Current Science*, vol. 91, no. 12, pp. 1700–1705, 2006.
- [25] N. T. Marques, G. B. Nolasco, and J. P. Leitão, "Factors affecting in vitro adventitious shoot formation on internode explants of *Citrus aurantium* L. cv. Brazilian," *Scientia Horticulturae*, vol. 129, pp. 176–182, 2011.
- [26] L. Y. Miyata, R. Harakava, L. C. L. Stipp, B. M. J. Mendes, B. Apezzato-da-Glória, and F. A. A. Mourao-Filho, "GUS expression in sweet oranges (*Citrus sinensis* L. Osbeck) driven by three different phloem-specific promoters," *Plant Cell Reports*, vol. 31, no. 11, pp. 2005–2013, 2012.
- [27] E. U. Khan, X.-Z. Fu, and J.-H. Liu, "Agrobacterium-mediated genetic transformation and regeneration of transgenic plants using leaf segments as explants in Valencia sweet orange," *Plant Cell, Tissue and Organ Culture*, vol. 109, no. 2, pp. 383–390, 2012.
- [28] P. Fávero, F. A. de Alves Mourão Filho, L. C. L. Stipp, and B. M. J. Mendes, "Genetic transformation of three sweet orange cultivars from explants of adult plants," *Acta Physiologiae Plantarum*, vol. 34, no. 2, pp. 471–477, 2012.
- [29] E. Pons, J. E. Peris, and L. Peña, "Field performance of transgenic citrus trees: assessment of the long-term expression of *uidA* and *nptII* transgenes and its impact on relevant agronomic and phenotypic characteristics," *BMC Biotechnology*, vol. 12, no. 12, p. 41, 2012.
- [30] B. Çevik, R. F. Lee, and C. L. Niblett, "Agrobacterium-mediated transformation of grapefruit with the wild-type and mutant RNA-dependent RNA polymerase genes of *Citrus tristeza* virus," *Turkish Journal of Agriculture and Forestry*, vol. 36, no. 2, pp. 195–206, 2012.
- [31] F. R. Muniz, A. J. De Souza, L. C. L. Stipp et al., "Genetic transformation of *Citrus sinensis* with *Citrus tristeza* virus (CTV) derived sequences and reaction of transgenic lines to CTV infection," *Biologia Plantarum*, vol. 56, no. 1, pp. 162–166, 2012.
- [32] N. Soler, M. Plomer, C. Fagoaga et al., "Transformation of Mexican lime with an intron-hairpin construct expressing untranslatable versions of the genes coding for the three silencing suppressors of *Citrus tristeza* virus confers complete resistance to the virus," *Plant Biotechnology Journal*, vol. 10, pp. 597–608, 2012.
- [33] M. Dutt, G. Ananthkrishnan, M. K. Jaromin, R. H. Brlansky, and J. W. Grosser, "Evaluation of four phloem-specific promoters in vegetative tissues of transgenic citrus plants," *The Tree Physiology*, vol. 32, no. 1, pp. 83–93, 2012.
- [34] S. N. Mondal, M. Dutt, J. W. Grosser, and M. M. Dewdney, "Transgenic citrus expressing the antimicrobial gene *Attacin E (attE)* reduces the susceptibility of "Duncan" grapefruit to the citrus scab caused by *Elsinoë fawcettii*," *European Journal of Plant Pathology*, vol. 133, no. 2, pp. 391–404, 2012.
- [35] A. Al-Bachchu, S. B. Jin, J. W. Park et al., "Agrobacterium-mediated transformation using embryogenic calli in satsuma mandarin (*Citrus unshiu* Marc.) cv. Miyagawa Wase," *Horticulture Environment Biotechnology*, vol. 52, no. 2, pp. 170–175, 2011.
- [36] V. Orbović, M. Dutt, and J. W. Grosser, "Seasonal effects of seed age on regeneration potential and transformation success rate in three citrus cultivars," *Scientia Horticulturae*, vol. 127, no. 3, pp. 262–266, 2011.
- [37] V. Orbović, P. Soria, G. A. Moore, and J. W. Grosser, "The use of *Citrus tristeza* virus (CTV) containing a green fluorescent protein gene as a tool to evaluate resistance/tolerance of transgenic citrus plants," *Crop Protection*, vol. 30, no. 5, pp. 572–576, 2011.
- [38] Y. He, S. Chen, A. Peng et al., "Production and evaluation of transgenic sweet orange (*Citrus sinensis* Osbeck) containing bivalent antibacterial peptide genes (Shiva A and Cecropin B) via a novel *Agrobacterium*-mediated transformation of mature axillary buds," *Scientia Horticulturae*, vol. 128, no. 2, pp. 99–107, 2011.
- [39] C. A. Reyes, A. De Francesco, E. J. Peña et al., "Resistance to Citrus psorosis virus in transgenic sweet orange plants is triggered by coat protein-RNA silencing," *Journal of Biotechnology*, vol. 151, no. 1, pp. 151–158, 2011.
- [40] J. Fan, X. Liu, S.-X. Xu, Q. Xu, and W.-W. Guo, "T-DNA direct repeat and 35S promoter methylation affect transgene expression but do not cause silencing in transgenic sweet orange," *Plant Cell, Tissue and Organ Culture*, vol. 107, no. 2, pp. 225–232, 2011.
- [41] L. Yang, C. Hu, N. Li, J. Zhang, J. Yan, and Z. Deng, "Transformation of sweet orange [*Citrus sinensis* (L.) Osbeck] with pthA-nls for acquiring resistance to citrus canker disease," *Plant Molecular Biology*, vol. 75, no. 1, pp. 11–23, 2011.
- [42] X.-Z. Fu, E. U. Khan, S.-S. Hu, Q.-J. Fan, and J.-H. Liu, "Overexpression of the betaine aldehyde dehydrogenase gene from *Atriplex hortensis* enhances salt tolerance in the transgenic trifoliolate orange (*Poncirus trifoliata* L. Raf.)," *Environmental and Experimental Botany*, vol. 74, no. 1, pp. 106–113, 2011.
- [43] S.-X. Xu, X.-D. Cai, B. Tan, and W.-W. Guo, "Comparison of expression of three different sub-cellular targeted GFPs in transgenic Valencia sweet orange by confocal laser scanning microscopy," *Plant Cell, Tissue and Organ Culture*, vol. 104, no. 2, pp. 199–207, 2011.
- [44] M. Dutt, M. Vasconcellos, and J. W. Grosser, "Effects of antioxidants on *Agrobacterium*-mediated transformation and accelerated production of transgenic plants of Mexican lime (*Citrus aurantifolia* Swingle)," *Plant Cell, Tissue and Organ Culture*, vol. 107, no. 1, pp. 79–89, 2011.
- [45] M. Dutt, J. Madhavaraj, and J. W. Grosser, "Agrobacterium tumefaciens-mediated genetic transformation and plant regeneration from a complex tetraploid hybrid citrus rootstock," *Scientia Horticulturae*, vol. 123, no. 4, pp. 454–458, 2010.

- [46] A. Ballester, M. Cervera, and L. Peña, "Selectable marker-free transgenic orange plants recovered under non-selective conditions and through PCR analysis of all regenerants," *Plant Cell, Tissue and Organ Culture*, vol. 102, no. 3, pp. 329–336, 2010.
- [47] M. L. P. De Oliveira, V. J. Febres, M. G. C. Costa, G. A. Moore, and W. C. Otoni, "High-efficiency *Agrobacterium*-mediated transformation of citrus via sonication and vacuum infiltration," *Plant Cell Reports*, vol. 28, no. 3, pp. 387–395, 2009.
- [48] J. M. Barbosa-Mendes, F. D. A. A. M. Filho, A. B. Filho, R. Harakava, S. V. Beer, and B. M. J. Mendes, "Genetic transformation of *Citrus sinensis* cv. Hamlin with *hrpN* gene from *Erwinia amylovora* and evaluation of the transgenic lines for resistance to citrus canker," *Scientia Horticulturae*, vol. 122, no. 1, pp. 109–115, 2009.
- [49] Z. Tong, B. Tan, J. Zhang, Z. Hu, W. Guo, and X. Deng, "Using precocious trifoliolate orange (*Poncirus trifoliata* [L.] Raf.) to establish a short juvenile transformation platform for citrus," *Scientia Horticulturae*, vol. 119, no. 3, pp. 335–338, 2009.
- [50] B. Tan, D.-L. Li, S.-X. Xu, G.-E. Fan, J. Fan, and W.-W. Guo, "Highly efficient transformation of the GFP and MAC12.2 genes into precocious trifoliolate orange (*Poncirus trifoliata* [L.] Raf.), a potential model genotype for functional genomics studies in *Citrus*," *Tree Genetics and Genomes*, vol. 5, no. 3, pp. 529–537, 2009.
- [51] A. Ballester, M. Cervera, and L. Peña, "Evaluation of selection strategies alternative to *nptII* in genetic transformation of citrus," *Plant Cell Reports*, vol. 27, no. 6, pp. 1005–1015, 2008.
- [52] M. C. Zanek, C. A. Reyes, M. Cervera et al., "Genetic transformation of sweet orange with the coat protein gene of *Citrus psorosis* virus and evaluation of resistance against the virus," *Plant Cell Reports*, vol. 27, no. 1, pp. 57–66, 2008.
- [53] A. Rodríguez, M. Cervera, J. E. Peris, and L. Peña, "The same treatment for transgenic shoot regeneration elicits the opposite effect in mature explants from two closely related sweet orange (*Citrus sinensis* (L.) Osb.) genotypes," *Plant Cell, Tissue and Organ Culture*, vol. 93, no. 1, pp. 97–106, 2008.
- [54] A. Ballester, M. Cervera, and L. Peña, "Efficient production of transgenic citrus plants using isopentenyl transferase positive selection and removal of the marker gene by site-specific recombination," *Plant Cell Reports*, vol. 26, no. 1, pp. 39–45, 2007.
- [55] G. Ananthkrishnan, V. Orbović, G. Pasquali, M. Čalović, and J. W. Grosser, "Transfer of *Citrus tristeza* virus (CTV)-derived resistance candidate sequences to four grapefruit cultivars through *Agrobacterium*-mediated genetic transformation," *In Vitro Cellular and Developmental Biology—Plant*, vol. 43, no. 6, pp. 593–601, 2007.
- [56] C. Chen, Q. Zheng, X. Xiang et al., "Development of pGreen-derived GFP binary vectors for *Citrus* transformation," *HortScience*, vol. 42, no. 1, pp. 7–10, 2007.
- [57] C. Fagoaga, F. R. Tadeo, D. J. Iglesias et al., "Engineering of gibberellin levels in citrus by sense and antisense overexpression of a GA 20-oxidase gene modifies plant architecture," *Journal of Experimental Botany*, vol. 58, no. 6, pp. 1407–1420, 2007.
- [58] O. Batuman, *Transformation of Citrus plants with constructs related to citrus tristeza virus (ctv) sequences [Ph.D. thesis]*, Hebrew University, 2006.
- [59] R. L. Boscariol, M. Monteiro, E. K. Takahashi et al., "Attacin A gene from *Trichoplusia ni* reduces susceptibility to *Xanthomonas axonopodis* pv. citri in transgenic *Citrus sinensis* 'Hamlin,'" *Journal of the American Society for Horticultural Science*, vol. 131, no. 4, pp. 530–536, 2006.
- [60] F. A. Azevedo, F. A. A. Mourão Filho, B. M. J. Mendes et al., "Genetic transformation of rangpur lime (*Citrus limonia* Osbeck) with the bO (bacterio-opsin) gene and its initial evaluation for *Phytophthora nicotianae* resistance," *Plant Molecular Biology Reporter*, vol. 24, no. 2, pp. 185–196, 2006.
- [61] B. Çevik, R. F. Lee, and C. L. Niblett, "Genetic transformation of *Citrus paradisi* with antisense and untranslatable RNA-dependent RNA polymerase genes of *Citrus tristeza* closterovirus," *Turkish Journal of Agriculture and Forestry*, vol. 30, no. 3, pp. 173–182, 2006.
- [62] M. Ahmad and B. Mirza, "An efficient protocol for transient transformation of intact fruit and transgene expression in *Citrus*," *Plant Molecular Biology Reporter*, no. 23, pp. 419–420, 2005.
- [63] W. Guo, Y. Duan, O. Olivares-Fuster et al., "Protoplast transformation and regeneration of transgenic Valencia sweet orange plants containing a juice quality-related pectin methylesterase gene," *Plant Cell Reports*, vol. 24, no. 8, pp. 482–486, 2005.
- [64] H. B. C. Molinari, J. C. Besspalhok, A. K. Kobayashi, L. F. P. Pereira, and L. G. E. Vieira, "*Agrobacterium tumefaciens*-mediated transformation of Swingle citrumelo (*Citrus paradisi* Macf. x *Poncirus trifoliata* L. Raf.) using thin epicotyl sections," *Scientia Horticulturae*, vol. 99, no. 3-4, pp. 379–385, 2004.
- [65] L. Peña, R. M. Pérez, M. Cervera, J. A. Juárez, and L. Navarro, "Early events in *Agrobacterium*-mediated genetic transformation of *Citrus* explants," *Annals of Botany*, vol. 94, no. 1, pp. 67–74, 2004.
- [66] A. Domínguez, M. Cervera, R. M. Pérez et al., "Characterisation of regenerants obtained under selective conditions after *Agrobacterium*-mediated transformation of citrus explants reveals production of silenced and chimeric plants at unexpected high frequencies," *Molecular Breeding*, vol. 14, no. 2, pp. 171–183, 2004.
- [67] M. Kayim, T. L. Ceccardi, M. J. G. Berretta, G. A. Barthe, and K. S. Derrick, "Introduction of a citrus blight-associated gene into Carrizo citrange [*Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.] by *Agrobacterium*-mediated transformation," *Plant Cell Reports*, vol. 23, no. 6, pp. 377–385, 2004.
- [68] W. A. B. Almeida, F. A. A. Mourao Filho, B. M. J. Mendes, A. Pavan, and A. P. M. Rodriguez, "*Agrobacterium*-mediated transformation of *Citrus sinensis* and *Citrus limonia* epicotyl segments," *Scientia Agricola*, vol. 60, no. 1, pp. 23–29, 2003.
- [69] J. C. Besspalhok Filho, A. K. Kobayashi, L. F. P. Pereira, R. M. Galvão, and L. G. E. Vieira, "Transient gene expression of β -glucuronidase in *Citrus* thin epicotyl transversal sections using particle bombardment," *Brazilian Archives of Biology and Technology*, vol. 46, no. 1, pp. 1–6, 2003.
- [70] D. D. Li, W. Shi, and X. X. Deng, "Factors influencing *Agrobacterium*-mediated embryogenic callus transformation of Valencia sweet orange (*Citrus sinensis*) containing the *pTA29*-barnase gene," *Tree Physiology*, vol. 23, no. 17, pp. 1209–1215, 2003.
- [71] W. A. B. Almeida, F. A. A. Mourão Filho, L. E. Pino, R. L. Boscariol, A. P. M. Rodriguez, and B. M. J. Mendes, "Genetic transformation and plant recovery from mature tissues of *Citrus sinensis* L. Osbeck," *Plant Science*, vol. 164, no. 2, pp. 203–211, 2003.
- [72] N. A. Chávez-Vela, L. I. Chávez-Ortiz, and E. P.-M. Balch, "Genetic transformation of sour orange using *Agrobacterium rhizogenes*," *Agrociencia*, vol. 37, no. 6, pp. 629–639, 2003.
- [73] V. J. Febres, C. L. Niblett, R. F. Lee, and G. A. Moore, "Characterization of grapefruit plants (*Citrus paradisi* Macf.)

- transformed with *Citrus tristeza* closterovirus genes,” *Plant Cell Reports*, vol. 21, no. 5, pp. 421–428, 2003.
- [74] R. P. Niedz, W. L. McKendree, and R. G. Shatters Jr., “Electroporation of embryogenic protoplasts of sweet orange (*Citrus sinensis* (L.) Osbeck) and regeneration of transformed plants,” *In Vitro Cellular and Developmental Biology—Plant*, vol. 39, no. 6, pp. 586–594, 2003.
- [75] C. Yu, S. Huang, C. Chen, Z. Deng, P. Ling, and F. G. Gmitter Jr., “Factors affecting *Agrobacterium*-mediated transformation and regeneration of sweet orange and citrange,” *Plant Cell, Tissue and Organ Culture*, vol. 71, no. 2, pp. 147–155, 2002.
- [76] D. D. Li, W. Shi, and X. X. Deng, “*Agrobacterium*-mediated transformation of embryogenic calluses of Ponkan mandarin and the regeneration of plants containing the chimeric ribonuclease gene,” *Plant Cell Reports*, vol. 21, no. 2, pp. 153–156, 2002.
- [77] L. Peña, M. Martín-Trillo, J. Juárez, J. A. Pina, L. Navarro, and J. M. Martínez-Zapater, “Constitutive expression of *Arabidopsis* LEAFY or APETALA1 genes in citrus reduces their generation time,” *Nature Biotechnology*, vol. 19, no. 3, pp. 263–267, 2001.
- [78] R. Ghorbel, A. Dominguez, L. Navarro, and L. Peña, “High efficiency genetic transformation of sour orange (*Citrus aurantium*) and production of transgenic trees containing the coat protein gene of *Citrus tristeza* virus,” *Tree Physiology*, vol. 20, no. 17, pp. 1183–1189, 2000.
- [79] G. A. Moore, D. Luth, M. McCaffery, V. J. Febres, S. M. Garnsey, and C. L. Niblett, “*Agrobacterium*-mediated transformation of grapefruit (*Citrus paradisi* Macf.) with genes from citrus tristeza virus,” in *Proceedings of the 1st International Citrus Biotechnology Symposium*, ISHS Acta Horticulturae 535, 2000.
- [80] D. Piestun, O. Batuman, X. Che et al., “Truncated versions of the *Citrus tristeza* virus (CTV) replicase and basta resistance genes incorporated in transgenic Troyer citrange,” in *Proceedings of the 1st International Citrus Biotechnology Symposium*, ISHS Acta Horticulturae 535, 2000.
- [81] A. Domínguez, J. Guerri, M. Cambra, L. Navarro, P. Moreno, and L. Peña, “Efficient production of transgenic citrus plants expressing the coat protein gene of *Citrus tristeza* virus,” *Plant Cell Reports*, vol. 19, no. 4, pp. 427–433, 2000.
- [82] G. H. Fleming, O. Olivares-Fuster, S. Fatta Del-Bosco, and J. W. Grosser, “An alternative method for the genetic transformation of sweet orange,” *In Vitro Cellular and Developmental Biology—Plant*, vol. 36, no. 6, pp. 450–455, 2000.
- [83] A. M. P. Koltunow, S. B. Protopsaltis, and N. Nito, “Regeneration of west indian limes (*Citrus aurantifolia*) containing genes for decreased seed,” in *Proceedings of the 1st International Citrus Biotechnology Symposium*, ISHS Acta Horticulturae 535, 2000.
- [84] Z. N. Yang, I. L. Ingelbrecht, E. Louzada, M. Skaria, and T. E. Mirkov, “*Agrobacterium*-mediated transformation of the commercially important grapefruit cultivar Rio Red (*Citrus paradisi* Macf.),” *Plant Cell Reports*, vol. 19, no. 12, pp. 1203–1211, 2000.
- [85] M. Cervera, J. A. Pina, J. Juárez, L. Navarro, and L. Peña, “*Agrobacterium*-mediated transformation of citrange: factors affecting transformation and regeneration,” *Plant Cell Reports*, vol. 18, no. 3–4, pp. 271–278, 1998.
- [86] J. E. Bond and M. L. Roose, “*Agrobacterium*-mediated transformation of the commercially important citrus cultivar Washington navel orange,” *Plant Cell Reports*, vol. 18, no. 3–4, pp. 229–234, 1998.
- [87] J.-L. Yao, J.-H. Wu, A. P. Gleave, and B. A. M. Morris, “Transformation of citrus embryogenic cells using particle bombardment and production of transgenic embryos,” *Plant Science*, vol. 113, no. 2, pp. 175–183, 1996.
- [88] L. Peña, M. Cervera, J. Juárez et al., “High efficiency *Agrobacterium*-mediated transformation and regeneration of citrus,” *Plant Science*, vol. 104, no. 2, pp. 183–191, 1995.
- [89] L. Peña, M. Cervera, J. Juárez et al., “*Agrobacterium*-mediated transformation of sweet orange and regeneration of transgenic plants,” *Plant Cell Reports*, vol. 14, no. 10, pp. 616–619, 1995.
- [90] T. Hidaka and M. Omura, “Transformation of Citrus protoplasts by electroporation,” *Journal of the Japanese Society Horticulture Science*, vol. 62, no. 2, pp. 371–376, 1993.
- [91] G. A. Moore, C. C. Jacono, J. L. Neidigh, S. D. Lawrence, and K. Cline, “*Agrobacterium*-mediated transformation of *Citrus* stem segments and regeneration of transgenic plants,” *Plant Cell Reports*, vol. 11, no. 5–6, pp. 238–242, 1992.
- [92] A. Vardi, S. Bleichman, and D. Aviv, “Genetic transformation of citrus protoplasts and regeneration of transgenic plants,” *Plant Science*, vol. 69, no. 2, pp. 199–206, 1990.
- [93] V. Orbovic and J. W. Grosser, “Citrus: sweet orange (*Citrus sinensis* L. Osbeck “Valencia”) and Carrizo citrange [*Citrus sinensis* (L.) Osbeck X *Poncirus trifoliata* (L.) Raf.],” in *Agrobacterium Protocols-Methods in Molecular Biology*, K. Wang, Ed., pp. 177–189, Humana Press, New York, NY, USA, 2006.
- [94] E. E. Hood, S. B. Gelvin, L. S. Melchers, and A. Hoekema, “New *Agrobacterium* helper plasmids for gene transfer to plants,” *Transgenic Research*, vol. 2, no. 4, pp. 208–218, 1993.
- [95] M. C. Christey, “Use of Ri-mediated transformation for production of transgenic plants,” *In Vitro Cellular and Developmental Biology—Plant*, vol. 37, no. 6, pp. 687–700, 2001.
- [96] M. D. Chilton, D. A. Tepfer, A. Petit, C. David, F. Casse-Delbart, and J. Tempé, “*Agrobacterium rhizogenes* inserts T-DNA into the genomes of the host plant root cells,” *Nature*, vol. 295, no. 5848, pp. 432–434, 1982.
- [97] J. V. Shanks and J. Morgan, “Plant “hairy root” culture,” *Current Opinion in Biotechnology*, vol. 10, no. 2, pp. 151–155, 1999.
- [98] S. M. Choi, S. H. Son, S. R. Yun, O. W. Kwon, J. H. Seon, and K. Y. Paek, “Pilot-scale culture of adventitious roots of ginseng in a bioreactor system,” *Plant Cell, Tissue and Organ Culture*, vol. 62, no. 3, pp. 187–193, 2000.
- [99] R. Wongsamuth and P. M. Doran, “Hairy root as an experimental system for production of antibodies,” in *Hairy Roots, Culture and Applications*, M. P. Doran, Ed., pp. 89–97, Harwood, Amsterdam, The Netherlands, 1997.
- [100] T. V. Nedelkoska and P. M. Doran, “Hyperaccumulation of cadmium by hairy roots of *Thlapsi caerulescens*,” *Biotechnology and Bioengineering*, vol. 67, no. 5, pp. 607–615, 2000.
- [101] V. Kumar, A. Sharma, B. C. N. Prasad, H. B. Gururaj, and G. A. Ravishankar, “*Agrobacterium rhizogenes* mediated genetic transformation resulting in hairy root formation is enhanced by ultrasonication and acetosyringone treatment,” *Electronic Journal of Biotechnology*, vol. 9, no. 4, pp. 1–9, 2006.
- [102] M. Kusaba, “RNA interference in crop plants,” *Current Opinion in Biotechnology*, vol. 15, no. 2, pp. 139–143, 2004.