Chapter 15
Insight to Biotechnological Advances in the Study of Beneficial Plant-Microbe Interaction with Special Reference to *Agrobacterium tumefaciens*

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Abstract *Agrobacterium tumefaciens* is the most scientifically investigated and proved cellular organism that has a natural capability to transfer genetic material between the kingdoms of life, from prokaryotes to eukaryotes. Recent advancement in biotechnological tools and techniques helps us to understand plant-microbe interactions because plants are closely associated with microorganisms that influence plants' overall fitness. With the rapid decline of natural resources and continuous increase in the population of developing countries, there is an utmost need for new tools and technologies that supplement the agriculture system and provide novel opportunities for ensuring global food and nutritional security. Till now, *Agrobacterium* has been widely used as a vector to genetically transform the plants with agronomically important traits. Apart from the role of *Agrobacterium tumefaciens* in plant genetic engineering, it also served plant biologist to scientifically investigate and reveal basic biological processes such as regulation of gene expression, gene identification and mapping, cell-cell recognition, cell-to-cell transport mechanism, nuclear import, and recombination mechanism and to study mutagenesis within plant cells. In this chapter, we mainly emphasized on importance of a systems understanding of plant-microbe interactions, with a special reference to *Agrobacterium tumefaciens*—as natural plant genetic engineer, signal transduction and host immune response, quorum sensing and quenching, plant genes involved in susceptibility/resistance, factor affecting *Agrobacterium* plant transformation, recent advances/application in plant biology research, and omics approaches for better understanding plant-microbe interaction complexity.

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A. Varma et al. (eds.), *Plant Microbe Symbiosis*,
https://doi.org/10.1007/978-3-030-36248-5_15

15.1 Introduction

Plants in their natural characteristic surroundings/habitats are encompassed by a large number of microorganisms that directly and indirectly interact with plants. Some microbes interact with plants in a mutually beneficial manner or otherwise colonize the plant only for their own benefits. In addition, microbes can also indirectly affect plants by severely changing their growth environment conditions. By using recent advancement in biotechnological approaches, molecular genetics and system biology approaches also provide insight to understand the true nature of plant-microbe interaction, basic biological processes, regulated gene expression, signal transduction mechanism, and plant immune signaling network and significantly offer novel strategies to augment quantitatively and qualitatively crop productivity and protection in an environment-friendly manner (Ishaq 2017; Pathak et al. 2018). Today, in the global world, the major challenges in agricultural biotechnology are to increase crop productivity and crop protection in adverse environmental conditions, manage resistance to pests and disease and herbicide tolerance, improve genetic engineering technologies to enhance public perception, and improve harvest index and nutrient cycling in agricultural ecosystems (Kossmann 2012; Prasad et al. 2018). Detailed scientific investigation on beneficial plant-microbe interactions possibly helps to develop microbial inoculants which can be used as plant strengtheners, phytostimulators, biopesticides, and biofertilizers. Advancement in understanding plant biology, novel genetic resources, and modification process and omics technologies revolutionized our concepts of sustainable food production, cost-effective alternative energy strategies, and novel biomaterial production that significantly contribute to revolutionize our agriculture system under changing climatic and environmental conditions (Moshelion and Altman 2015).

Agrobacterium tumefaciens gene tranfer method still prevailing as one of the oldest and most widely used method for genetic manipulation of various economically and horticulturally important monocot and dicot plants by optimized efficient and reliable tissue culture protocol, in planta transformation procedure, and more recently *Agrobacterium* T-DNA-derived nano-complex method for recalcitrant crop species (Ziemienowicz 2014; Kumar et al. 2018a). However, various factors of bacterial, host, and environmental origin affect the transformation efficiency which needs to be critically optimized and addressed. Till date, many economically important crop species or elite varieties have been developed using transgenic technology by the use of *Agrobacterium tumefaciens*-mediated gene transfer techniques. Genetic transformation of plants using *Agrobacterium*-mediated gene transfer is a key technique for plant molecular breeding to introduce agronomically important desirable characteristics/traits into the existing plant genome while preserving genetic identity of plants. The broad compensation of transgenic crops for a society to resolve the food and nutritional security issues has been well recognized and is addressed by added benefits such as tolerance to various biotic and abiotic stresses, herbicide tolerance, virus resistance, higher nutritional value, and enhancement of the fruit shelf life (Shukla et al. 2018). As the biotechnological advancement in the area of plant biotechnology and plant genetic

engineering technologies, biotech crops/genetically modified crops are considered as the fastest adopted crop technology in current modern agriculture era, as per the latest update by the International Service for the Acquisition of Agri-biotech Applications (ISAAA). In 2017, biotech crops were planted on 189.8 million hectares area by 24 countries, and from the initial plantation of 1.7 million hectares in 1996, there was an ~112-fold increase in 2017. Soybean, maize, cotton, and canola are the most planted biotech crops in 2017. Biotech soybean occupied an area of 94.1 million hectares followed by biotech maize (59.7 million hectares), biotech cotton (24.1 million hectares), and biotech canola (10.2 million hectares) globally in 2017. Apart from soybean, maize, cotton, canola, and alfalfa, the following biotech crops, i.e., papaya, potato, sugar beet, eggplant, squash, and apple, were also planted in different countries. There was a 9% increase in the global market value of biotech crops in 2017 than from 2016, i.e., US$17.2 billion (ISAAA 2017).

15.2 *Agrobacterium tumefaciens*: Natural Plant Genetic Engineer

Agrobacterium tumefaciens is also called a natural plant genetic engineer because of its natural ability to transfer genetic material between the kingdoms of life, from prokaryotes to eukaryotes. *Agrobacterium tumefaciens* is a rod-shaped, motile, and Gram-negative soil bacterium having tumor-inducing (Ti) plasmid, which causes crown gall disease in plants (Tzfira and Citovsky 2008; Gelvin 2009, 2010; Ziemienowicz 2014). Ti plasmid maintains most of the Ti-plasmid genes in transcriptionally inactive state; their expression is activated by the presence of suitable plant host. *Agrobacterium* enters into the plants through wounded tissue, and phenolic compound acetosyringone and monosaccharides are released by wounded plant cell, which activate the expression of virulence genes, although these genes are silent until induced. Vir A is a sensor protein, and its periplasmic domain binds to a phenolic compound such as acetosyringone to activate cytoplasmic protein kinase domain leading to Vir A autophosphorylation. For phosphonyl group transfer from Vir A to Vir G genes (transcriptional regulator), Vir G genes activate all the other virulence genes by binding to a 12 bp DNA element called the Vir box located upstream of the Vir operon. *Agrobacterium* chromosomal virulence genes (*chv E*) bind to a certain plant-derived sugar and also promote autophosphorylation of Vir A and enhance its signaling. The Vir region is ~40 kb, and it includes several distinct operons. The first step in T-DNA transfer includes the action of single-stranded endonucleases that cleave T-DNA at the border sequence. The *Vir D* genes (Vir D_1, helicase activity; Vir D_2, endonucleases activity) and Vir D_2 site-specific endonucleases recognize 25 bp T-DNA border sequence, and Vir D_2 nicks only the bottom strand at each border sequence and releases the single-stranded T-DNA molecule. Vir D_2 remains covalently attached to 5′ end of T-DNA throughout the transfer to host nucleus and is thought to protect T-DNA

from exonucleolytic attack. Vir B operon encodes 11 proteins that along with Vir D_4 forms type IV secretion system (T4SS). The T-DNA-Vir D_2 complex moves into the plant cell through a large protein complex called type IV secretion system (T4SS) that spans in the bacterial outer and inner membranes and possibly the plant cell wall. T-DNA-Vir D_2 and other proteins in the Vir E region move through T4SS into the plant cytoplasm. A possible explanation is that Vir D_2 and Vir E_2 contribute to T-DNA movement into the nucleus because it has a nuclear localization signal that directs the complex to plant nucleus. A plant importin α (also known as karyopherin) also contributes to the movement of T-DNA complex into the nucleus. Once the T-DNA complex enters the nucleus, it is thought to attach to the plant nuclear DNA through interaction with nucleosomal proteins. The exact mechanism of DNA insertion into the plant genome is not fully resolved, but it occurs via a process known as nonhomologous or illegitimate recombination (Anand et al. 2008; Christie et al. 2014; Srivastava et al. 2016; Willig et al. 2018).

Agrobacterium tumefaciens as a natural plant genetic engineer is used as a plant transformation vector, which requires four major key steps that are disarming process (bacterium had to be made nonpathogenic), introduction of selected desirable gene(s) along with selectable markers genes into the T-DNA region, in vitro manipulation of large-size tumor-inducing (Ti) plasmid, and efficient method development for regeneration of whole plants from transformed cells. Due to the natural genetic engineering ability of *Agrobacterium tumefaciens*, it has been successfully commercially exploited to create transgenic plants using plant genetic engineering approaches to enhance crop productivity, nutritional value, and crop protection by increased tolerance to biotic and abiotic stresses and also helps in the reduction of harmful agrochemicals (Gelvin 2010; Mine et al. 2014; Christie et al. 2014). *Agrobacterium*-mediated gene transfer also conferred improved growth and grain yield in rice plant by enhancing the biosynthesis of an iron chelator at low iron bioavailability conditions (Takahashi et al. 2001). Using *Agrobacterium*-mediated transformation for the production of herbicide-tolerant crops such as soybean, canola, cotton, maize, sugar beet, and rapeseed oil resulted in the reduction of harmful agrochemicals (Slater et al. 2008; Jube and Borthakur 2009; Ziemienowicz 2014; Srivastava et al. 2016; Kumar and Srivastava 2016).

15.3 Signal Transduction and Host Immune Response

Agrobacterium is the mostly studied and scientifically investigated phytopathogen till date and proved for its ability to genetically transform host plants by transferring and integrating T-DNA of its tumor-inducing (Ti) plasmid or a foreign desirable gene such as agronomically important traits (Lacroix and Citovsky 2013). Gohlke and Deeken (2014) have reported how plants respond to *Agrobacterium tumefaciens* causative agent of crown gall disease. *Agrobacterium tumefaciens* recognition and signal transduction pathway responses to plant-derived signaling molecules for understanding complexity of *Agrobacterium*-plant interaction have been largely

studied over several decades (Subramoni et al. 2014). For transferring and integrating its T-DNA of tumor-inducing (Ti) plasmid, *Agrobacterium* perceives and recognizes plant-derived signals to activate its virulence genes, which are responsible for T-DNA transfer into the plant nucleus. The expression of genes inside the plant hosts leads to the production of plant growth regulators, i.e., auxins {indole-3-acetic acid (IAA) and cytokinin}, and opines which resulted in tumor formation. *Agrobacterium* makes use of opines as sole nutrient sources as well as signaling molecule to activate quorum sensing (QS) for increased virulence and opine metabolism.

For active defense response in plants/host immune response against microbial pathogens, signal transduction pathway plays a central role (Hwang et al. 2015a). Recognition of *Agrobacterium* as pathogen by host plants resulted in plant defense response elicitation. However, plants do not have any specialized immune cells, but showed the sophisticated innate defense system. Plants have various receptor molecules which recognize conserved pathogen-associated molecular patterns (PAMPs). Pathogen-associated molecular patterns are structurally and sequence characterized, highly conserved within divergent classes of pathogens, such as bacteria/fungi, and also often found in nonpathogenic microbes that's why they are also called microbe-associated molecular patterns (MAMPs), which elicit sets of defense mechanisms (Ausubel 2005). The most characterized pathogen-associated molecular patterns are peptides derived from flagellin (flg22) and the elongation factor *EF-Tu* (*elf18*) (Gómez-Gómez et al. 2001). Flagellin (particularly flagellin peptide flg22) is recognized by a specific receptor kinase (*FLS2*) and induces the expression of various defense-related genes to prompt resistance against pathogenic bacteria (Chinchilla et al. 2006). *EF-Tu* is the most abundant protein present in many bacteria and upon cell membrane integrity disruption released into the extracellular spaces (Kunze et al. 2004; Zipfel et al. 2006; Nicaise et al. 2009). However, *Agrobacterium* flagellin protein (N-terminal conserved domain) is not recognized by the host plant and fails to induce defense response and does not trigger an immune response (Zipfel et al. 2004; Yuan and Williams 2014).

Agrobacterium has developed numerous strategies to evade the host's immune responses. Ditt et al. (2001) have investigated the plant response to *Agrobacterium tumefaciens* infection and its transformation in inoculated *Ageratum conyzoides* plant cultures by comparing cDNA amplified fragment length polymorphism (AFLP) pattern. From amplified cDNA fragment pattern, differential regulated gene expression was studied after co-cultivation (24 and 48 h) with *Agrobacterium.* To confirm the cDNA-AFLP differential pattern sequence, similarities were also carried out which revealed the role of amplified genes in the signal transduction of plant defense response. However, various scientific findings in a number of host species such as *Arabidopsis thaliana*, rice, and rye grass indicated that *Agrobacterium*-induced defenses limit transformation efficiency (Ditt et al. 2001; Zipfel et al. 2006; Taj et al. 2014; Zhang et al. 2013). To detect pathogenic and nonpathogenic microbes, extra- and intracellular receptors on host cell trigger signal transduction mechanism resulting in immediate physiological changes such as production of damaging reactive oxygen species (ROS), Ca^{2+} fluxes (secondary

messenger molecule), extracellular alkalization, and hypersensitive response to localized programmed cell death (Boller and Felix 2009). Initial signal transduction events to regulate these responses modulate defense gene expression in mitogen-activated protein kinase (MAPK) pathway (Asai et al. 2002). Upregulation of hormone (salicylic acid, jasmonate, and ethylene)-specific defense gene is also being expressed and induces systemic defenses in plants (Jones and Dangl 2006; Spoel and Dong 2012; Yuan and Williams 2014; Hwang et al. 2015b).

15.4 Quorum Sensing and Quenching

Agrobacterium tumefaciens induces crown gall formation generally on a wide range of dicotyledonous plants and pathogenicity determinants of this bacterium mostly borne on tumor-inducing (Ti) plasmid. The conjugative transfer of the plasmid genes and expression of genes are regulated by quorum sensing (QS) (Dessaux and Faure 2018). Based upon accumulation of an acyl homoserine lactone (AHL), *A. tumefaciens* regulates the expression of genes in a population density-dependent manner, such kind of regulations were named as quorum sensing, and signals are called quorum signals, autoinducers, or quoromones. When *A. tumefaciens* infects the host plants, then quorum sensing is induced, and it varies among different strains, but here it is being discussed about the octopine-utilizing agrobacterial strains (Zhang et al. 2002). Transfer of T-DNA into the plant cells leads to the production of octopine which is recognized by *A. tumefaciens* transcriptional regulator named as octopine catabolism regulator (OccR). Once recognized, transcription activation of octopine catabolism operon (consists of genes involved in octopine uptake and catabolism) takes place. In this operon, *traR* gene encodes a transcription factor bound to acyl homoserine lactone (AHL) as quorum signals. Acyl homoserine lactone activates an operon particularly *traI* gene (encodes an enzyme for the synthesis of AHL) and then binds more avidly to *traR*, forming a positive feedback regulatory loop. Various other *traR* regulated genes involved in Ti-plasmid replication and conjugal transfer (Yuan and Williams 2014). Recent investigations have shown that plants are capable of recognizing bacterial quormone signals and responding to these signals through their various defense mechanisms and developmental stages, and thus in initiating quormone degradation processes, this process is collectively called quorum quenching (Dessaux and Faure 2018). Quorum quenching involves the exclusion of the quorum signal from the environment. Acyl homoserine lactone (AHL) lactonase encoded by *attM* of *Agrobacterium* is believed to degrade and quench the quormone signal. Plant defense response compounds such as salicylic acid and gamma-Aminobutyric acid which accumulate in crown gall tumors caused by *Agrobacterium tumefaciens* induced the expression of a*ttM*, which contributed quorum quenching to the plant and bacterium. Dessaux and Faure (2018) reported that quorum-sensing elements/signal molecule in conjunction with quorum quenching (QQ) activates a complex-integrated "go/no go system" that finely controls the Ti-plasmid transfer in response to various

environmental cues. This finely tuned go/no go system of quorum sensing and quenching permits the bacteria to sense its presence or absence in a gall, in a decaying or living tumor, and in stressed plant tissues.

15.5 Plant Genes Involved in Susceptibility/Resistance to *Agrobacterium* Transformation

Gelvin (2010) and Yuan and Williams (2014) have reported different identified proteins such as *hat* (hypersusceptible to *Agrobacterium* transformation) and *rat* (resistant to *Agrobacterium* transformation) genes, which conferred hypersensitivity and resistance to *Agrobacterium* transformation. Resistant to *Agrobacterium* transformation; *rat* genes encode a protein that interacted with transferred DNA (T-DNA) integration and a VirB-interacting protein that facilitate contact between the type IV secretion systems (T4SSs) are large protein complexes contains a channel through which proteins or protein-DNA complexes can be translocated, which traverse the cell envelope of many bacteria and the host cell. Zhu et al. (2003) and Gelvin (2010) also discussed about various other identified genes involved in bacterial attachment/biofilm formation (arabinogalactan protein *AtAGP17*, cellulose synthase-like *CslA-09* and *CslB-05*, and plant defense reaction proteins), cytoplasmic trafficking (microtubules/kinesin, actin, myosin, and cyclophilin), nuclear targeting [importin α, importin β/transportin, CAK2M kinase, protein phosphatase 2C (*PP2C*), *VIP1*, caspase, GALLS interacting protein (*GIPa*)], transgene expression (histones H2A, H3-11, and H4), and susceptibility to transformation (*Myb* transcription factor). Comprehensive investigation of the plant's contributions to the infection process/disease procedure can help to identify and distinguish strategies/methods to protect plants, such as walnut (*Juglans regia*), grapevines, and almond (*Prunus dulcis*), that are vulnerable to crown gall disease and furthermore prompt more reliable and proficient plant transformation methods, especially for important monocotyledonous crops (Yuan and Williams 2014).

15.5.1 Factor Affecting Agrobacterium *Plant Transformation*

An efficient, successful *Agrobacterium* plant transformation using desirable gene (s) for agronomically important traits largely depends upon the optimization of various factors which determine the rate of transformation frequency in different crop species (Ziemienowicz 2014; Kumar and Srivastava 2016; Kumar et al. 2018a). Various factors which are known to affect the *Agrobacterium* plant transformation frequency include plant species, explant type (hypocotyl, cotyledon, root, leaf, and stem), agrobacterial strain (LBA4404, EHA101, C58, AGL1), vector plasmid (pCAMBIA,

pGreen, pGA, pCG, pGPTV, Bi-BAC), culture medium compositions (micro- and macronutrient concentration, sugars, plant growth regulators), preculturing time, co-cultivation period (1–5 days; mostly: 24, 48, 60, 72 h), temperature of co-cultivation range 19–30 °C (optimal temp. dicots, 19–20 °C; monocots, 24–25 °C), pH of co-cultivation medium (acidic pH: 5.2, 5.5, 5.6, 5.8, or 6.0) and concentration of bacterial inoculum ($1\sim10^6$–$1\sim10^{10}$ cfu/ml), antibiotics (cefotaxime, carbenicillin, and kanamycin), selectable markers [neomycin phosphotransferase (*nptII*), phosphinothricin acetyltransferase (*pat*), and hygromycin phosphotransferase (*hpt*)], and effect of different inducers like acetosyringone. Extensive work to study the effects of all these factors has been carried out by various researchers during the *Agrobacterium* plant genetic transformation studies (Kavitah et al. 2010; Sood et al. 2011; Ziemienowicz 2014; Verma et al. 2014; Kumar et al. 2015, 2017, 2018a, b, c; Kumar and Srivastava 2015, 2016; Srivastava et al. 2016; Gambhir et al. 2017; Parmar et al. 2017; Shaunak et al. 2018).

Guo et al. (2012) reported that plant transformation efficiency in tomato varies depending on the selection marker and thus on the concentration of selective agent such as kanamycin and carbenicillin. Ziemienowicz (2014) reported the timentin effect on in vitro shoot regeneration and its use for the suppression of *Agrobacterium tumefaciens* bacterial growth in *Agrobacterium*-mediated genetic transformation of tobacco (*Nicotiana tabaccum*) and concluded that timentin may be an alternative antibiotic for the effective suppression of *A. tumefaciens* in genetic transformation. Kumar et al. (2017) studied the effects of antibiotics kanamycin and cefotaxime to determine the aptness of kanamycin resistance as a selectable marker and for cefotaxime in controlling excessive agrobacterial growth during *Agrobacterium* genetic transformation studies in broccoli (*Brassica oleracea* L. var. *italica* cv. Solan Green Head) using cultured hypocotyl, cotyledon, leaf, and petiole tissues. To find out the minimum concentration of kanamycin required for the selection of putative transformed cells during *Agrobacterium* transformation, increasing doses of kanamycin (10, 20, 30, 40, and 50 mg/l) to leaf and petiole explants were given. Leaf and petiole explants exhibited decreased in fresh weight as kanamycin concentration increased, resulting in full or partial inhibition of shoot regeneration. The nontransformed tissues did not survive on the selective medium containing kanamycin. A significant or nonsignificant negative correlation occurred between kanamycin concentration and explant fresh weight over the time. Cefotaxime also affects the plant regeneration potential and transformation efficiency. Similar findings were reported by Sharma (2010, 2014), Husaini (2010), Sharma et al. (2011), Aggarwal (2011), Ahmad et al. (2012), and Gambhir et al. (2017). Therefore, selection and identification of transgenic events from non transgenics are crucial steps of genetic transformation and kanamycin and cefotaxime proved as an effective selective agents in improving selection and transgenic regenerants efficiency.

In vitro manipulation of cultured explants was reported to improve the competency of plant cells for efficient T-DNA delivery and the subsequent recovery of transformed plant cells; however, it is largely species-dependent (Ziemienowicz 2014). Opabode (2006) had observed increased T-DNA delivery in rice explants when treated with sucrose, whereas desiccation improved the T-DNA delivery, and

stable transformation was recorded in sugarcane, maize, wheat, and soybean. Cardoza and Stewart (2003) and Opabode (2006) also observed that preculturing condition, co-cultivation time, and *Agrobacterium* density affect T-DNA delivery and integration in canola. Bacterial culture (*Agrobacterium*) density higher than $1–10^{10}$ cfu/ml usually damaged the cultured plant cells/tissues and resulted in poor transformation efficiency (Ziemienowicz 2014; Kumar and Srivastava 2016).

Different selectable marker genes such as *nptII* (neomycin phosphotransferase-II), *hpt* (*hygromycin phosphotransferase*), and *pat* (phosphinothricin acetyltransferase) under the control of constitutive promoters, i.e., CaMV35S, work efficiently for the selection of desirable transgene expression in *Agrobacterium*-transformed cells from the nontransformed cells in various crop species (Kumar and Srivastava 2016; Kumar et al. 2017). But use of antibiotic selectable marker genes(s) has been strongly opposed, criticized, and facing problems among consumer acceptance for genetically modified crops, due to biosafety concerns. Moreover, selectable marker gene(s) apart from transformed cell selections does not have any functional relevance inside the plant cell, and the constitutive expression of antibiotic marker gene-encoded proteins might affect the plant metabolism (Parmar et al. 2017). So as to increase the consumer acceptance for genetically modified crops and to minimize the biosafety concerns, recent advent in molecular biotechnology led to the development of clean-gene technology or marker-free transgenic technology. Different strategies have been used by various researchers to eliminate the selection marker gene for marker-free transgenic plant development such as co-transformation, homologous recombination, use of multi-auto-transformation vectors, transposition system, and site-specific recombinations (Gleave et al. 1999; Puchta 2000; Tuteja et al. 2012).

15.6 Recent Advances/Application in Plant Biology Research

Agrobacterium tumefaciens-mediated transformation has been remarkably considered as an indispensable tool in plant biology research for economically and horticulturally important crop plants. Significant findings have been achieved in the past using *Agrobacterium tumefaciens*-mediated transformation method for studies of gene expression, gene function, and protein localization. For gene identification and gene mapping studies using reverse genetics approaches, transferred DNA (T-DNA) has also been successfully employed to generate mutants. Its random insertion and integration into the genome resulted in the interruption of gene with a known DNA sequence and can be used for gene identification. Initially large-scale T-DNA insertion libraries were developed in model crop plants, i.e., *Arabidopsis* and rice (*Oryza sativa*), in late 1990 (Yuan and Williams 2014). The unifying properties of *A. tumefaciens* strains to transfer T-DNA to fungi and yeast also extend this technique to generate insertion mutant libraries. Till now, *Agrobacterium*-mediated transformation remains the only genetic tool available in many medicinally important fungal species (Yuan and Williams 2014).

Recently, in spite of the numerous ongoing improvements in plant biotechnology and development of efficient alternative gene transfer techniques, *Agrobacterium tumefaciens* still prevails as the oldest and widely used successful cellular organism for plant transformation. *Agrobacterium tumefaciens* is an indispensable tool in the targeted gene delivery of selected desirable gene(s) such as agronomically important traits which are not naturally present in the plant genotypes/wild germplasm with high efficiency for the production of transgenic plants. For the production of biotic (pest resistant, bacterial, fungal, and virus resistant) and abiotic stress (heat, cold, flood, and heavy metals)-tolerant plants, resistance to herbicide, increasing nutritional status (vitamins, lipids, amino acids, and proteins), recombinant therapeutic (protein, vaccine, antibodies) production, introduction of new traits (flower color modification, fruit ripening), metabolic pathway regulations (production of sugars, essential oil, nutrient accumulation), and bioremediation processes (pollution control), numbers of targeted desired gene(s) of interest available incorporated into host plants using *Agrobacterium tumefaciens*-mediated transformation procedure (Srivastava et al. 2016; Kumar and Srivastava 2016; Parmar et al. 2017). However, regardless of the significant achievement of *Agrobacterium*-mediated genetic transformation in a number of plant species ranging from model plants, cereal crops, medicinal plants, woody species, nuts and fruit crops, ornamental plants, turf grasses, legume crops, vegetable crops, and forest plant species using plant genetic engineering techniques, still some limitations of host range and development of efficient regeneration protocols are there, which need to be addressed.

For efficient reliable, high-throughput *Agrobacterium* transformation, in planta transformation procedures have been optimized by Feldmann and Marks (1987) in the model plant *A. thaliana* initially. Application of this developed method relies on minimized labor cost and expenses, does not depend upon tissue culture regeneration which is time-consuming, requires a lot of standardization and optimization procedures, and limits down the mutagenesis rate and somaclonal variations during in vitro regenerated cultures. Another highly efficient transformation procedure has also been developed by advancement in the technique such as floral dip method in *Arabidopsis* (Clough and Bent 1998) and vacuum infiltration (Bechtold and Pelletier 1998) in cereal crops, i.e., rice and maize (Chumakov et al. 2006) and wheat (Mamontova et al. 2010). Successful in planta transformation method has been introduced to design time- and labor-efficient methods for the production of transgenic plants by various researchers to transform a number of plant/crop species such as safflower (Rao and Rohini 1999), radish (Curtis 2003), *Brassica napus* (oil seed crop) (Wang et al. 2003), mulberry (Ping et al. 2003), cotton (Keshamma et al. 2008), and groundnut (Rohini and Rao 2008).

Recent strategy using *Agrobacterium* T-DNA-derived nano-complex has been used to transform recalcitrant crop plant species (Ziemienowicz 2014). Chugh et al. (2009) used this technique in preparing in vitro nano-complex that consisted of *Agrobacterium* transferred DNA (T-DNA) region, virulence protein ($VirD_2$), and single-stranded DNA-binding protein (RecA) then successfully delivered to wheat (triticale microspores) using Tat2 cell-penetrating peptide. This recently used novel approach resulted in single transgene copy integration events into the genome of triticale plants and also prevented DNA degradation. Chugh et al. (2009) also

observed integration of intact copies of the transgene and expression in all the regenerated transgenic plants, when triticale microspores were transfected with the prepared nano-complex. Using *Agrobacterium* nano-complex, the mediated method of transgene delivery has been proven highly efficient particularly in the crop species which are difficult to transform by other methods (Ziemienowicz et al. 2012). As cell-penetrating peptides were shown to transfer their DNA and proteins to monocot as well as dicot plants, this approach will be remarkably highly valuable for plant biology research (Chen et al. 2007; Chugh and Eudes 2008; Chugh et al. 2010).

15.7 Omics Approaches for Understanding Plant-Microbe Interaction Complexity

Omics-based approaches such as genomics, transcriptomics, proteomics, metabolomics, ionomics, and phenomics have accelerated the biological research and give insight to reveal the molecular mechanisms of plant-microbe interaction, insect resistance to pesticides, and plant tolerance to herbicides for better pest management, saving the time and expenses of producing better-quality food crops that are resistant to various stresses and exhibit a high nutritional value (Willig et al. 2018). Imam et al. (2016) have reported the use of various "omics" tools to understand beneficial and pathogenic effects of microbes and crop improvement with the recent advancement made in sequencing technologies. Interpreting plant-microbe interactions is a promising aspect to comprehend the advantages and the pathogenic effect of microbes using various "microbiomics" approaches which has impressively fast-tracked the ongoing research in biological sciences. Omics sciences enable a systems biology approach toward understanding the complex interfaces between genes, proteins, and metabolites within the resulting phenotype (Van Emon 2016). Plant-microbe interactions with special reference to *Agrobacterium tumefaciens*, which is an alpha-proteobacterium of the family *Rhizobiaceae*, affected a wide range of plants and act as a natural genetic engineer that makes it of great concern to the agriculture industry (Schenk et al. 2008). With the rapid change in climatic conditions, use of omics approaches and information on plant-pathogen interaction also broaden our ideas and perspective of a wide range of interaction (Torto-Alalibo et al. 2009; Knief 2014). Presently proteomics in blend with bioinformatics and computational biology approaches are generally utilized strategy to translate plant-pathogen association by the isolation, characterization, and expression profiling of the entire set of proteins inside a cell under specific conditions and time (Jayaraman et al. 2012; Imam et al. 2016). Proteomics approaches also have been applied to study protein-protein interactions involved in plant defense reactions and map protein modification which determines the difference between a wild-type and genetically modified organism. Similarly, metabolite-based approach, i.e., metabolomics, is also used to determine the nutritional difference between traditional and genetically modified crops, to identify plant defense metabolites, and to study differences at molecule/metabolite level between the healthy and diseased

plant. So utilization of interventions of omics science and technologies significantly augments crop productivity, crop protection, and crop management practices in modern agriculture (Willig et al. 2018; Pathak et al. 2018; Singh et al. 2019).

15.8 Conclusion

Plant-associated microorganisms fulfill important function of plant health and growth and remarkably contributed to provide disease resistance, enhance stress tolerance, aid nutrient availability and uptake, and promote biodiversity. With biotechnological advancement, significant progress has been made in the past and ongoing for better understanding of plant-microbe interactions so that better strategies can be implemented and the problem of global food and nutrition security can be resolved. *Agrobacterium*-mediated transformation is still a prevailing important biotechnological tool for the transfer of desirable characteristics to crop plants and supplements the classical plant breeding practices to enhance agricultural production. Despite the significant achievement of *Agrobacterium*-mediated genetic transformation in plant genetic engineering, some problems still need to be addressed such as transformation of recalcitrant economically important crops, host range, development of efficient plant regeneration protocol, and introduction of multiple stacked traits, transgene stability, and inheritance in further generation without gene loss. Better understanding of host-pathogen interactions and detailed investigation of plant proteins involved in assisting T-DNA delivery into the host plant genome, elucidation of *Agrobacterium* signaling pathways and regulatory mechanism, and identification and characterization of plant genes and proteins essential for *Agrobacterium*-mediated transformation will provide new insights useful for plant genetic engineering. In the twenty-first century, there is an urge for the implementation of a systems bio-agriculture integrated approach to achieve significant improvement in agriculture to solve the issue of global food and nutritional security.

Acknowledgments The senior author (PK) thankfully acknowledges the award of National Postdoctoral Fellowship, Science and Engineering Research Board, Department of Science and Technology, Government of India, New Delhi, India.

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