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REVIEW

Agrobacterium-mediated genetic transformation of plants: the role of host

O. KARAMI[1]*, M. ESNA-ASHARI[2], G. KARIMI KURDISTANI[3] and B. AGHAVAISI[1]

Department of Biotechnology[1]*, and Department of Horticulture*[2]*, Bu-Ali Sina University,*
6517833131 Hamadan, Iran
Young Researchers Clop, Islamic Azad University, 256325756 Sanandaj, Iran[3]

Abstract

Agrobacterium-mediated genetic transformation is the most widely used technology to obtain overexpression of recombinant proteins in plants. Molecular events that occur within *Agrobacterium* during interactions with host plants have been studied extensively, and now we have a reasonable understanding the key factors involved in the regulation of T-DNA nuclear import and genomic integration. By contrast, very little is known about the events that take place in the host cells during genetic transformation by *Agrobacterium*. Here, we describe the plant-related factors including genotype, genes, proteins, competency of target tissues and phenolic compounds that participate in *Agrobacterium*-mediated genetic transformation and discuss their possible roles in this process. Because *Agrobacterium* probably adapts existing cellular processes for its life cycle, identifying the processes in host cells during *Agrobacterium* infection might contribute to better understanding of basic biological processes as cell communication, intracellular transport and DNA repair and recombination as well as to expanding the host range of *Agrobacterium* as a genetic engineering tool.

Additional key words: T-DNA, *vir* gene, VIR protein.

Introduction

The ability of *Agrobacterium* to genetically transform a wide variety of plant species has earned it a place of honour in basic plant research and modern plant biotechnology. Transformation results from the production of a single-stranded copy (T-strand) of transferred DNA (T-DNA) molecule by the bacterial virulence machinery, its transfer into the host cell followed by integration into the host genome (for recent reviews, see Gelvin 2003, McCullen and Binns 2006). *Agrobacterium*-mediated genetic transformation is a multi-step process which begins with recognition and sensing a wounded host cell by a virulent *Agrobacterium* and ends with the expression of its T-DNA integrated in the transformed cell's genome. It has been previously reviewed that *Agrobacterium* deploys a large number of proteins and uses several molecular machines to initiate and execute early steps of the transformation process (Gelvin 2003, Christie *et al*. 2005, McCullen and Binns 2006). Briefly, proteins encoded by the bacterial chromosomal virulence and tumour-inducing plasmid virulence genes (*vir*) mediate recognition of and attachment to the host cell as well as production of a mobile T-strand-protein complex (T-complex) and its export into it. Once inside the host cell cytoplasm, several VIR proteins and host factors act together to deliver the T-complex into the host cell nucleus to integrate into its genome.

Both *Agrobacterium*-based systems and direct gene transfer *via* microprojectile bombardment have successfully been used in genetic transformation of plants. Although the method of introducing DNA into cells by

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Abbreviations: AS - acetosyringone; BA - benzyladenine; 2,4-D - 2,4-dichlorophenoxyacetic acid; GUS - β-glucuronidase; HR - homologous recombination; MAPK - mitogen-activated protein kinase; MDIBOA - 2-hydroxy-4,7-dimethoxybenzoxazin; miRNA - microRNA; NHEJ - non-homologues end-joining; NHR - non-homologous recombination; NLS - nuclear localization signal; PAMP - pathogen associated molecular patterns; PRR - pattern recognition receptors; SA - salicylic acid; SAR - systemic acquired resistance; SCF - Skp1-cullin-F-box; T-complex - T-strand-protein complex; T-DNA - transferred DNA; VIR - virulence.

* Corresponding author; fax: (+91) 83163372, e-mail: hiva@basu.ac.ir

microprojectile bombardment has revolutionized the field of genetic transformation of crop plants, a major drawback of this system is the considerable variation seen in stability, integration, and expression of the introduced transgene. *Agrobacterium*-mediated transformation system, on the other hand, facilitates the precise integration of a small number of genes into the plant genome and shows a greater degree of transgene stability (Komari and Kubo 1999, Shou *et al*. 2004).

This system is influenced by a number of factors such as bacterial strains, plasmids, tissue culture environment, media for explant culture, co-cultivation duration, acetosyringone (AS), explant wounding, selective marker and vector and competency of target plant tissues for infection (Li *et al*. 1997, Salas *et al*. 2001, Zambre *et al*. 2003, Yu *et al*. 2002, Olhoft *et al*. 2003, Wu *et al*. 2003, Cheng *et al*. 2004, Bříza *et al*. 2008, Cho *et al*. 2008). Unlike the role of bacterial factors, the host factors in transformation process has remained obscure for nearly a century, and only recently have we begun to understand how *Agrobacterium* hijacks host factors and cellular processes during transformation process (Tzfira *et al*. 2002, 2004, 2006, Lacroix *et al*. 2005, Tao *et al*. 2004, Zhu *et al*. 2003, Roberts *et al*. 2003, Ditt *et al*. 2006, Anand *et al*. 2007, Loyter *et al*. 2005, Baek and Shapleigh 2005). Identification of such factors and studies of these processes hold great promise for the future of plant biotechnology and genetic engineering, as they might help developing conceptually new techniques and approaches needed today to expand the host range of *Agrobacterium* and to control the transformation process and its outcome through the production of transgenic plants. In this review, we focus on plant cell factors that participate in *Agrobacterium*-mediated genetic transformation and discuss their possible roles in the process.

Plant species and genotype

Wild types of *Agrobacterium* species are known as the causative agents of the ‘crown gall’ disease in a rather limited number of economically important plant species (Burr *et al*. 1998), while *Agrobacterium* under laboratory conditions, can transform other eukaryotic species, ranging from fungi to human cells (Lacroix *et al*. 2006a), which holds great promise for the future of biotechnology. Currently, *Agrobacterium*-mediated transformation is extensively used to produce transgenic plants in both dicotyledons and monocotyledons including the major economic crops, vegetables, ornamental, medicinal and pasture plants.

Various plant species differ greatly in their susceptibility to *Agrobacterium* infection (Anderson and Moore 1979, De Cleene and DeLey 1976, Porter1991, Cheng *et al*. 2004). Even within a species, different cultivars or ecotypes may show different degrees of susceptibility to tumorigenesis by particular *Agrobacterium* strains. These differences have been noted in maize (Ritchie *et al*. 1993), various legumes (Hood *et al*. 1987, Owens and Cress 1984), aspen (Beneddra *et al*. 1996), pine (Bergmann and Stomp 1992), tomato (Van Roekel *et al*. 1993), *Arabidopsis* (Nam *et al*. 1997) and grape (Lowe and Krul 1991). The ability of particular *Agrobacterium* strains to transform plant cells is defined by mechanisms necessary for attachment and DNA-transfer, and the ability of plants to produce different inducer molecules. The differences in the *vir* gene expression in different hosts affect their sensitivity to *Agrobacterium* infection. Low level of *vir* gene expression can make a plant recalcitrant by virtue of the inability of the bacterium to synthesize and transfer sufficient T-strand DNA essential for a successful infection.

Agrobacterium-mediated transformation of higher plants is now well-established for dicotyledonous species. In recent years, the frequency of gene transfer to monocotyledonous species has also been greatly improved. Successful *Agrobacterium*-mediated transformation has been reported in rice (Hiei *et al*. 1994, Hiei *et al*. 1997, Kant *et al*. 2007, Toki *et al*. 1997), maize (Ishida *et al*. 1996), barley (Tingay *et al*. 1997, Shrawat *et al*. 2007), wheat (Cheng *et al*. 1997) and sorghum (Zhao *et al*. 2000, Carlos *et al*. 2004, Carvalho *et al*. 2004). However, the difference in the competence of *Agrobacterium* to infect genotypes or species has been a major drawback in the genetic transformation of elite monocotyledonous cultivars, especially in extending the host range to commercial cultivated plants. Amongst cereals, rice appears to be the least genotype dependent, as more than 40 genotypes of Japonica, Indica and Javonica rice have been transformed so far. While in other major cereals, only a few model genotypes have successfully been used in the *Agrobacterium*-mediated transformation. For example, maize cv. A188 or its hybrids, wheat cv. Bobwhite, barley cvs. Golden Promise and Igri and sugarcane cv. Ja 60-5 could be mentioned. Although transgenic plants have recently been recovered from elite cultivars or lines of sorghum (Zhao *et al*. 2000), maize (Gordon-Kamm *et al*. 2002) and barley (Wang *et al*. 2001), the overall transformation frequency is lower than that with model cultivars. Therefore, it becomes important to make elite cultivars amenable to tissue culture and to improve their regenerability by manipulating existing tissue culture system.

The difference in the susceptibility of genotypes to *Agrobacterium* could be due to the presence of an inhibition system in *Agrobacterium* sensory machinery. Inhibitors like 2-hydroxy-4,7-dimethoxybenzoxazin (MDIBOA), which is the major organic exudate of maize seedling roots, specifically inhibits induction of *vir* gene expression through unknown mechanism (Zhang *et al*. 2000, Maresh *et al*. 2006). It has also been shown that the ability of *Agrobacterium* strains to infect recalcitrant plants was determined by the *virA* locus (Heath *et al*. 1997). The relative difference in the resistance of agronomically important plant species to *Agrobacterium*-mediated genetic transformation may be due to the presence of such inhibitors, rather than insufficient activation of the *Agrobacterium* virulence machinery by host cell exudates. It seems that naturally occurring inhibitors directed against signal perception by the *virA/virG* two-component regulatory system play an important role in host defence (Zhang *et al*. 2000). MDIBOA is not the only natural inhibitor of *vir* gene induction because indole-3-acetic acid has also shown to inhibit *vir* gene induction (Liu and Nester 2006).

Plants perceive *Agrobacterium* and the transferred transgenes as foreign invaders and use their defence systems to battle the infection process and expression of foreign genes. Plant defence could interfere at any step of *Agrobacterium*-mediated transformation, starting with attachment of bacteria to the plant cell and ending with gene expression and stable integration of T-DNA into the plant genome. Recent studies show that *Agrobacterium* interferes with plant defence gene expression (Veena *et al*. 2003, Ditt *et al*. 2005) and that the *Arabidopsis cep1* mutant, which constitutively expresses defence-related genes, is more resistant to *Agrobacterium* infection (Zhu *et al*. 2003). Recognition of microbes in higher eukaryotes depends on an array of recognition receptors (PRRs). These PRRs recognize characteristic molecular structures shared by large groups of microbes, the so-called pathogen associated molecular patterns (PAMPs). PAMPs play key roles as activators of the innate immune response in animals (Akira and Takeda 2004) and, analogously, as elicitors of defence responses in plants (Nürnberger *et al*. 2004). Recently, it was demonstrated that the application of PAMPs such as flagellin and EF-Tu can activate plant immunity (Zipfel and Felix 2005, Ingle *et al*. 2006, Jones and Dangl 2006). PAMP perception probably reduces the *Agrobacterium* induced genetic transformation, because an *Arabidopsis* mutant in the *efr* gene, which encodes a receptor kinase essential for perception of the bacterial EF-Tu PAMP, was super-susceptible to transformation (Zipfel *et al*. 2006).

In contrast, the systemic acquired resistance response of the host plant is likely inhibited by *Agrobacterium* infection based on the observations that infected *Arabidopsis* plants exhibited reduction in salicylic acid (SA) accumulation and *pr1* and *pr5* gene expression, which were even lower than their already low levels in healthy plants (Gaspar *et al*. 2004). These *Agrobacterium* effects on systemic acquired resistance (SAR) may involve the host lysine-rich arabinogalactan protein AtAGP17, because a mutant in the *atagp17* gene (*rat1*) retained its pathogenesis-related (PR)1 and PR5 protein expression levels in the presence of *Agrobacterium* and became resistant to the infection (Durrant and Dong 2004, Gaspar *et al*. 2004). SA is predominantly associated with resistance against plant pathogens, and triggering SAR (Grant and Lamb 2006). Anand *et al*. (2007) have shown that, SA reduced ‘crown gall’ disease caused by *A. tumefaciens* in *Nicotiana benthamiana*. SA also shares structural similarity with other natural inhibitors of *vir* gene induction, such as indole-3-acetic acid (Liu and Nester 2006). *Agrobacterium* has evolved to counteract the RNA silencing response of the host. Intriguingly, the interrelationship between *Agrobacterium* infection and RNA silencing is very complex, because on one hand, the development of *Agrobacterium*-induced tumours requires suppression of RNA silencing mediated by short interfering RNAs, and on the other hand, it mandates a functional microRNA (miRNA)-mediated silencing, as miRNA-deficient plants are almost immune to infection (Dunoyer *et al*. 2006).

Plant species may differ in their temporal competence for transformation following wounding. Braun (1947) was the first who noted this window of competence in *Vinca rosea*. Bacteria were applied to cut plant surfaces various times after wounding. When the plants were inoculated within 3 d of wounding, tumor induction was relatively efficient. Inoculation 4 d after wounding resulted in only a few percent of the plants developing tumors, and after 5 d, tumorigenesis was absent. However, tomato remained susceptible to tumorigenesis up to two weeks after wounding (Braun 1954). Davis *et al*. (1991) showed that 6 d after wounding tomato plants still retained approximately 25 % of the susceptibility in comparison to the plants that inoculated immediately after wounding. However, susceptibility could be increased at later times by the addition of SA (Stachel *et al*. 1985), but SA-treated plants never achieved susceptibility equal to untreated ones inoculated directly after wounding. Davis *et al*. (1991) also stated that, although suberinization of the cell walls, which may present a physical barrier to transformation, occurred 4 d after wounding, suberinized cells still retained high transformation susceptibility. The authors thus concluded that factors additional to suberinization must play a role in temporal competence for transformation.

Competency of target plant tissues and cells

Most strategies utilizing *Agrobacterium*-mediated gene delivery are performed under *in vitro* conditions, and require plant tissues competent for transformation as well as a tissue culture system for regenerating entire plants. A variety of explants could be used as target material for *Agrobacterium*-mediated transformation. These include embryonic cultures, immature embryos, mature seed-derived calli, meristems, shoot apices, excised leaf blades, roots, cotyledons, stem segments and callus suspension cultures. The type of explant is very important as it must be suitable for regeneration allowing the recovery of whole transgenic plants. In fact, it is the totipotency of plant cells that underlies most plant transformation systems.

Several investigators have shown that, various tissues, organs, and cell types within a plant may differ in their susceptibility to *Agrobacterium* transformation (Repellin *et al*. 2001, Zhang *et al*. 2008). Ritchie *et al*. (1993) showed that in maize transformation occurred in mesocotyl segments originating from the intercalary meristem region. Cells showing transient expression exhibited a tendency for preferential location on the scutellum side near the place of embryo axis connection, which was also observed by McCormac *et al*. (1998) in intact barley embryos. In sorghum, source of the explant had also a significant effect on the transformation rate (Zhao *et al*. 2000) and immature embryos from field-grown plants showed a higher transformation frequency than immature embryos from glasshouse-grown plants. Schlappi and Hohn (1992) demonstrated only embryos in which the shoot apical meristem had begun to differentiate showed competence, and the timing of this window differed among the three maize cultivars examined. De Kathen and Jacobsen (1995) showed that, only dedifferentiating cells near the vascular system of cotyledon and epicotyl regions of *Pisum sativum* were susceptible to *Agrobacterium* transformation. In cotyledon and leaf tissues of *Arabidopsis*, only dedifferentiating mesophyll cells were competent for transformation. In root tissue, competent cells were found in dedifferentiating pericycle. These cells were small, isodiametric, and had prominent nuclei and dense cytoplasm (Sangwan *et al*. 1992). Embryogenic callus derived from mature seeds has been reported to be the best tissue for *Agrobacterium*-mediated transformation in some plant species (Hiei *et al*. 1994, Cheng *et al*. 1997, 2003).

Variation in response of plant tissues to *Agrobacterium* has been attributed in part to differences in the ability of this bacterium to attach the plant cells or to differences in T-DNA transfer machinery (Lippincott *et al*. 1977, Nam *et al*. 1997, Yanofsky *et al*. 1985). It is generally accepted that only plants with an appropriate wound response will develop large populations of wound-adjacent cells that are competent for transformation (Potrykus 1991). However, more significantly, cell death is observed in cultures of many plant tissues following exposure to *Agrobacterium*. Modification of transformation parameters can increase the probability of stably transforming some recalcitrant cell types. However, cell death following *Agrobacterium* infection still remains a significant limitation (Gelvin 2003). Tissue browning and necrosis following exposure to *Agrobacterium* occurs in many monocot and dicot plants, including poplar (De Block 1990), grape (Perl *et al*. 1996, Pu and Goodman 1992), sorghum (Carvalho *et al*. 2004, Gao *et al*. 2005), wheat (Parrott *et al*. 2002), tomato, pepper and lettuce (Van der Hoorn *et al*. 2000; Wroblewski *et al*. 2005). *Agrobacterium* transformation triggers expression of many genes in the host cell, including components of plant defence machinery (Ditt *et al*. 2001, Veena *et al*. 2003). On pathogen infection, one of the earliest defence mechanisms activated is the production of reactive oxygen species, responsible for activating programmed cell death (Parrott *et al*. 2002). Co-cultivation of *Agrobacterium* with maize or wheat tissues has been resulted in a process closely analogous to apoptosis in animal cells (Hansen 2000). Parrott *et al*. (2002) reported that, after *Agrobacterium* infection, wheat embryos and root cells rapidly produced hydrogen peroxide, displayed as alteration in cell wall composition and resulted in cellular necrosis and subsequent cell death. A correlation between the reduction in cell death and the improvement of transformation frequency has been demonstrated in rice (Enríquez-Obregón *et al*. 1998), sugarcane (Enríquez-Obregón *et al*. 1997), sorghum (Zhao *et al*. 2000) and maize (Ishida *et al*. 1996). Parrott *et al*. (2002) also observed that, lowering the H_2O_2 content significantly reduced the extent of embryo and root cell death in wheat after *Agrobacterium* transformation. It has also been found that *Agrobacterium*-induced necrosis observed in *Poaceae* can be alleviated by the use of necrosis inhibiting agents, such as silver nitrate (Hansen and Durham 2000). Anti-necrotic treatment of the target tissues may result in increasing transformation efficiency (Enríquez- Obregón *et al*. 1997). One report indicated that maize callus infected with *Agrobacterium* undergoes a rapid hypersensitive reaction, and this response was suppressed by expression of two baculovirus genes, *p35* and *iap* (Hansen 2000). However, tissue browning and necrosis after *Agrobacterium* infection are still major obstacles in genetic transformation of plants.

Competence for transformation may either be absent or low in recalcitrant explants, however, it can be enhanced by phytohormone treatments (Valvekens *et al*. 1988, Sangwan *et al*. 1992, Geier and Sangwan 1996, Villemont *et al*. 1997). An explant becomes susceptible to *Agrobacterium* when it is precultured on medium containing phytohormones (Potrykus 1990, Valvekens *et al*. 1988, Sangwan *et al*. 1992, Chateau *et al*. 2000, Saini, and Jaiwal 2007). The choice of growth regulator was the most important factor affecting transformation efficiency measured as frequency of transient expression and stable integration. In many monocots, 2,4-dichlorophenoxyacetic acid (2,4-D)-derived calli and the presence of it in the co-cultivation medium enhances transformation efficiency (Rashid *et al*. 1996, Wu *et al*. 2003). In *Typha latifolia*, a significantly higher percentage of calli has generated using picloram and showed transient β-glucuronidase (GUS) activity (Nandakumar *et al*. 2004). In barley, dicamba in the callus induction and maintenance media was generally promoting transient expression and subsequent stable transformation (Trifonova *et al*. 2001). In kenaf, pre-culturing the explants for 2 d in benzyladenine (BA) containing medium, was found to enhance the transient GUS expression (Herath *et al*. 2005).

Phytohormone treatment activates cell division and dedifferentiation in many tissues. The stimulation of cell division by phytohormones suggests that, efficient *Agrobacterium* transformation may occur at a particular stage of the plant cell cycle (Chateau *et al*. 2000). Villemont *et al*. (1997) investigated the role of plant cell cycle in *Agrobacterium*-mediated transformation of *Petunia* mesophyll cells. Cycling cells with no phytohormone treatments, could not be transformed either transiently or stably to express a T-DNA-encoded *gusA* transgene. Similarly cells treated with mimosine, which blocks the cell cycle in late G1 phase, could not be transformed. In addition, the cycling cells that showed the highest transformation competence were those that showed a very high S and G2 phase/M phase ratio. The authors concluded that T-DNA could be taken up, translocated to the nucleus, and expressed in the cells conducting DNA synthesis but in the absence of cell division, and thus that *Agrobacterium*-mediated transient transformation requires DNA synthesis corresponding to S phase. Subsequent cell division was necessary for T-DNA integration and stabilization of transformation. Development of a direct assay for T-strand uptake and nuclear translocation that does not depend upon T-DNA-encoded gene expression is required to resolve these two alternatives.

A significant factor that enhances transformation of crop species is dessication of explants prior to, or post, *Agrobacterium* infection. Arencibia *et al*. (1998) reported that, air-drying sugarcane suspension cells prior to inoculation slightly improved T-DNA delivery and subsequently increased transformation efficiency. Similarly, air-drying calli derived from rice suspension cultures showed the transformation efficiency 10-fold or more as compared to the control without air-drying (Urushibara *et al*. 2001). Cheng *et al*. (2003) reported that desiccation of pre-cultured immature embryos, suspension culture cells, embryonic calli of wheat, and embryogenic calli of maize greatly enhanced T-DNA delivery and plant tissue recovery after co-culture, leading to increased stable transformation frequency. This treatment was not only effective in monocot species, but also improved T-DNA delivery in recalcitrant dicot species such as soybean suspension cells (Cheng and Fry 2000). Although the molecular mechanism of desiccation during co-culture remains unclear, it is known that desiccation suppresses the growth of *Agrobacterium*.

Phenolic compounds

In *Agrobacterium*-mediated transformation systems, physical wounding of explants is commonly done as this greatly influences transformation efficiency (Rashid *et al*. 1996). Not only does the wound site act as an entry point for bacterium but also results in the release of phenolic substances necessary for *vir* gene activation (Joubert *et al*. 2002). Phenolic compounds, like SA, released by wounded cells have been found to be essential for induction of the virulence genes. *A. tumefaciens* virulence genes are induced by plant signals through the VIRA-VIRG two-component regulatory system. The VIRA protein is activated by the signals like acidic pH, phenolic compounds, and some monosaccharides (Winans *et al*. 1988, Doty *et al*. 1996). Activated VIRA in turn promotes the phosphorylation of the response regulator VIRG protein leading to the upregulation of all *vir* promoters (Jin *et al*. 1990). The expression induction of *vir* genes in response to host-released phenolic compounds has been identified and reviewed (Spencer *et al*. 1990, Johnson *et al*. 1998). The increase of transformation efficiency, based on the application of additional SA, have also been reported in apple (James *et al*. 1993), rice (Aldemita and Hodges 1996), soybean (Santarem *et al*. 1998), cotton (Sunilkumar and Rathore 2001) and barley (Kumlehn *et al*. 2006). However, SA has not enhanced the transformation efficiency in plum (Mannie *et al*. 1991), poplar (Confalonieri *et al*. 1997), and tea (Mondal *et al*. 2001). It was also reported that, SA either increases or decreases transformation in pea (Duval and Shetty 2001, Nadolska-Orczyk and Orczyk 2000).

In monocots, where such compounds are not synthesized, addition of SA during plant-bacteria interaction supports the gene transfer (Usami *et al*. 1987, Wu *et al*. 2003, Cheng *et al*. 1997, Hiei *et al*. 1997, Kumlehn *et al*. 2006). Although SA has not been an essential for *Agrobacterium*-mediated transformation in barley (Tingay *et al*. 1997, Fang *et al*. 2002, Shrawat *et al*. 2007), the omission of SA led to transformation events failure in several other monocots (Ishida *et al*. 1996, Rashid *et al*. 1996, Nandakumar *et al*. 2004). Difference in SA requirement for successful transformation of cereals may be due to the difference in the inoculation and co-cultivation duration and also in the competence of target tissues. However, some explants of monocot species could be efficiently transformed without the aid of external *vir* inducing chemicals. For example, meristematic sections of sugarcane pre-treated with an antinecrotic mixture (Enriquez-Obregon *et al*. 1999), and pre-cultured immature embryos and embryogenic calli of wheat co-cultured under desiccation conditions could efficiently be transformed (Cheng *et al*. 2003).

Turk *et al*. (1991) indicated that, the optimal induction of the *vir* genes in pTiC58 and pTib6 require SA at pH 5.8 and 5.3. Fortin *et al*. (1992) reported that 60 μM SA inhibited the growth of T37 and C58 strains, at pH 5.8, but the growth of other strains was not affected. Therefore, the optimum concentration of SA for transformation is also a function of the *A. tumefaciens* strain used and the pH during co-cultivation.

Genes and proteins

Identification and molecular characterization of the plant genes involved in successful *Agrobacterium*-mediated transformation have opened up new avenues for better understanding of the plant response to *Agrobacterium* infection. However, little is known about the role of plant genes and their products in the transformation process.

Agrobacterium utilizes specific receptors on the host cell wall. Two *Arabidopsis* ecotypes, Bl-1 and Petergof, and two T-DNA-insertion mutants of the WT ecotype, designated *rat1* and *rat3* (resistant to *Agrobacterium*), are deficient in *Agrobacterium* binding to their root explants (Nam *et al*. 1997, 1999). While the specific genes responsible for the reduction of in *Agrobacterium* binding in Bl-1 and Peterg have still remained unknown, the *rat1* and *rat3* mutations were found to affect an arabinogalactan and other potential cell-wall proteins, respectively. Vitronectins are family of proteins utilized as specific receptors by different pathogenic bacteria in mammalian cells (Paulsson and Wadstrom 1990). Attachment of *Agrobacterium* cells to plant tissues could be inhibited by human vitronectin antibodies and *Agrobacterium* mutants, which are defective in their attachment ability to plant cells, showed a reduction in binding to vitronectin, so plant vitronectin-like molecules have been suggested to play a role in *Agrobacterium* attachment to its host cells (Wagner and Matthysse 1992). Another host factor, a cellulose synthase-like protein CSLA9, might ber involved in *Agrobacterium* attachment. Disruption of the *csla9* gene in *Arabidopsis* plants resulted in a limited reduction in *Agrobacterium* attachment to inoculated roots (Zhu *et al*. 2003a). Hwang and Gelvin (2004) have recently identified three *Arabidopsis* proteins that interact with the main T-pilus protein, VirB2. These proteins include VirB2 interactors BTI1, BTI2 and BTI3 with unknown functions, and a membrane associated GTPase and AtRAB8 (Hwang and Gelvin 2004). The presence of these proteins has been required for an efficient transformation.

AtKAP-A belongs to a family of proteins named as importins, which are known to be involved in nuclear translocation of proteins containing nuclear localization signal (NLS) sequences (Ballas *et al.* 1997). The ability of AtKAP-A to stimulate the nuclear import of VirD2 in immobilized yeast cells, the interaction of VirD2 with three other members of the *Arabidopsis* importin family (Bakó *et al*. 2003), and the observation that an *Arabidopsis* mutant in one of the importin *a* genes is resistant to *Agrobacterium* infection (Zhu *et al*. 2003b) further support the idea that *Agrobacterium* utilizes the importin *a*-dependent nuclear import machinery of the host cell for nuclear uptake of the invading T-complex.

Increased plant susceptibility to *Agrobacterium* infection by over-expression of the *Arabidopsis* nuclear protein VIP1 was demonstrated by Tzfira *et al*. (2002). Their results indicated that VIP1, which specifically interacts with VirE2 (Tzfira *et al*. 2001), VIP1 and VIP1-VirE2 complexes, accumulates in the nucleus in living plant cells (Tzfira *et al*. 2001, 2004a, Lacroix *et al*. 2005, Li *et al*. 2005). Djamei *et al*. (2007) shown that, VIP1 is a transcription factor which is a direct target of the *Agrobacterium*-induced mitogen-activated protein kinase (MAPK) MPK3. Upon phosphorylation by MPK3, VIP1 delocalizes from cytoplasm to nucleus to regulate expression of the pathogenesis-related gene *pr1*. MAPK-dependent phosphorylation of VIP1 is necessary for VIP1-mediated *Agrobacterium* T-DNA transfer, indicating that *Agrobacterium* abuses the MAPK-targeted VIP1 defence signalling pathway for nuclear delivery of the T-DNA complex.

Once inside the nucleus, the T-strand must be delivered to site of its future integration in the host chromatin. While the exact sequence of events that mediate this intranuclear transport and chromatin targeting is still unknown, various plant factors and several molecular mechanisms have been implicated in these concluding steps of the transformation process. Specifically, CAK2M and TATA box-binding protein, VIP1 binding VirE2 (Tzfira *et al*. 2001) and core histones binding VIP1 (Li *et al*. 2005, Loyter *et al*. 2005) may function in chromatin targeting of the T-complex.

At least partial uncoating the T-DNA from its escorting proteins is necessary for exposing the T-strand to the host DNA repair machinery which will complement it to the double-stranded form and integrate the latter into the host genome. Potentially, this is achieved by the targeted proteolysis machinery of the host cell. The first indication of targeted proteolysis involvement in the transformation process came from the studies of VirF, a bacterial host range factor (Regensburg-Tuink and Hooykaas 1993) exported into the host cell (Vergunst *et al*. 2000). Tzfira *et al*. (2004) have shown that bacterial VirF, which localizes to the plant cell nucleus along with the T-DNA complex, is an F-box protein that interacts with VIP1 and destabilizes both VIP1 and VirE2, presumably by targeting them for proteasomal degradation. Tzfira *et al.* (2004) found that, VirF does not interact with or destabilize VirD2, which suggests that VirD2 may remain bound to the T-DNA until a later stage. There is some evidence showing that, VirD2 plays an additional role in guiding the efficiency or precision of genomic integration (Tinland *et al*. 2000). VirF also interacts with the *Arabidopsis* SKP1 homolog ASK1 and thus may form a Skp1-cullin-F-box (SCF) complex with ASK1 (Schrammeijer *et al*. 2001). Later studies identified VIP1 as one of the cellular substrates for VirF and demon-strated that, VirF destabilizes VIP1 and its cognate VirE2 when co-expressed in yeast cells or in plant, and that, in yeast, this destabilization requires the presence of Skp1 (Tzfira *et al*. 2004a). Because VirE2 represents the major protein component of the T-complex, its targeted proteolysis by the SCFVirF complexes may represent a mechanism for T-DNA uncoating prior to or during its integration into the host genome (Tzfira *et al*. 2004a). Consistent with this hypothesis, both VirF and ASK1 localize to the plant cell nucleus, the cellular compartment in which the T-DNA uncoating is expected to occur; furthermore, early T-DNA expression was specifically inhibited by a proteasomal inhibitor (Tzfira *et al*. 2004a).

T-DNA integration is the last and perhaps the most host dependent step of the transformation process (Tzfira *et al*. 2004b). Host factors are required for complementation of the T-strand molecule to double stranded DNA, for production of DNA breaks in the host genome and for ligation of the T-DNA molecule into these breaks. The *Arabidopsis* histone H2A, which displays higher expression levels in tissues more susceptible to *Agrobacterium* infection (Yi *et al*. 2002), is essential for T-DNA integration in somatic cells (Mysore *et al*. 2000). Thus, in addition to its role in directing the T-complex molecules to the integration site (Li *et al*. 2005, Loyter *et al*. 2005), H2A may be involved in relaxing the host DNA structure (Mysore *et al*. 2000). *Agrobacterium* infection of yeast mutants in specific DNA repair genes allowed identification of *ku70*, *rad50*, *mre11*, *xrs2*, *lig4* and *sir4* as key proteins in T-DNA integration *via* non-homologous (illegitimate) recombi-nation (NHR) pathway (Van Attikum and Hooykaas 2003), and demonstrated that, *rad51* and *rad52*, but not *rad50*, *mre11*, *xrs2*, *lig4* or *ku70* are essential for T-DNA integration by homologous recombination (HR) (Van Attikum *et al*. 2001). Furthermore, *ku*70 (Van Attikum *et al*. 2001) and *rad52* (Van Attikum and Hooykaas, 2003) were found to be the key determinants for T-DNA integration *via* HR or NHR respectively, and double mutation of the *ku70* and *rad52* genes resulted in complete blockage of T-DNA integration (Van Attikum and Hooykaas 2003). Another yeast DNA repair protein, *rad54*, promoted a high-frequency gene targeting in transgenic plants (Shaked *et al*. 2005). In plant cells, T-DNA integration occurs mainly through NHR, even when the T-DNA shares high homology with the host genome, indicating that *Agrobacterium* may be exclusively using the host non-homologues end-joining (NHEJ) DNA repair machinery during the integration step. In fact, the critical role of KU80, a key participant of NHEJ which usually functions in a complex with KU70 and DNA protein kinase (Tzfira *et al*. 2004b, Lacroix *et al*. 2006b), during T-DNA integration in *Arabidopsis* somatic tissues was recently revealed by the observations that *Arabidopsis* insertional mutants in the *ku80* gene are defective in T-DNA integration in somatic cells, and that complexes between KU80 and double-stranded T-DNA molecules can be immuno-precipitated from *Agrobacterium*-infected plants (Li *et al*. 2005b). The role of KU80 during transformation of germ-line cells, however, is less clear as it has been reported to be both required (Friesner and Britt 2003) and dispensable (Gallego *et al*. 2003) for T-DNA integration. Similarly, the role of the *Arabidopsis* LIG4 ligase, another NHEJ participant, in the transformation process remains controversial; LIG4 was dispensable for T-DNA integration in somatic *Arabidopsis* cells (Van Attikum *et al*. 2003), but it was essential for T-DNA integration in germ-line cells (Friesner and Britt 2003).

Although the knowledge of plant-inducing changes in bacterial gene expression has been crucial to our understanding this interaction, much less is known about overall changes in gene expression in the host. *Agrobacterium* infection triggers changes in the gene expression pattern of host cells, inducing or repressing specific sets of plant genes (Ditt *et al*. 2001, Veena *et al*. 2003). Ditt *et al*. (2001) found the altered expression of a number of plant transcripts within host cells after 24 and 48 h of interaction with *Agrobacterium*. They also showed that, proteins encoded by these genes had a putative role in plant signal transduction and the defence response. Using suppressive subtractive hybridization and DNA microarrays, Veena *et al*. (2003) identified numerous plant genes that were differentially expressed during the early stages of *Agrobacterium*-mediated transformation. The genes identified in their study included those involved in defence responses, cell division and growth, primary and secondary metabolism and chaperones. The majority of these genes showed expression induction during the early stages of infection with various strains of *Agrobacterium*. This study also demonstrated the involvement of T-DNA and/or VIR proteins as factors that resulted in the differential expression of these genes during *Agrobacterium* infection. However, in comparison with Ditt *et al*. (2001), who investigated infection by transfer-competent *Agrobacterium* strain lasting for 24 and 48 h, Veena *et al*. (2003) showed that, the expression of defence response genes was significantly decreased during the later stages of infection. Interestingly, cells infected with transfer-deficient *Agrobacterium* strains showed significant re-induction of these genes during the later stages of transformation. These results suggest that, the suppression of the host defence response is a prerequisite to successful plant transformation. Recently, a number of plant genes that were expressed during different stages of *Arabidopsis*-*Agrobacterium* interactions have also been identified using oligonucleotide microarray (Ditt *et al*. 2006). A delayed plant response in which some general defence genes are activated and proliferative genes are suppressed has been demonstrated in this study. It is possible that the robust set of genes we have described may be the consequence of the action of different regulatory pathways. Most likely, one pathway is defined by the set of Frankiebox- and Telobox-regulated genes. Other pathways may respond to different components of *Agrobacterium* and to different stages of its association with plant cells.

References

Akira, S., Takeda, K.: Toll-like receptor signalling. - Nat. Rev. Immunol. **4**: 499-511, 2004.

Aldemita, R.R., Hodges, T.K.: *Agrobacterium tumefaciens*-mediated transformation of *japonica* and *indica* rice varieties. - Planta **199**: 612-617, 1996.

Anand, A., Vaghchhipawala, Z., Ryu, C.M., Kang, L., Wang, K., Del-Pozo, O., Martin, G.B., Mysore, K.S.: Identification and characterization of plant genes involved in *Agrobacterium*-mediated plant transformation by virus-induced gene silencing. - Mol. Plant Microbe Interact. **20**: 41-52, 2007.

Anderson, A., Moore, L.: Host specificity in the genus *Agrobacterium*. - Phytopathology **69**: 320-323, 1979.

Arencibia, AD., Carmona, E.R.C., Tellez, P., Chan, M.T., Yu, S.M., Trujillo, L.E., Oramas, P.: An efficient protocol for sugarcane (*Saccharum spp*. L.) transformation mediated by *Agrobacterium tumefaciens*. - Transgenic Res. **7**: 213-222, 1998.

Baek, S.H., Shapleigh, J.P.: Expression of nitrite and nitric oxide reductases in free-living and plant-associated *Agrobacterium tumefaciens* C58 cells. - Appl. environ. Microbiol. **71**: 4427-36, 2005.

Bakó, L., Umeda, M., Tiburcio, A.F., Schell, J., Koncz, C.: The VirD2 pilot protein of *Agrobacterium*-transferred DNA interacts with the TATA box-binding protein and a nuclear protein kinase in plants. - Proc. nat. Acad. Sci. USA **100**: 10108-10113, 2003.

Ballas, N., Citovsky, V.: Nuclear localization of signal binding protein from *Arabidopsis* mediates nuclear import of *Agrobacterium* VirD2 protein. - Proc. nat. Acad. Sci. USA **94**: 10723-10728, 1997.

Beneddra, T., Picard, C., Nesme, X.: Correlation between susceptibility to crown gall and sensitivity to cytokinin in aspen cultivars. - Phytopathology **86**: 225-231, 1996.

Bergmann, B., Stomp, A.M.: Effect of host plant genotype and growth rate on *Agrobacterium tumefaciens*-mediated gall formation in *Pinus radiata*. - Phytopathology **82**: 1457-1462, 1992.

Braun, A.: Thermal studies on the factors responsible for tumour induction in crown gall. - Amer. J. Bot. **34**: 234-240, 1947.

Braun, A.: The physiology of plant tumors. - Annu. Rev. Plant Physiol. **5**: 133-192, 1954.

Bříza, J., Pavingerová, D., Přikrylová, P., Gazdová, J., Vlasák, J., Niedermeierová, H.: Use of phosphomannose isomerase-based selection system for *Agrobacterium*-mediated transformation of tomato and potato. - Biol. Plant. **52**: 453-461, 2008.

Burr, T.J., Bazzi, C., Sule, S., Otten, L.: Crown gall of grape: biology of *Agrobacterium vitis* and the development of disease control strategies. - Plant Dis. **82**: 1288-1297, 1998.

Carlos, H., Carvalho, S., Zehr, U.B., Gunaratna, N., Anderson, J., Kononowicz, H.H., Hodges, T.K., Axtell, J.D.: *Agrobacterium*-mediated transformation of sorghum: factors that affect transformation efficiency. - Genet. mol. Biol. **27**: 259-269, 2004.

Carvalho, C.H.S., Zehr, U.B., Gunaratna, N., Anderson, J.M., Kononowicz, H.H., Hodges, T.K., Axtell, J.D.: *Agrobacterium*-mediated transformation of sorghum: factors that affect transformation efficiency. - Genet. mol. Biol. **27**: 259-269, 2004.

Chateau, S., Sangwa, R.S., Sangwan-Norreel, B.S.: Competence of *Arabidopsis thaliana* genotypes and mutants for *Agrobacterium tumefaciens*-mediated gene transfer: role of phytohormones. - J. exp. Bot. **51**: 1961-1968, 2000.

Cheng, M., Fry, J.E.: An improved efficient *Agrobacterium*-mediated plant transformation method. - Int. Patent Publ. WO 0034/491, 2000.

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

Cheng, M., Hu, T., Layton, J.I., Lium, C.N., Fry, J.E.: Desiccation of plant tissues post *Agrobacterium* infection enhances T-DNA delivery and increases stable transformation efficiency in wheat. - *In Vitro* cell. dev. Biol. Plant. **39**: 595-604, 2003.

Cheng, M., Lowe, B.A., Spencer, M., Ye, X., Armstrong, C.L.: Factors influencing *Agrobacterium*-mediated transformation of monocotyledonous species. - *In Vitro* cell. dev. Biol. Plant. **40**: 31-45, 2004.

Cho, M.-A., Moon, C.-Y., Liu, J.-R., Choi, P.-S.: *Agrobacterium*-mediated transformation in *Citrullus lanatus*. - Biol. Plant. **52**: 365-369, 2008.

Christie, P.J., Atmakuri, K., Krishnamoorthy, V., Jakubowski, S., Cascales, E.: Biogenesis, architecture, and function of bacterial type IV secretion systems. - Annu. Rev. Microbiol. **59**: 451-485, 2005.

Confalonieri, M., Balestrazzi, A., Cella, R.: Genetic transformation of *Populus deltoides* and *Populus euramericana* clones using *Agrobacterium tumefaciens*. - Plant Cell Tissue Organ Cult. **48**: 53-61, 1997.

Davis, M.E., Miller, A.R., Lineberger, R.D.: Temporal competence for transformation of *Lycopersicon esculentum* Mill. cotyledons by *Agrobacterium tumefaciens*: relation to wound-healing and soluble plant factors. - J. exp. Bot. **42**: 35-64, 1991.

De Block, M.: Factors influencing the tissue culture and *Agrobacterium tumefaciens*-mediated transformation of hybrid aspen and poplar clones. - Plant Physiol. **93**: 1110-

1116, 1990.

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

De Kathen, A., Jacobsen, H.-J.: Cell competence for *Agrobacterium*-mediated DNA transfer in *Pisum sativum* L. - Transgen. Res. **4**: 184-191, 1995.

Ditt, R.F., Kerr, K.F., Figueiredo, P., Delrow, J., Comai, L., Nester, E.W.: The *Arabidopsis thaliana* transcriptome in response to *Agrobacterium tumefaciens*. - Mol. Plant Microbe Interact. **19**: 665-681, 2006.

Ditt, R.F., Nester, E.W., Comai, L.: Plant gene expression response to *Agrobacterium tumefaciens*. - Proc. nat. Acad. Sci. USA **98**: 10954-10959, 2001.

Ditt, R.F., Nester, E., Comai, L.: The plant cell defense and *Agrobacterium tumefaciens*. - FEMS Microbiol. Lett. **247**: 207-213, 2005.

Djamei, A., Pitzschke, A., Nakagami, H., Rajh, I., Hirt, H.: Trojan horse strategy in *Agrobacterium transformation*: abusing MAPK defense signaling. - Science **318**: 453-456, 2007.

Doty, S.L., Yu, N.C., Lundin, J.I., Heath, J.D., Nester, E.W.: Mutational analysis of the input domain of the *VirA* protein of *Agrobacterium tumefaciens*. - J. Bacteriol. **4:** 961-970, 1996.

Dunoyer, P., Himber, C., Voinnet, O.: Induction, suppression and requirement of RNA silencing pathways in virulent *Agrobacterium tumefaciens* infections. - Nat. Genet. **38**: 258-262, 2006.

Durrant, W.E., Dong, X.: Systemic acquired resistance. - Annu. Rev. Phytopathol. **42**: 185-209, 2004.

Duval, B., Shetty, K.: The stimulation of phenolics and antioxidant activity in pea (*Pisum sativum*) elicited by genetically transformed anise root extract. - J. Food Biochem. **25**: 361-377, 2001.

Enriquez-Obregon, G.A., Prieto-Samsonov, D.L., De la Riva, G.A., Perez, M.I., Selman Housein, G., Vazquez-Padron, R.I.: *Agrobacterium*-mediated Japonica rice transformation: a procedure assisted by an antinecrotic treatment. - Plant Cell Tissue Organ Cult. **59**: 1659-168l, 1999.

Fang, Y.D., Akula, C., Altpete, F.: *Agrobacterium*-mediated barley (*Hordeum vulgare* L.) transformation using green fluorescent protein as a visual marker and sequence analysis of the T-DNA::barley genomic DNA junctions. - J. Plant Physiol. **159**: 1131-1138, 2002.

Fortin, C., Nester, E.W., Dion, P.: Growth inhibition and loss of virulence in cultures of *Agrobacterium tumefaciens* treated with acetosyringone. - J. Bacteriol. **174**: 5676- 5685, 1992.

Friesner, J., Britt, A.B.: Ku80- and DNA ligase IV-deficient plants are sensitive to ionizing radiation and defective in T-DNA integration. - Plant J. **34**: 427-440, 2003.

Gallego, M.E., Bleuyard, J.Y., Daoudal-Cotterell, S., Jallut, N., White, C.I.: Ku80 plays a role in nonhomologous recombination but is not required for T-DNA integration in *Arabidopsis*. - Plant J. **35**: 557-565, 2003.

Gao, Z., Xie, X., Ling, Y., Muthukrishnan, S., Liang, G.H.: *Agro-bacterium tumefaciens*-mediated sorghum trans-formation using a mannose selection system. - Plant Biotechnol. J. **3**: 591-599, 2005.

Gaspar, Y.M., Nam, J., Schultz, C.J., Lee, L.Y., Gilson, P.R., Gelvin, S.B., Bacic, A.: Characterization of the *Arabidopsis* lysine-rich arabinogalactan-protein AtAGP17 mutant (*rat1*) that results in a decreased efficiency of *Agrobacterium* transformation. - Plant Physiol. **135**: 2162-2171, 2004.

Geier, T., Sangwan, R.S.: Histology and chemical segregation reveal cell-specific differences in the competence for regeneration and *Agrobacterium*-mediated transformation in *Kohleria* internode explants. - Plant Cell Rep. **15**: 386-390, 1996.

Gelvin, S.B.: *Agrobacterium*-mediated plant transformation: the biology behind the ‘gene-jockeying’ tool. - Microbiol. mol. Biol. Rev. **67**: 16-37, 2003.

Gordon-Kamm, W., Dilkes, B.P., Lowe, K., Hoerster, G., Sun, X., Oss, M., Church, L., Bunde, C., Farrell, J., Maddock, S., Synder, J., Ykes, L., Li, Z., Woo, Y.M., Idney, D., Larkins. B.A.: Stimulation of the cell cycle and maize transformation by disruption of the plant retinoblastoma pathway. - Proc. nat. Acad. Sci. USA **99**: 11975-11980, 2002.

Grant, M., Lamb, C.: Systemic immunity. - Curr. Opin. Plant Biol. **9**: 414-420, 2006.

Hansen, G.: Evidence for *Agrobacterium*-induced apoptosis in maize cells. - Mol. Plant-Microbe Interact. **13**: 649-657, 2000.

Hansen, G., Durham, N.C.: Plant transformation methods. - US Patent No. 6,162,965, 2000.

Heath, J.D., Boulton, M.L., Raineri, D.M., Doty, S.L., Mushegian, A.R., Harles, T.C., Davies, J.W., Nester, E.W.: Discrete regions f the sensor protein virA determine the strain-specific ability of *A*grobacterium to agroinfect maize. - Mol. Plant-Microbe Interact. **10**: 221-227, 1997.

Herath, S.P., Suzuki, T., Hattori, K.: Factors influencing *Agrobacterium*-mediated genetic transformation of kenaf. - Plant Cell Tissue Organ Cult. **82**: 201-206, 2005.

Hiei, Y., Komari, T., Kubo, T.: Transformation of rice mediated by *Agrobacterium tumefaciens*. - Plant mol. Biol. **35**: 205-218, 1997.

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

Hood, E.E., Fraley, R.T., Chilton, M.-D.: Virulence of *Agrobacterium tumefaciens* strain A281 on legumes. - Plant Physiol. **83**: 529-534, 1987.

Hwang, H.H., Gelvin, S.B.: Plant proteins that interact with VirB2, the *Agrobacterium tumefaciens* pilin protein, mediate plant transformation. - Plant Cell **16**: 3148-3167, 2004.

Ingle, R.A., Carstens, M., Denby, K.J.: PAMP recognition and the plant-pathogen arms race. - Bioessays **28**: 880-889, 2006.

Ishida, Y., Saito, H., Ohta, S., Hiei, Y., Komari, T., Kumashiro, T.: High efficiency transformation of maize (*Zea mays* L.) mediated by *Agrobacterium tumefaciens*. - Nat. Biotechnol. **14**: 745-750, 1996.

James, D.J., Uratsu, S., Cheng, J., Negri, P., Viss, P., Dandekar, A.M.: Acetosyringone and osmoprotectants like betaine or proline synergistically enhance *Agrobacterium*-mediated transformation of apple. - Plant Cell Rep. **12**: 559-563, 1993.

Jin, S., Roitisch, T., Christie, P.J., Nester, E.W.: The regulatory VirG protein specifically binds to a *cis* acting regulatory sequence involved in transcriptional activation of *Agrobacterium tumefaciens* virulence genes. - J. Bacteriol. **172**: 531-537, 1990.

Johnson, T.M., Das, A.: Organization and regulation of expression of the *Agrobacterium* virulence genes. - In: Spaink, H.P., Kondorosi, A., Hooykaas, P.J.J. (ed.): The Rhizobiaceae: Molecular Biology of Model Plant-

Associated Bacteria. Pp. 265-279. Kluwer Academic Publishers, Dordrecht 1998.

Jones, J.D.G., Dangl, J.L.: The plant immune system. - Nature **444**: 323-329, 2006.

Joubert, P., Beaupère, D., Lelièvre, P., Wadouachi, A., Sangwan, R.S., Sangwan-Norreel, B.S.: Effects of phenolic compounds on *Agrobacterium vir* genes and gene transfer induction a plausible molecular mechanism of phenol binding protein activation. - Plant Sci. **162**: 733-743, 2002.

Kant, P., Kant, S., Jain, R.K., Chaudhury, V.K.: *Agrobacterium*-mediated high frequency transformation in dwarf recalcitrant rice cultivars. - Biol. Plant. **51**: 61-68, 2007.

Karami, O., Deljou, A., Kordestani, G.K.: Secondary somatic embryogenesis of carnation (*Dianthus caryophyllus*). - Plant Cell Tissue Organ Cult. **92**: 273-280, 2008.

Karami, O., Deljou, A., Mahmodi Pour, A.: Repetitive somatic embryogenesis in carnation on picloram supplemented media. - Plant Growth Regul. **51**: 33-39, 2007.

Komari, T., Kubo, T.: Methods in genetic transformation: *Agrobacterium tumefaciens*. - In: Vasil, I.K. (ed.): Molecular Improvement of Cereal Crops. Pp. 43-83. Kluwer Academic Publishers, Dordrecht 1999.

Kumlehn, J., Serazetdinora, L., Hensel, G., Becker, D., Loerz, H.: Genetic transformation of barley (*Hordeum vulgare* L.) *via* infection of androgenetic pollen culture with *Agrobacterum tumefaciens*. - Plant Biotechnol. J. **4**: 251-258, 2006.

Lacroix, B., Li, J., Tzfira, T., Citovsky, V.: Will you let me use your nucleus? How *Agrobacterium* gets its T-DNA expressed in the host plant cell. - Can. J. Physiol. Pharmacol. **84**: 333-345, 2006b.

Lacroix, B., Tzfira, T., Vainstein, A., Citovsky, V.: A case of promiscuity: *Agrobacterium*'s endless hunt for new partners. - Trends Genet. **22**: 29-37, 2006a.

Lacroix, B., Vaidya, M., Tzfira, T., Citovsky, V.: The VirE3 protein of *Agrobacterium* mimics a host cell function required for plant genetic transformation. - EMBO J. **24**: 428-437, 2005.

Li, J., Krichevsky, A., Vaidya, M., Tzfira, T., Citovsky, V.: Uncoupling of the functions of the *Arabidopsis* VIP1 protein in transient and stable plant genetic transformation by *Agrobacterium*. - Proc. nat. Acad. Sci. USA **102**: 5733-5738, 2005.

Li, X-Q., Liu, C.-N., Ritchie, S.W., Peng, J.Y., Gelvin, S.B., Hodgcs, TK.: Factors influencing *Agrobacterium*-mediated transient expression of *gusA* in rice. - Plant mol. Biol. **20**: 1037-1048, 1992.

Lippincott, B.B., Whatley, M.H., Lippincott, J.A.: Tumor induction by *Agrobacterium* involves attachment of bacterium to a site on host plant-cell wall. - Plant Physiol. **59**: 388-390, 1977.

Liu, P., Nester, E.W.: Indoleacetic acid, a product of transferred DNA, inhibits *vir* gene expression and growth of *Agrobacterium tumefaciens* C58. - Proc. nat. Acad. Sci. USA **103**: 4658-4662, 2006.

Lowe, B.A., Krul, W.R.: Physical, chemical, developmental, and genetic factors that modulate the *Agrobacterium-Vitis* interaction. - Plant Physiol. **96**: 121-129, 1991.

Loyter, A., Rosenbluh, J., Zakai, N., Li, J., Kozlovsky, S.V., Tzfira, T., Citovsky, V.: The plant VirE2 interacting protein 1. A molecular link between the *Agrobacterium* T-complex and the host cell chromatin? - Plant Physiol **138**: 1318-1321, 2005.

Mannie, S., Morgens, P.H., Scorza, R., Cordts, J.M., Callahan, A.M.: *Agrobacterium*-mediated transformation of plum (*Prunus domestica* L.) hypocotyl slices and regeneration of transgenic plant. - Natur. Biotechnol. **9**: 853-857, 1991.

Maresh, J., Zhang, J., Lynn, D.G.: The innate immunity of maize and the dynamic chemical strategies regulating two-component signal transduction in *Agrobacterium tumefaciens*. - ACS Chem. Biol. **1**: 165-175, 2006.

McCormac, A.C., Wu, H., Bao, M., Wang, Y., Xu, R., Elliot, C.M., Chen, D.F.: The use of visual marker genes as cell specific reporters of *Agrobacterium*-mediated T-DNA delivery to wheat (*Triticum aestium* L.) and barley (*Hordeum vulgare* L.). - Euphytica **99**: 17-25, 1998.

McCullen, C.A., Binns, A.N.: *Agrobacterium tumefaciens* and plant cell interactions and activities required for interkingdom macromolecular transfer. - Annu. Rev. cell. dev. Biol. Plant **22**: 101-127, 2006.

Mondal, T.K., Bhattacharya, A., Ahuja, P.S., Chand, P.K.: Transgenic tea (*Camellia sinensis* (L) O. Kuntze cv. Kangra Jat) plants obtained by *Agrobacterium* mediated transformation of somatic embryos. - Plant Cell Rep. **20**: 712-720, 2001.

Mysore, K.S., Nam, J., Gelvin, S.B.: An *Arabidopsis* histone H2A mutant is deficient in *Agrobacterium* T-DNA integration. - Proc. nat. Acad. Sci. USA **97**: 948-953, 2000.

Nadolska-Orczyk, A., Orczyk, W.: Study of the factors influencing *Agrobacterium*-mediated transformation of pea (*Pisum sativum* L.). - Mol. Breed. **6**: 185-194, 2000.

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

Nam, J., Mysore, K.S., Zheng, C., Knue, M.K., Matthysse, A.G., Gelvin, S.B.: Identification of T-DNA tagged *Arabidopsis* mutants that are resistant to transformation by *Agrobacterium*. - Mol. gen. Genet. **261**: 429-438, 1999.

Nandakumar, R., Chen, L., Rogers, S.M.D.: Factors affecting the *Agrobacterium*-mediated transient transformation of the wetland monocot, *Typha latifolia*. - Plant Cell Tissue Organ Cult. **79**: 31-38, 2004.

Olhoft, P.M., Flagel, L.E., Donovan, C.M., Somers, D.A.: Efficient soybean transformation using hygromycin B selection in the cotyledonary-node method. - Planta **216**: 723-735, 2003.

Owens, L.D., Cress, D.E.: Genotypic to variability of soybean response *Agrobacterium* strains harboring the Ti or Ri plasmids. - Plant Physiol. **77**: 87-94, 1984.

Parrott, D.L., Anderson, A.J., Carman, J.G.: *Agrobacterium* induces plant cell death in wheat (*Triticum estivum* L.). - Physiol. mol. Plant Pathol. **60**: 59-69, 2002.

Paulsson, M., Wadstrom, T.: Vitronectin and type-I collagen binding by *Staphylococcus aureus* and coagulase-negative streptococci. - FEMS Microbiol. Immunol. **65**: 55-62, 1990.

Perl, A., Lotan, O., Abu-Abeid, M., Holland, D.: Establishment of an *Agrobacterium*-mediated genetic transformation system for grape (*Vitis vinifera* L.): the role of antioxidants during grape-*Agrobacterium* interaction. - Natur. Biotechnol. **14**: 624-628, 1996.

Porter, J.R.: Host range and implications of plant infection by *Agrobacterium rhizogenes*. - Crit. Rev. Plant Sci. **10**: 387-421, 1991.

Potrykus, I.: Gene transfers to plants: assessment of published approaches and results. - Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 205-225, 1991.

Potrykus, I.: Gene transfer to cereal: an assessment. - Natur. Biotechnol. **8**: 535-542, 1990.

Pu, X.A., Goodman, R.N.: Induction of necrogenesis by *Agrobacterium tumefaciens* on grape explants. - Physiol. mol. Plant Pathol. **41**: 245-254, 1992.

Rashid, H., Yokoi, S., Toriyama, K., Hinata, K.: Transgenic plant production mediated by *Agrobacterium* in *indica* rice. - Plant Cell Rep. **15**: 727-730, 1996.

Regensburg-Tuink, A.J., Hooykaas, P.J.J.: Transgenic *N. glauca* plants expressing bacterial virulence gene *virF* are converted into hosts for nopaline strains of *A. tumefaciens*. - Nature **363**: 69-71, 1993.

Repellin, A., Baga, M., Jauhar, P.P., Chibbar, R.N.: Genetic enrichment of cereal crops via alien gene transfer: new challenges. - Plant Cell Tissue Organ. Cult. **64**: 159-183, 2001.

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

Roberts, R.L., Metz, M., Monks, D.E., Mullaney, M.L., Hall, T., Nester, E.W.: Purine synthesis and increased *Agrobacterium tumefaciens* transformation of yeast and plants. - Proc. nat. Acad. Sci. USA **100**: 6634-6639, 2003.

Salas, M.G., Park, S.H., Srivatanakul, M., Smith, R.H.: Temperature influence on stable T-DNA integration in plant cells. - Plant Cell Rep. **20**: 701-705, 2001.

Saini, R., Jaiwal, P.K.: *Agrobacterium tumefaciens*-mediated transfromation of blackgram: an assessment of factors influencing the efficiency of uidA gene transfer. - Biol. Plant. **51**: 75-79, 2007.

Sangwan, R.S., Bourgeois, Y., Brown, S., Vasseur, G., Sangwan-Norreel, B.S.: Characterization of competent cells and early events of *Agrobacterium*-mediated genetic transformation in *Arabidopsis thaliana*. - Planta **188**: 439-456, 1992.

Santarem, E.R., Trick, H.N., Essig, J.S., Finer, J.J.: Sonication-assisted *Agrobacterium*-mediated transformation of soybean immature cotyledons: optimization of transient expression. - Plant Cell Rep. **17**: 752-759, 1998.

Schlappi, M., Hohn, B.: Competence of immature maize embryos for *Agrobacterium*-mediated gene transfer. - Plant Cell **4**: 7-16, 1992.

Schrammeijer, B., Risseeuw, E., Pansegrau, W., Regensburg-Tuink, T.J., Crosby, W.L., Hooykaas, P.J.: Interaction of the virulence protein VirF of *Agrobacterium tumefaciens* with plant homologs of the yeast Skp1 protein**.** - Curr. Biol. **11**: 258-262, 2001.

Shou, H., Frame, B.R., Whitham, S.A., Wang, K.: Assessment of transgenic maize events produced by particle bombardment or *Agrobacterium*-mediated transformation. - Mol. Breed. **13**: 201-208, 2004.

Shrawat, A.K., Beckera, D., Lörz, H.: *Agrobacterium tumefaciens*-mediated genetic transformation of barley (*Hordeum vulgare* L.). - Plant Sci. **172**: 281-290, 2007.

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

Stachel, S.E., Messens, E., Van Montagu, M., Zambryski, P.: Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in *Agrobacterium tumefaciens*. - Nature **318**: 624-629, 1985.

Sunilkumar, G., Rathore, K.S.: Transgenic cotton: factors influencing *Agrobacterium*-mediated transformation and regeneration. - Mol. Breed. **8**: 37-52, 2001.

Tao, Y., Rao, P.K., Bhattacharjee, S., Gelvin, S.B.: Expression of plant protein phosphatase 2C interferes with nuclear import of the *Agrobacterium* T-complex protein VirD2. - Proc. nat. Acad. Sci. USA **101**: 5164-5169, 2004.

Tingay, S., McElroy, D., Kalla, R., Fieg, S., Wang, M., Thornton, S., Brettell, R.: *Agrobacterium tumefaciens*-mediated barley transformation. - Plant J. **11**: 1369-1376, 1997.

Tinland, B., Schoumacher, F., Gloeckler, V., Bravo-Angel, A.M., Hohn, B.: The *Agrobacterium tumefaciens* virulence D2 protein is responsible for precise integration of T-DNA into the plant genome. - EMBO J. **14**: 3585-3595, 1995.

Toki, S.: Rapid and efficient *Agrobacterium*-mediated transformation in rice. - Plant mol. Biol. Rep. **15**: 16-21, 1997.

Trifonova, A., Madsen, S., Olesen, A.: *Agrobacterium*-mediated transgene delivery and integration into barley under a range of *in vitro* culture conditions. - Plant Sci. **161**: 871-880, 2001.

Turk, S.C.H.J., Melchers, L.S., Den Dulk-Ras, H., Regensburg-Tuink, A.J.G., Hooykaas, P.J.J.: Environmental conditions differentially affect *vir* gene induction in different *Agrobacterium* strains. Role of the virA sensor protein. - Plant mol. Biol. **16**: 1051-1059, 1991.

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

Tzfira, T., Citovsky, V.: Partners-in-infection: host proteins involved in the transformation of plant cells by *Agrobacterium*. - Trends cell. Biol. **12**: 121-129, 2002.

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

Tzfira, T., Vaidya, M., Citovsky, V.: VIP1, an *Arabidopsis* protein that interacts with *Agrobacterium* VirE2, is involved in VirE2 nuclear import and *Agrobacterium* infectivity. - EMBO J. **20**: 3596-3607, 2001.

Tzfira, T., Vaidya, M., Citovsky, V.: Increasing plant susceptibility to *Agrobacterium* infection by overexpression of the *Arabidopsis* nuclear protein VIP1. - Proc. nat. Acad. Sci. USA **99**: 10435-10440, 2002.

Tzfira, T., Vaidya, M., Citovsky, V.: Involvement of targeted proteolysis in plant genetic transformation by *Agrobacterium*. - Nature **431**: 87-92, 2004a.

Urushibara, S., Tozawa, Y., Kawagishi-Kobayashi, M., Wakasa, K.: Efficient transformation of suspension-cultured rice cells mediated by *Agrobacterium tumefaciens*. - Breed. Sci. **5**: 33-38, 2001.

Usami, S., Morikawa, S., Takebe, I., Machida, Y.: Absence of monocotyledonous plants of the diffusible plant factors inducing T-DNA circulation and *vir* gene expression in *Agrobacterium*. - Mol. gen. Genet. **290**: 221-226, 1987.

Valvekens, D., Van Montagu, M., Van-Lijsebettens, M.: *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* root explants by using kanamycin selection. - Proc. nat. Acad. Sci. USA **85**: 5536-5540, 1988.

Van Attikum, H., Hooykaas, P.J.J.: Genetic requirements for the targeted integration of *Agrobacterium* T-DNA in *Saccharomyces cerevisiae*. - Nucl. Acids Res. **102**: 12265-12269, 2003.

Van Attikum, H., Bundock, P., Hooykaas, P.J.J.: Non-homologous end-joining proteins are required for *Agrobacterium* T-DNA integration. - EMBO J. **20**: 6550-6558, 2001.

Van der Hoorn, R.A.L., Laurentm, F., Roth, R., De Wit, P.J.G.M.: Agroinfiltration is a versatile tool that facilitates comparative analyses of Avr9/Cf-9-induced and Avr4/Cf-4-induced necrosis. - Mol. Plant-Microbe Interact. **13**: 439-446, 2000.

Van Roekel, J.S.C., Damm. B., Melchers, L.S., Hoekema, A.: Factors influencing transformation frequency of tomato (*Lycopersicon esculentum*). - Plant Cell Rep. **12**: 644-647, 1993.

Veena, J.H., Doerge, R.W., Gelvin, S.B.: Transfer of T-DNA and Vir proteins to plant cells by *Agrobacterium tumefaciens* induces expression of host genes involved in mediating transformation and suppresses host defense gene expression. - Plant J. **35**: 219-226, 2003.

Vergunst, A.C., Schrammeijer, B., Den Dulk-Ras, A., De Vlaam, C.M.T., Regensburg-Tuink, T.J., Hooykaas, P.J.J.: VirB/D4-dependent protein translocation from *Agrobacterium* into plant cells. - Science **290**: 979-982, 2000.

Villemont, E., Dubois, F., Sangwan, R.S., Vasseur, G., Bourgeois, Y., Sangwan-Norreel, B.S.: Role of the host cell cycle in the *Agrobacterium*-mediated genetic transformation of *Petunia*: evidence of an S-phase control mechanism for T-DNA transfer. - Planta **201**: 160-172, 1997.

Wagner, V.T., Matthysse, A.G.: Involvement of vitronectin-like protein in attachment of *Agrobacterium tumefaciens* to carrot suspension culture cells. - J. Bacteriol. **174**: 5999-6003, 1992.

Wang, M.-B., Abbott, D.C., Upadhyaya, N.M., Jacobsen, J.V., Aterhouse, P.M.: *Agrobacterium tumefaciens*-mediated ransformation of an elite Australian barley cultivar with virus resistance and reporter genes. - Aust. J. Plant Physiol. **28**: 149-156, 2001.

Winans, S.C., Kerstetter, R.A., Nester, E.W.: Transcriptional regulation of the *virA* and *virG* genes of *Agrobacterium tumefaciens*. - J. Bacteriol. **170**: 4047-4054, 1988.

Wroblewski, T., Tomczak, A., Michelmore, R.: Optimization of *Agrobacterium*-mediated transient assays of gene expression in lettuce, tomato and *Arabidopsis*. - Plant Biotechnol. J. **3**: 259-273, 2005.

Wu, H., Sparks, C., Amoah, B., Jones, H.D.: Factors influencing successful *Agrobacterium*-mediated genetic transformation of wheat. - Plant Cell Rep. **21**: 659-668, 2003.

Yanofsky, M., Montoya, A., Knauf, V., Lowe, B., Gordon, M., Nester, E.: Limited-host-range plasmid of *Agrobacterium tumefaciens*: molecular and genetic analyses of transferred DNA. - J. Bacteriol. **163**: 341-348, 1985.

Yu, C., Huang, S., Chen, C., Deng, Z., Ling, P., Gmitter, F.G.: Factors affecting *Agrobacterium*-mediated transformation and regeneration of sweet orange and citrange. - Plant Cell Tissue Organ Cult. **71**: 147-155, 2002.

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

Zhang, G.N., Jia, J.F., Hao, J.G., Wang, X.R., He, T.: Plant regeneration from mesophyll protoplasts of *Agrobacterium rhizogenes*-transformed *Astragalus melilotoides.* - Biol. Plant. **52**: 373-376, 2008.

Zhang, J., Boone, L., Kocz, R., Zhang, C., Binns, A.N., Lynn, D.G.: At the maize/*Agrobacterium* interface: natural factors limiting host transformation. - Chem. Biol. **7**: 611-621, 2000.

Zhao, Z., Cai, T., Tagliani, L., Miller, M., Wang, N., Pang, H., Rudert, M., Schroeder, S., Hondred, D., Seltzer, J., Pierce, D.: *Agrobacterium*-mediated sorghum transformation. - Plant mol. Biol. **44**: 789-798, 2000.

Zhu, Y., Nam, J., Carpita, N.C., Matthysse, A.G., Gelvin, S.B.: *Agrobacterium*-mediated root transformation is inhibited by mutation of an *Arabidopsis* cellulose synthase-like gene. - Plant Physiol. **133**: 1000-1010, 2003a.

Zhu, Y., Nam, J., Humara, J.M., Mysore, K., Lee, L.Y., Cao, H., Valentine, L., Li, J., Kaiser, AD., Kopecky, A.L., Hwang, H.H., Bhattacharjee, S., Rao, P.K., Tzfira, T., Rajagopal, J., Yi, H., Yadav, B.S., Crane, Y.M., Lin. K., Larcher, Y., Gelvin, M.J., Knue, M., Ramos, C., Zhao, X., Davis, S.J., Kim. S.I., Ranjith-Kumar, C.T., Choi, Y.J., Hallan, V.K., Chattopadhyay, S., Sui, X., Ziemienowicz, A., Matthysse, A.G., Citovsky, V., Hohn, B., Gelvin, S.B.: Identification of *Arabidopsis rat* mutants. - Plant Physiol. **132**: 494-505, 2003b.

Zipfel, C., Felix, G.: Plants and animals: a different taste for microbes? - Curr. Opin. Plant Biol. **8**: 353-360, 2005.

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J.D.G., Boller, T., Felix, G.: Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. - Cell **125**: 749-760, 2006.

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