Journal of Forest Science
Vol. 25, No. 3, pp. 195~204, December 2009

Agrobacterium tumefaciens-Mediated Genetic Transformation: Mechanism and Factors

Nitish Kumar[1*], K.G. Vijayanand[2], Muppala P. Reddy[3], Amritpal S. Singh[1], and Subhash Naraynan[1]

[1]*Department of Agricultural Biotechnology, Anand Agricultural University, Anand-388110, Gujarat, India*
[2]*Discipline of wasteland research, Central Salt & Marine Chemicals Research Institute (Council of Scientific and Industrial Research, New Delhi), Bhavnagar, Gujarat-364002, India*
[3]*Plant Stress Genomics and Technology Center, King Abdullah University of Science and Technology, Thuwal-23955-6900, Kingdom of Saudi Arabia*

ABSTRACT : *Agrobacterium*-mediated genetic transformation has been widely used for the production of genetically modified transgenic plants to obtain specific desired traits. Most of the molecular mechanisms that underlie the transformation steps have been well elucidated over the years. However, a few steps, such as nuclear targeting, T-DNA integration, and *Agrobacterium*-plant proteins involved remain largely obscure and are still under extensive studies. This review describes the major steps involved in the molecular mechanism of *Agrobacterium*-mediated transformation and provides insight in the recent developments in studies on the *Agrobacterium*-mediated genetic transformation system. Some factors affecting the transformation efficiency are also briefly discussed.

Keywords : Agrobacterium tumefaciens, Genetic transformation, Mechanism and Factor

INTRODUCTION

Cultivation and improvement of crops have been the key to agriculture and civilization. Improvement of crops is a major target for scientist due to explosion in population, social demands, health requirements, environmental stresses and ecological considerations. Two major approaches i.e., conventional breeding and biotechnological approach have been suggested. Conventional plant breeding techniques have limitations as these depend on sexual compatibility and often take 10-15 years to release a new variety due to extensive backcrossing. Biotechnological crop improvement thus, appears to be the only time effective, alternative approaches wherein transgenic production will be the most important in achieving the above parameters. Recombinant DNA technology and tissue culture, together with the recent gene transfer methods like biolistic, electroporation, micro-injection, Poly ethylene glycol, silicon carbide fibre and liposomes now enable to target gene into plants even from distantly related organism like bacteria, virus, animals and even humans (Crossway et al., 1986; Fraley, 1986; Fromm et al., 1987; De la pena et al., 1987; Klein et al., 1987; Kaeppler et al., 1990; Zhang et al., 1997). Of these, *Agrobacterium*-mediated transformation is preferred method of gene transfer for reasons like simplicity, cost effectiveness, little re-arrangement of transgene, ability to transfer relatively long DNA segments (Hamilton et al., 1997), and preferential integration of foreign genes into transcriptionally active regions (Konez et al., 1989; Ingelbrecht et al., 1991) thereby ensuring proper expression of transgenes in plants (Hernandez et al., 1999) as compared to other methods. The present review, therefore will briefly but critically discuss recent findings and thoughts in these areas with particular emphasis on *Agrobacterium*-mediated genetic transformation.

* Corresponding author: (E-mail) nitishbt1@rediffmail.com

Agrobacterium-mediated gene transfer

Agrobacterium is a gram-negative, soil-dwelling bacterium, which infects plant cells near wounds, usually at the junction between the root and stem (crown) in a wide range of plant species. There are about 331 genera with 643 species all of which contain a large circular plasmid called as tumor inducing or Ti plasmid in case of *A. tumefaciens* and root inducing or Ri plasmid in *A. rhizogenes*. These plasmids contain genes for (a) virulence (b) catabolism of specific opines (c) host-directed opine synthesis and (d) synthesis of bacterial-type plant hormones. *Agrobacterium*-mediated gene transfer involves incubation of cells or tissues with the bacterium (co-cultivation), followed by regeneration of plants from the transformed cells. For plant species that are readily amenable to tissue culture, *Agrobacterium*-mediated gene transfer, the first widely adopted methods of developing transgenic plants, remains the most popular technique. Probably the greatest advantage of the system is that it offers the potential to generate transgenic cells at relatively high frequency, without a significant reduction in plant regeneration rates. The system is simple, inexpensive and in many cases efficient. Moreover the DNA transferred to the plant genome is defined, it does not normally undergo any major rearrangements and it integrates into the genome as a single copy (Walden and Wingender, 1995). During infection, the bacterium transfers a small section of its own genetic material (T-DNA) into the genome of the host plant's cell (Zambryski, 1992; Tzfira et al., 2004; Tzfira and Citovsky, 2006). Once inserted, the bacterial genes are expressed by infected cells of that plant. During the infection process, first the plant cell begins to proliferate and form tumors and then synthesize an arginine derivative called opine. The opine synthesized is usually nopaline or octopine depending on the strain involved. These opine are catabolized and used as energy sources by the infecting bacteria. By understanding and manipulating this process of infection or transformation, scientists have been able to harness these powerful and sophisticated vectors to transfer specific cloned genes of major importance. Initially, monocotyledons were considered outside the host range of *Agrobacterium*. However, advances in understanding of the biology of the infection process, availability of gene promoters suitable to monocotyledons (Wilmink et al., 1995) as well as selectable markers have improved transformation of monocotyledons (Smith and Hood, 1995). Transgenic plants of Citrus (Moore et al., 1992), rice (Hiei et al., 1994) and maize (Ritchie et al., 1993) have been produced via *Agrobacterium*-mediated transformation. However, success of *Agrobacterium*-mediated transformation depends on the cultivar (Robinson and Firoozabady, 1993), the choice of explant (Robinson and Firoozabady, 1993; Jenes et al., 1993), the *Agrobacterium* strain (Gelvin and Liu, 1994; Kumar, 2003); the conditions of co-cultivation, the selection method and the mode of plant regeneration (Opabode, 2006; Kumar, 2009). *Agrobacterium* co-cultivation has been successfully used for the transformation of leaves, roots, hypocotyls, petioles, cotyledons (Zambryski, 1992; Hooykaas and Beijersbergen, 1994; Li et al., 2008; Kumar 2009; Padmanabhan and Sahi, 2009), pollen-derived embryos (Sangwan et al., 1991), seeds (Feldmann and Marks, 1987) and even plants (Cheng et al., 1997). The bacteria parasitize on the plants through transfer and integration of a part of the plasmid that is called as the "transfer DNA" or the T-DNA. The T-DNA that is transferred into the plant cells contains genes which encode proteins involved in the biosynthesis of opines and plant-type phyto-hormones. The opine incite conjugal transfer of bacterial plasmid to neighboring bacteria (genetic colonization) and favor their proliferation. T-DNA is a small section of the plasmid DNA, about 23 kb in size, which makes up about 10% of the Ti or Ri plasmids. This stretch of DNA is flanked by 25 bp repeated sequences, which are recognized by the endonucleases encoded by the vir genes. Within the T-DNA, two distinct regions TL and TR have been identified. The T-DNA of nopaline strains can integrate as a single segment, whereas octopine strains frequently integrate as two segments TL and TR. TL carries the genes controlling auxin and cytokinin biosynthesis and is always present when tumors are formed. Failures of TR to integrate results in the loss of opine biosynthesis (Webb and Morris, 1992). The vir (virulence) region of Ti plasmid contains the genes

which mediate the process of T-DNA transfer. Vir gene action generates and processes a T-DNA copy and facilitates T-DNA movement out of the bacterium and into the plant cell. Helper plasmids for non-oncogenic plant transformation have been developed to utilize the vir gene functions with T-DNAs containing genes of choice (Hood et al., 1993). The removal of the oncogenes from the Ti plasmid results in disarmed strains of *A. tumefaciens* (Klee et al., 1987). The oncogenes of *Agrobacterium* are replaced by reporter genes/screenable marker genes (e.g. b-glucuronidase gene (gus), luciferase (luc) gene for analyzing gene expression. Genes conferring resistance to antibiotics (e.g. neomycin phosphotransferase II (*nptII*), hygromycin phosphotransferase (*hpt*), phosphinothricin acetyl transferase (bar) are used to allow selection between transgenic and non transgenic cells. Also oncogenes have been replaced by genes of economic importance. Plants are usually transformed with relatively simple constructs, in which the gene of interest is coupled to a promoter of plant, viral or bacterial origin. Some promoters confer constitutive expression while others may be selected to permit tissue specific expression. The cauliflower mosaic virus (CaMV) 35S RNA promoter is often used because it directs high levels of expression in most plant tissues.

Mechanism of *Agrobacterium* infection, T-DNA transfer and integration

Plant species differ greatly in their susceptibility to infection by *Agrobacterium tumefaciens* or *rhizogenes*. Even within a species, different cultivars or ecotypes may show different degree of susceptibility. These differences have been noted in a variety of plant species. The subject matter has been reviewed (Gelvin, 2003; Tzfira and Citovsky, 2006). Though environmental or physiological factors are attributed for these differences, genetic basis for susceptibility has been described in *Arabidopsis* (Nam et al., 1997). *Agrobacterium* attaches to plant cells in a polar manner in a two-step process. The first step is likely mediated by a cell-associated acetylated, acidic capsular polysaccharide (Reuhs et al., 1997). The second step involves the elaboration of cellulose fibrils by the bacterium, which enmeshes large numbers of bacteria at the wound surface (Matthysse et al., 1982). The interaction between *Agrobacterum* spp. and plant involves a complex series of chemical signals communicated between the pathogen and the host cells. These signals include neutral and acidic sugars, phenolic compounds, opines (crown gall specific molecules synthesized by transformed plants), Vir (virulence) proteins and the T-DNA (Gelvin, 2003). Baker et al. (1997) has described the chemical signaling in plant-microbe interactions. The T-DNA transfer process initiates when *Agrobacterium* perceives certain phenolic compounds from wounded plant cells (Hooykass and Beijersbergen, 1994) which serves as inducers or coinducers of the bacterial *vir* genes. Phenolic chemicals such as acetosyringone and related compounds are perceived via the VirA sensory proteins (Doty et al., 1996). Most of the induced Vir proteins are directly involved in T-DNA processing from the Ti plasmid and the subsequent transfer of T-DNA from the bacterium to plant. Among them VirD2 and VirE2 contain plant active nuclear localization signal sequences (NLS) (Herrera-Estrella et al., 1990, Tzifira and Citovsky, 2006). VirD2 protein is directly involved in processing the T-DNA from the Ti plasmid. It nicks the Ti plasmid at 25-bp directly repeated sequences, called T-DNA borders that flank the TDNA (Veluthambi et al., 2003). Thereafter, it strongly associates with 5' end of the resulting DNA molecule (Filichkin and Gelvin, 1993) through tyrosin (Vogel and Das, 1992). VirD2 contains two nuclear localization signal (NLS) sequences (Herrera-estrella et al., 1990) whereas VirE2 contains two separate bipartite nuclear localization signal (NLS) regions that can target linked reporter proteins to plant cell nuclei (Citovsky et al., 1994). Many plant species are still recalcitrant to *Agrobacterium* transformation. This recalcitrance does not result from a lack of T-DNA transfer or nuclear targeting, rather its integration into the genome of regenerable cells appears to be limiting. In the future, it may be possible to overexpress endogenous genes involved in the integration process or to introduce homologous genes from other species, and thereby affect higher rates of stable transformation (Gelvin, 2003).

Factors affecting *Agrobacterium* infection and transformation efficiency

Ever since the first genetically engineered *Agrobacterium* was used to produce a transgenetic plant (Hooykaas and Schilperoort, 1992; Mantis et al., 1992; Sheng and Citovsky, 1996; De la Riva et al., 1998; Wei et al., 2000; Zupan et al., 2000; Gelvin 2003; Tzfira and Citovsky, 2006), a wide variety of plants have been genetically modified for crop improvement and many of them have been commercialized. However, production of such transgenic plants involves the modification of a number of parameters due to the affinity of *Agrobacterium* to specific host plants only. The different factors that have been optimized are discussed below.

Bacterial strain / vector

The fact that different strains have different capacity of transform tissues or plants are well documented (Kumar, 2003; Opabode, 2006). The nopaline strains in general have better potential to infect woody species as compared to the octopine ones (Ahuja, 1987). The difference may be due to the lack of "overderive" sequences in the commonly used binary vectors that are derived from pBin19. Overderive sequence is more essential for octopine strains than the nopaline ones. The other differences may be due to the chromosomal virulence genes (chvs) which are related to the attachment of *Agrobacterium* to the plant cell walls. The octopine strains are specifically characterized by the virF gene, or a host range determinant that is induced by acetosyringone (Jarchow et al., 1991). The nopaline strains are more effective than the octopine strains and have been demonstrated in case of grapes wherein the GV3101 strain was more superior (Berres et al., 1992). The strains play a significant role in transformation efficiency has been further proved in the Novel Orange *Citrus sinensis* (Bond and Roose, 1988). Bacterial strains and vectors are known to affect transformation efficiency of plants. Thus, when Hiei et al. (1994) tested different combinations of two strains and three binary vectors in rice, only the strain LBA 4404 (pTOK233) was the most efficient. Surprisingly, the combination of the super virulent strain EHA101 and the super-binary vector plG121Hm were less efficient than the LBA4404 (plG12Hm) alone. Hamilton et al. (1997) also showed that *Agrobacterium* could transfer DNA fragments as large as 150 kb into the plant genome by employing the principle of bacterial artificial chromosome (BAC) into the binary vectors, thereby generating the so-called binary BAC (BiBAC). Veluthambi et al. (2003) reviewed the use of new series of vectors like the small and stable pPZPs, the pCAMBIAs with single cloning sites and the pART series with multiple cloning sites.

Pre-culture/wounding

Transformation efficiency is also considerably affected by pre-culturing and co-cultivation period (Barik et al., 2007; Kumar, 2009). Pre-culturing induces cell division in explant and makes them more receptive to *Agrobacterium* and is largely dependent on the time of pre-culture. Explant pre culture has been reported to be a useful procedure in *A. tumefaciens*-mediated transformation of several plant species (Lawrence and Koundal 2000; Barik et al., 2005; Xu et al., 2009). Four days of pre-culture required for the leaf dises of *Jatropha* curcas (Kumar, 2009). The time period required for pre-culture was genotype dependent in case of almonds (Tsi et al., 1994). However, negative effect of pre-culture on woody plant transformation was also observed in almonds. Pre-culturing of leaf pieces for two days was found to reduce the transformation efficiency to 10 % in *Cyphomandra betacea* (Altkinsons and Gardner, 1993), and tea (Mondal et al., 2002).

Bacterial density and growth phase

Agrobacterium cell density, as well as the stage of bacterial is also important for genetic transformation (Mathysse, 1986). The late log phase is considered to be the most suitable for transformation in a majority of plants (Mondal, 1999, Kuamr, 2003; Kumar, 2009). However, at a high density regeneration of plant tissue is generally inhibited

by bacterial-induced stress and controlling the overgrowth of bacteria during co-cultivation becomes difficult. In citrus, the bacterial density of $4x10^7$ cells/ml as compared to $4x10^8$ cells/ml yielded the maximum (20.6 %) transformation efficiency (Pena et al., 1995). At the late-log phase, corresponding to OD_{600}= 0.6, the maximum transformation efficiency (21.55 %) was observed in case of *J. curcas* (Kumar, 2009). Moreover, O.D. values higher than 0.6 at A_{600nm} indicating the late log phase were not desirable for the co-cultivation of almond leaf discs (Archilletti et al., 1995). Transformation efficiency in black poplar (Confalonieri et al., 1995) and grapevine (Baribault et al., 1990) however, was not affected by bacterial density.

Inducers of vir genes

Several stimuli that are known to induce the vir genes includes - low concentrations of phenolic compounds acetosyringone, hydroxyl-acetosyringone (Sheng and Citovsky, 1996), pH (> 5.7) of the medium (Bolton et al., 1986; Kumar, 2009), carbon source as sucrose and glucose (Seo et al., 2002; Kumar 2003), culture conditions during co-cultivation like temperature (Stachel et al., 1986), darkness and osmoticum (Koichi et al., 2002). However, some reports showed that light rather than dark and temperature as low as 22°C or even lower were crucial to higher transformation efficiency (Zambre et al., 2003). Of the different type of stimuli, the effects of phenolics on vir gene induction has been most thoroughly studied and have thus been tabulated (Table 1). Acetosyringone is known to improve the transformation efficiency in a large number of plant species (Godwin et al., 1991; Opabode, 2006; Kumar, 2009). Acetosyringone has been found to be effective at a wide range of concentrations (20 μM-100 μM) in a number of plant species. Even in the two different cultivars of the same species of grapevine, different concentrations were required i.e. 20 μM (Baribault et al., 1990) and 100 μM (Colby et al., 1991). While 20 μM acetosyringone was effective for peaches (Smigocki and Hammerschlag, 1991), 100 μM was required for trifoliate oranges (Hiramatsu-Kaneyoshi et al., 1994;), *J. curcas* (Li et al., 2008; Kumar 2009) and 200 μM enhanced the transformation efficiency of hybrid

Table 1. Different phenolics compounds used in woody plant transformation

Phenolics compound	Species	Referances
Hydroxy-acetosyringone, Catechol, Pyrogallic acid, Chalcone derivation	Citrus, Almond, Walnut etc.	Asbhy et al., 1988 McGranahan et al., 1988 Gutierrez et al., 1997 Bond and Roose 1998 Miguel and Oliveira, 1999
Benzylacetones, Dibenzylacetones, Hydroxy-acetophenone, Acetovanillone syringaldehyde	Pinus, Mulbery, Foxglove, Guava etc.	Joubert et al., 1995 Humara et al., 1999 Agarwal et al., 2004 Saito et al., 2004 Rai et al., 2009
Syringic acid, Methyl easter	Grape wine, Kiwifruit, Orange fruit etc.	Spencer and Towers, 1988 Baribault et al., 1990 Bond and Roose 1998 Kobayashi et al., 1996 Torregrosa et al., 2002
Sinapinic acid, Vanillin, Ferulic acid,	Tea, Poplar, Rubber etc.	Kumar et al., 2004 Spencer et al., 1990 Parsons et al., 1986 Blanc et al., 2005
Acetosyringone	*Mango*, *Jatropha*, Castor, Peach etc.	Stachel et al., 1986 Scorza et al., 1990 Krishna and Singh, 2007 Kumar, 2009

poplar cv. NC-5339 (Howe et al., 1994). However, acetosyringone failed to bring about transformation in cultivars *Populus deltoids* and *Poplus euranericana* (Confalonieri et al., 1994) and tea (Mondal et al., 2002; Kumar, 2003; Kumar et al., 2004). The other important inducer 'glucose' has been reported to bring about transformation in apples (James et al., 1993) and strawberries (Shimoda et al., 1990). Although plant growth regulators have not been considered to be inducers, yet they have been reported to enhance the transformation efficiency in woody plants when used in the co-cultivation medium (Bondt et al., 1996). The presence of TDZ and NAA in the co-cultivation medium enhanced the recovery of transformed subterranean clover shoots probably because the peripheral cells at the cut surface of hypocotyl responded better when grown on a regeneration medium supplemented with TDZ (Sangwan et al., 1991). Similarly, 2, 4-D was also used in the co-cultivation medium during genetic transformation of tea (Sandal, 2003).

Plant variety and explant

The limited host range specificity of *Agrobacterium* is a well documented fact (Hawes et al., 1989). *Agrobacterium* has been reported to infect 643 host plants from 331 genera (DeCleene and DeLey, 1976). Anderson and Moore assayed 176 strains of *Agrobacterium* for pathogenicity on 11 dicotyledenous plants and found extensive host range variations between widely amongst the different cultivars or genotypes (Hawes et al., 1989). Different varieties of a single species were also found to respond differently to a particular bacterial strain. The host range of individual strains of *A. tumefaciens* is determined primarily by the Ti plasmid and can range from few to hundred species (Dandekar et al., 1988). Thus, *Agrobacterium*-mediated transformation is highly specific to plant species and cultivars (Kumar, 2003) and transformation efficiency may vary even with the same cultivars depending upon the explant (Wei et al., 2000). Although the actual biochemical basis for host range variations in *Agrobacterium* is not clear yet, two distinct regions of the Ti plasmid is now thought to contribute to the overall host specifically of the bacterium (Hooykaas and Schilperoot, 1992). These include loci (vir A) in the virulence region and another locus within the T-DNA. Besides, the T-DNA and the virulent genes, there is a certain undefined host factor that influence specificity to some extent (Hood et al., 1993). However, these factors also mediate susceptibility of plants to infection by recombinant strains and may vary even within different parts of the same plant (Martin et al., 1989). Thus, when the same explant (leaf and petiole) was used for different cultivars 'Meeker', 0.91 % for chilliwack and 8.1 % for 'Canby' etc. (Mathews et al., 1995). Considering the larger surface area for manipulation, easy availability and maintenance of true to type nature are considered to be attractive as explant for biotechnological crop improvement and have been used extensively.

Conclusion and perspective

As *Agrobacterium*-mediated plant transformation has become the most used method to introduce foreign genes to obtain a desired phenotype in a variety of crops, the fundamental knowledge underlying the molecular mechanism of *Agrobacterium*-mediated plant transformation has been a hot topic since many years. The important events including the bacterial attachment to the plant cell, vir gene activation, T-DNA processing, nuclear targeting and T-DNA integration have been quite well studied, although the role of the host cellular proteins involved in the transformation process remains largely obscure and is still under extensive investigation. A better understanding of all molecular events in the process as well as the plant proteins involved could be exploited for the further improvement of *Agrobacterium*-mediated plant transformation. In addition, the knowledge on the factors that influence the transformation efficiency is also crucial. The detailed knowledge on the factors limiting the transformation efficiency will broaden the range of the crop species that can be transformed by *A. tumefaciens* especially for the recalcitrant species.

References

Agarwal, S., Kanwar, K., Saini, N., Jain, R. K. 2004. *Agrobacterium* tumefaciens mediated genetic transformation and regeneration of *Morus alba* L. Scientia Horti. 100, 183-191.

Ahuja, M. R. 1987. Gene transfer in Forest trees. In: Genetic Manipulation of Woody plants. (Hanover J.W. and Keathley D.E. eds.). Plenum Press, New York, 25-41.

Archilletti, T., Lauri, P., Damiano, C. 1995. *Agrobacterium* mediated transformation of almond leaf pieces. Plant Cell Rep. 14, 267-272.

Ashby, A. M., Watson, M. D., Loake, G. J., Shaw, C. H. 1988. Ti-plasmid specific chemotaxis of Agrobacterium tumefaciens C58C1 towards vir inducing phenolic compounds and factors from monocotyledons and dicotyledonous plants. J Bact. 170, 4181-4187.

Atkinson, G. R., Gardner, C. R. 1993. Regeneration of transgenic tamarillo plants. Plant Cell Rep. 12, 347-351.

Baker, B., Zambryski, P., Staskawicz, B., Dinesh-Kumar, S. P. 1997. Signalling in Plant- Microbe Interaction. Science 276, 726-733.

Baribault, T.J., Skene, K. G. M., Cain, P. A., Scott, N. S. 1990. Transgenic grapevines: Regeneration of shoots expressing β-glucuronidase. J Exp Bot. 229, 1045-1049.

Barik, D. P., Mohapatra, U., Chand, P. K. 2005. Transgenic grasspea (*Lathyrus sativus* L.): Factors influencing *Agrobacterium*-mediated transformation and regeneration. Plant Cell Rep. 24, 523-531.

Berrs, R., Otten, L., Tinland, B., Clog-Malgarini, E., Walter, B. 1992. Transformation of *Vitis* tissue by different strains of *Agrobacterium tumefaciens* containing the T-6b gene. Plant Cell Rep. 11, 192-195.

Blanc, G., Baptiste, C., Oliver, G., Martin, F., Montoro, P. 2006. Efficient *Agrobacterium tumefaciens*-mediated transformation of embryogenic calli and regeneration of *Hevea brasiliensis* Müll Arg. Plants. Plant Cell Rep. 24, 724-733.

Bolton, G. W., Nester, E. W., Gordon, M. P. 1986. Plant phenolic compounds induce expression of the *Agrobacterium tumefaciens* loci needed for virulence. Science 232, 983-985.

Bond, J. E., Ross, M. L 1998. *Agrobacterium*-mediated transformation of commercially important citrus cultivars Washington Naval Orange. Plant Cell Rep, 18, 229-237.

Cheng, M., Fry, J. E., Pang, S., Zhou, H., Hironaka, C. M., Duncan, D. R., Conner, T. W., Wan, Y. 1997. Genetic transformation of wheat mediated by *Agrobacterium tumefaciens*. Plant Physiol. 115, 971-980.

Citovsky, V., Wong, M. L., Zambryski, P. C. 1989. Cooperative interaction of *Agrobacterium* VirE2 protein with single stranded DNA: implications for the T-DNA transfer process. Pro. Nat. Acad. Sci. USA 86, 193-1197.

Colby, S. M., Juncosa, A. M., Meredith, C. P. 1991. Cellular differences in *Agrobaterium* susceptibility and regenerative capacity restrict the development of transgenic grapevines. J. Am. Soc. Hort. Sci. 116, 356-361.

Confalonieri, M., Balestrazzi, A., Bisoffi, S., Cella, R. 1995. Factor affecting *Agrobacterium tumefacions* mediated transformation in several black poplar clones. Plant Cell Tiss Organ Cult. 43, 215-222.

Crossway, A., Oaks, J., Irwine, J., Ward, B., Knauf, V., Shewmaker, C. 1986. Integration of foreign DNA following microinjection of tobacco mesophyll protoplasts. Mol. Gen. Genet. 202, 179-185.

Dandekar, A. M., Martin, L. A., McGranahan, G. 1988. Genetic transformation and foreign gene expression in walnut tissue. J. Am. Soc. Hort. Sci. 113, 945-949.

De Cleene, M., De Ley, J. 1976. The hosts range of crown gall. Bot. Rev. 42, 389-466.

De la Pena, A., Lorz, H., Schell, J. 1987. Transgenic rye plants obtained by injecting DNA into young floral tillers. Nature 325, 274-276.

De la Riva, G. A., Gonzalez-Cabrera, J., Vazquez-Padron, R., Ayra-Pardo, C. 1998. *Agrobacterium tumefaciens*: a natural tool for plant transformation. Elect. J. Biotech. 1, 118-123.

Doty, S. L., Yu, M. C., Lundin, J. I., Heath, J. D., Nester, E. W. 1996. Mutational analysis of the input domain of the VirA protein of *Agrobacterium tumefaciens*. J. Bact. 178, 961-970.

Feldmann, K. A., Marks, M. D. 1987. *Agrobacterium*-mediated transformation of germinating seeds of *Arabidopsis thaliana*: a non-tissue culture approach. Mol. Gen. Genet. 208, 1-9.

Filichkin, S. A., Gelvin, S. B. 1993. Formation of a putative relaxation intermediate during T-DNA processing directed by the *Agrobacterium tumefaciens* VirD1,D2 endonuclease. Mol. Micro. 8, 915-926.

Fraley, R. T., Rogers, S. G., Horsh, R. B. 1986. Genetic transformation in higher plants. Crit. Rev. Plant Sci. 4, 1-46.

Fromm, M., Callis, J., Taylor, I. P., Walbot, V. 1987. Electroporation of DNA and RNA into plant protoplast. Methods Enzym. 153, 351-365.

Gelvin, S. B. 2003. *Agrobacterium*-Mediated Plant Transformation: the Biology behind the "Gene-Jockeying" Tool. Micro. Mol. Biol. Rev. 67, 16-37.

Gelvin, S. B., Liu, C. N. 1994. In: Gelvin, S. B., Schilperoort, R. A. (eds) Plant Molecular Biology Manual, Kluwer Academic Publishers, Dordrecht 4, 1-3.

Goodwin, I., Todd, G., Ford-Loyd, B., Newbury, H. J. 1991. The effects of acetosyringone and pH on *Agrobacterium*-mediated transformation vary according to plant species. Plant Cell Rep. 9, 671-675.

Gutiérreze, M. A., Luth, D., Moore, G. A. 1997. 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 Rep.16, 745-753.

Hamilton, C., Frary, A., Lewis, C., Tanskley, S. D. 1997. Stable transfer of intact high molecular weight DNA into plant chromosomes. Proc. Nat. Acad. Sci. USA 93, 9975-9979.

Hernandez, J. B. P., Remy, S., Sauco, V. G., Swennen, R., Sagi, L. 1999. Chemotactic movement and attachment of *Agrobacterium tumefaciens* to banana cells and tissues. J Plant Physiol. 155, 245-250.

Herrera-Estrella, A., Van Montagu, M., Wang, K. 1990. A bacterial peptide acting as a plant nuclear targeting signals: The amino-terminal portion of *Agrobacterium* VirD2 protein directs

a b-galactosidase fusion protein into tobacco. Proc. Nat. Acad. Sci. USA 87, 9534-9537.

Hiei, Y., Ohta, S., Komari, T., Kumashiro, T. 1994. Effiecient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant J. 6, 271-282.

Hiramatsu-Kaneyoshi J, Kobayashi S, Nakamura Y, Shigemoto N and Doi Y (1994) A simple and efficient gene transfer system of trifoliage orange (*Poncirus trifoliate Raf.*) Plant Cell Reports 13: 541-545.

Hood, H. E., Gelvin, S. B., Melchers, L. S., Hoekema, A. 1993. New *Agrobacterium* helper plasmids for gene transfer to plants. Transgenic Res. 2, 208-218.

Hooykaas, P. J. J., Beijersbergen, A. G. M. 1994. The virulence. system of *Agrobacterium tumefaciens*. Ann. Rev. Phyto. 32, 157-179.

Hooykaas, P. J. J., Schilperoort, R. A. 1992. *Agrobacterium* and plant genetic engineering. Plant Mol. Biol. 19, 15-38.

Howe, G. T., Goldfarb, B., Strauss, S. H. 1994. *Agrobacterium*-mediated transformation of hybrid poplar suspension cultures and regeneration of transformed plants. Plant Cell Tiss. Organ Cult. 36, 59-71.

Humara, J. M., López, M., Ordas, R. J. 1999. *Agrobacterium* tumefaciens-mediated transformation of *Pinus pinea* L. cotyledons: an assessment of factors influencing the efficiency of uidA gene transfer. Planr cell rep. 19, 51-58.

Ingelbrecht, I., Breyne, P., Vancomperonolle, A., Van Montagu, J. M., Depicker, A. 1991. Transcriptional interferences in transgenic plants. Gene 109, 239-242.

James, D. J., Passey, A. J., Webster, A. D., Barbara, D. J., Dandekar, A. M., Uratsu, S. L., Viss, P. 1993. Transgenic apples and strawberries: Advances in transformation, induction of genes for insect resistance and field studies of tissue cultured plants. Sci. Hort. 336, 170-175.

Jarchow, E., Grimsley, N. H., Hohn, B. 1991. Vir F, the host range determining virulence genes of *Agrobacterium tumefaciens*, affects T-DNA transfer to Zea mays. Proc. Nat. Acad. Sci. USA 88, 10426-10430.

Jenes, B., Morre, H., Cao, J., Zhang, W., Wu, R. 1993. Techniques for gene transfer. In: Kung S. Wu R (eds) Trangenic plants, Academic Press, San Diego, 1, 125-146.

Joubert, P., Sangwan, R. S., Aovad, M. E. A., Beaupere, D., Sangwan-Norreel, S. B. 1995. Influence of phenolic compounds on Agrobacterium vir gene induction and onion gene transfer. Phytochem. 40, 1623-1628

Kaeppler, H. F., Gu, W., Somers, D. A., Rhines, H. W., Cockburn, A. F. 1990. Silicon carbide fiber-mediated DNA delivery into plant cells. Plant Cell Rep. 9, 415-418.

Klee, H., Horsch, R., Rogers, S. 1987. *Agrobacterium*-mediated plant transformation and its further applications to plant biology. Ann. Rev. Plant Physiol. 38, 467-486.

Klein, T. M., Wolf, E. D., Wu, R., Sanford, J. C. 1987. High velocity microprojectiles for delivering nucleic acids into living cells. Nature 327, 70-73.

Kobayashi, S., Nakamura, K., Kaneyoshi, J., Higo, H., Higo, K. 1996. Transformation of kiwifruit (*Actinidia chinensis*) and trifoliate orange (*Poncirus trifoliata*) with a synthetic gene encoding the human epidermal growth factor (hEGF). J. Jpn. Soc. Hortic. Sci. 64, 763-769.

Koichi, T., Bae, C. H., Seo, M. S., Song, I. J., Lim, Y. P., Song, P. S., Lee, H. Y. 2002. Overcoming ob Barriers to Transformation in Monocot Plants. J. Plant Biotech. 4, 135-141.

Konez, C., Martini, N., Mayerhofer, R., Konez-Kalman, Z., Korber, H., Redei, G. P., Schell, J. 1989. High frequency T-DNA mediated tagging in plants. Proc. Nat. Acad. Sci. USA 86, 8467-8471.

Krishna, H., Singh, S.K. 2007. Biotechnological advances in mango (*Mangifera indica* L.) and their future implication in crop improvement-a review. Biotech Adv. 25, 223-243.

Kumar, N. 2003. Studies on recalcitrance of leaf explants to *Agrobacterium*-mediated genetic transformation during the production of transgenic tea. M.Sc. Thesis, Himachal Pradesh Krishi Vishwavidyalaya, Palampur, H.P., India.

Kumar, N. 2009. Studies on regeneration and genetic transformation of *Jatropha curcas*. PhD Thesis, Bhavnagar University, Bhavnagar, Gujarat, india.

Kumar, N., Panday, S., Bhattacharya, A., Ahuja, P. S. 2004. Do leaf surface characteristics affect *Agrobacterium* infection in tea (*Camellia sinensis* (L.) O. Kuntze)? J Biosci. 29, 309-317.

Lawrence, P. K., Koundal, K. R. 2000. Simple protocol for *Agrobacterium tumefaciens*-mediated transformation of pigeonpea [*Cajanus cajan* (L.) Millsp.]. J Plant Biol. 27, 299-302.

Li, M., Li, H., Jiang, H., Pan, X., Wu, G. 2008. Establishment of an *Agrobacteriuim*-mediated cotyledon disc transformation method for *Jatropha curcas*. Plant Cell Tiss. Organ Cult. 92, 173-181.

Mantis, N. J., Winnans, S. C. 1992. The *Agrobacterium tumefaciens* vir genetranscriptional activator virG is transcriptionally induced by acid pH and other stress stimuli. J. of Bacteriology 174, 1189-1196.

Martin, G. C., Millar, A. N., Castle, L. A., Morris, J. W., Morris, R. O., Dandekar, A. M. 1989. Feasibility studies using β-glucuronidase as a gene fusion marker in Apple, Peach and Radish. J. Am. Soc. Hort. Sci. 115, 686-691.

Mathews, H., Wagoner, W., Cohen, C., Kellogg, J., Bestwick, R. 1995. Efficient genetic transformation of red raspberry *Rubus ideaus* L. Plant Cell Rep. 14, 471-476.

Mathysee, A. G. 1986. Initial interaction of *Agrobacterium tumefaciens* with plant host cell. Crit. Rev. Microbiol. 13, 281-307.

Mathysse, A. G., Gurlitz, R. H. G. 1982. Plant Cell range for attachment of *Agrobacterium tumefaciens* to tissue culture cells. Physiol. Plant Path. 21, 318-387.

McGranahan, G. H., Leslie, C. A., Uratsu, S. L., Martin, L. A., Dandekar, A. M. 1988. *Agrobacterium*-mediated transformation of Walnut somatic embryos and regeneration of transgenic plants. Biotech. 6, 800-804.

Miguel, C. M., Oliveira, M. M. 1999. Transgenic almond (*Prunus dulcis* Mill.) plants obtained by *Agrobacterium*-mediated transformation of leaf explants. Plant Cell Rep. 18, 387-393.

Mondal, T. K. 1999. Studies on RAPD markers for detection of genetic diversity, in vitro regeneration and *Agrobacterium*-mediated

genetic transformation of tea (*Camellia sinensis* (I). O Kuntze). Ph D. Thesis. Utkal University, Bhubneswar.

Mondal, T. K., Bhattacharya, T. K., Sood, A., Ahuja, P. S., Chand, P. K. 2002. Transgenic tea (*Camellia sinensis* (I.) O. Kuntze cv. Kangra Jat) plants obtained by *Agrobacterium*-mediated transformation of somatic embryos. Plant Cell Rep. 20, 712-720.

Moore, G. A., Jacono, C., Neidigh, J. L., Lawrence, S. D., Cline, K. 1992. *Agrobacterium*-mediated transformation of citrus stem segments and regeneration of transgenic plants. Plant Cell Rep. 11, 238-242.

Nam, J., Matthysse, A. G., Gelvin, S. B. 1997. Differences in susceptibility of Arabidopsis ecotypes to crown gall disease may result from a deficiency in T-DNA integration. Plant Cell 9, 317-333.

Opabode, J. T. 2006. *Agrobacterium*-mediated transformation of plants:emerging factors that influence efficiency. Biotech. Mol. Biol. Rev. 1, 12-20.

Padmanabhan P, Sahi SV (2009) Genetic transformation and regeneration of Sesbania *drummondii* using cotyledonary nodes. Plant Cell Rep 28: 31-40.

Parsons, T. J., Sinkar, V. P., Nester, E. W., Gordon, M. P. 1986. Transformation of poplar by *Agrobacterium tumefaciens*. *Biotech.* 4, 533-536.

Pena, L., Cervera, M., Juarez, J., Ortega, C., Pina, J. A., Duran-Vila, N., Navarro, L. 1995. High efficiency *Agrobacerium* mediated transformation and regeneration of *Citrus*. Plant Sci. 104, 183-191.

Rai, M. K., Asthana, P., Jaiswal, V. S., Jaiswal, U. 2010. Biotechnological advances in guava (*Psidium guajava* L.): recent developments and prospects for further research. Plant Cell rep. 24, 1-12.

Reuhs, B. L., Kim, J. S., Matthysse, A. G. 1997. Attachment of *Agrobacterium tumefaciens* to carrot cells and Arabidopsis wound sites is correlated with the presence of a cell-associated, acidic polysaccharide. J. Bact. 179, 5372-5379.

Ritchie, S. W., Lui, C. N., Sellmar, J. C., Kononowicz, H., Hodges, T. K., Gelvin, S. B. 1993. *Agrobacterium tumefaciens*-mediated expression of *gusA* in maize tissues. Transgenic Res. 2, 252-265.

Robinsons, K. E. P., Firoozabady, E. 1993. Transformation of floriculture crops. Sci. Hort. 55, 83-99.

Saito, K., Yamazaki, M., Shimomura, K., Yoshimatsu, K., Murakoshi, I. 1990. Genetic transformation of foxglove (*Digitalis purpurea*) by chimeric foreign genes and production of cardioactive glycosides. Plant cell rep. 9, 121-124.

Sandal, I. 2003. Developing transgenic tea (*Cammelia sinensis* (I) O. Kuntze) against biotic and abiotic stresses. Ph.D. thesis. Guru Nanak Dev University, Amritsar.

Sangwan, R. S., Ducrocq, C., Sangwan-Norrel, S. B. 1991. Effect of culture condition on *Agrobacterium*-mediated transformation in *Dutura*. Plant Cell Rep. 10, 90-93.

Sangwan, R.S., Ducrocq, C., Sangwan-Norreel, B. 1993. *Agrobacterium*-mediated transformation of pollen embryos in *Datura innoxia* and *Nicotiana tabacum*: production of transgenic haploid and fertile homozygous diploid plants. Plant Sci. 95, 99-115.

Seo, M. S., Bae, C. H., Choi, D. O., Rhim, S. L., Seo, S. C., Song, P. S., Lee, H. Y. 2002. Investigation of transformation efficiency of rice using *Agrobacterium tumefaciens* and high transformation of GPAT (glycerol-3-phosphate acyltransferase) gene relative to chilling tolerance. Kor. J. Plant Biotech. 29, 85-92.

Sheng, J., Citovsky, V. 1996. *Agrobacterium*-Plant cell DNA transport: Have virulence proteins will travel. The Plant Cell 8, 1609-1710.

Shimoda, N., Toyoda-Yamamoto, A., Nagamine, J., Usami, S., Katayama, M., Sakagami, N., Machida, Y. 1990. Control of expression of *Agrobacterium vir* genes by synergistic action of phenolic signal molecules and monosaccharides. Proc. Nat. Acad. Sci. USA 87, 6684-6688.

Smigocki, A. C., Hammerschlag, F. A. 1991. Regeneration of plants from peach embryo cells infected with a shooty mutant strain of *Agrobacterium*. J. Am. Soc. Hort. Sci. 116, 1092-1097.

Smith, R. H., Hood, E. E. 1995. *Agrobacterium tumefaciens* transformation of monocotyledons. Crop Sci. 35, 301-309.

Spencer, P. A., Tanaka, A., Towers, G. H. N. 1990. An *Agrobacterium* signal compound from grapevine cultivars. Phytochem. 29, 3785-3788.

Spencer, P. A., Towers, G. H. N. 1988. Specificity of signal compounds detected by *Agrobacterium tumefaciens*. Phytochem. 27, 2781-2785.

Stachel, S. E., Nester, E. W., Zambryski, P. C. 1986. A plant cell factor induces *Agrobacterium tumefaciens* vir gene expression. Proc. Nat. Acad. Sci. USA 83, 379-383.

Torregrosa, L., Iocco, P., Thomas, M. R. 2002. Influence of *Agrobacterium* Strain, culture medium, and cultivar on the transformation efficiency of *Vitis vinifera* L. Am. J. Enol. Vitic. 53, 183-190.

Tsi, C. J., Podila, K. J., Chiang, V. L. 1994. *Agrobacterium*-mediated transformation of quaking aspen (*Picea gluaca*) cultured *in vitro*. Plant Cell Rep. 14, 94-97.

Tzfira, T., Citovsky, V. 2006. *Agrobacterium*-mediated genetic transformation of plants: biology and biotechnology. Curr. Opi. Biotech. 17, 147-154.

Tzfira, T., Li, J., Lacroix, B., Citovsky, V. 2004. *Agrobacterium* T-DNA integration:molecules and models.Trends in Genet. 20, 375-383.

Veluthambi, K., Gupta, A. K., Sharma, A. 2003. The current status of plant transformation technologies. Curr Sci. 84, 368-380.

Vogel, A. M., Das, A. 1992. Mutational analysis of *Agrobacterium tumefaciens* virD2: tyrosine 29 is essential for endonuclease activity. J. Bact. 174, 303-308.

Vuylasteker, C., Dewaele, S., Rambour, S. 1998. Auxin induced lateral root formation in chicory. Ann Bot. 81, 449-454.

Walden, R., Wingender, R. 1995. Gene-transfer and plant regeneration techniques. Trends Biotech. 13, 324-331.

Webb, K. J., Morris, .P 1992. Methodologies of plant transformation. In: Gatehouse AMR, Hilder VA, Boulter D (eds) Plant Genetic Manipulation for Crop Protection. CAB Int, Wallingford, Oxon, UK, 7-43.

Wei, I., Guangqin, G., Guochang, Z. 2000. *Agrobacterium*-mediated transformation: state of the art and future prospect. Ch. Sci. Bull.

45, 1537-1546.

Wilmink, A., Van de van, B. C. E., Dons, H. J. M. 1995. Activity of constitutive promoters in various species from the Liliaceae. Plant Mol. Biol. 28, 949-955.

Wilson, P. J., Van Staden, J. 1990. Rhizocaline rooting cofactors and the concept of promotors and inhibitors of adventitious rooting-A review. Ann. Bot. 66, 479-490.

Xu, J., Wang, Y. Z., Yin, H. X. 2009. Efficient *Agrobacterium tumefaciens*-mediated transformation of *Malus zumi* (Matsumura) Rehd using leaf explant regeneration system. Elect. J. Biotech. 12, 1-8.

Zambre, M., Terryn, N., Clercq, J. D., Buck, S. D., Dillen, W., Montagu, M. V., Straeten, D., Angenon, G. 2003. Light strongly promotes gene transfer from *Agrobacterium tumefaciens* to plant cells. Planta 216, 580-586.

Zambryski, P. C. 1992. Chronicles from the *Agrobacterium*-plant cell DNA transfer story, Ann. Rev. Plant Physiol. Plant Mol. Biol. 43, 465-470.

Zhang, J., Xu, R., Elliott, M. C. 1987. *Agrobacterium*-mediated transformation of elite indica and japonica rice cultivars. Mol Biotech. 3, 223-226.

Zupan, J. R., Muth, T. R., Draper, O., Zambryski, P. 2000. The transfer of DNA from *Agrobacterium tumefaciens* into plants a feast of fundamental insight. The Plant J. 23, 11-28.

(Received November 4, 2009; Accepted December 26, 2009)