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A review on maize (*Zea mays* L.) transformation for expression of insecticidal proteins

M. GURUPRASAD[1], V. SRIDEVI[2], B. RAMESH[3], B. MANJUNATHA[4] AND M. SATISH KUMAR*[,5]

[1]*Department of Biochemistry*
Bharathiar University, Coimbatore-641 046 (Tamil Nadu), India
**(e-mail : pesmurari@gmail.com)*

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ABSTRACT

Biotechnology offers many techniques in case of human welfare i. e. crop improvement and nutrition. It has a potential progress in plant improvement by using molecular techniques. It also shows rapid progress in understanding the molecular, biochemical and physiological levels in plant improvement. The biotechnology tool shows how the plant gene transfers from bacteria to plant cell. By this tool, there is improvement in plant nutrition (golden rice) and pathogen resistant in crops. Today agriculture is facing many problems like cob borer and leaf smut disease. To overcome this problem, *Agrobacterium*-mediated transformation and biolostic methods are used for gene transfer for development of transgenic crop. So, we reviewed techniques like tissue culture, gene transfer, plant breeding and maintenace of germplasm. The tools of biotechnology were provided to investigator to develop a transgenic plant by tissue culture and gene transformation by *Agrobacterium*-mediated transformation and biolistic method. Later assessment was measured between *Agrobacterium*-mediated transformation and biolistic methods. So, in previous literature, the results evaluated that low copy number, expression and inheritance of gene had good transformation and more number of transgenic plants developed. Here, it was shown that low copy number, expression and inheritance were good in agrobacterium, when compared to biolistic gene transfer. These are due to more copy number which results in gene silencing. So, *Agrobacterium*- mediated transformation is better than the biolistic gene transfer.

Key words : *Agrobacterium* strain, biolistics, insecticidal proteins, maize (*Zea mays* L), transformation

Current Status of Transgenic Crop

The introduction of GM crops was in 1990. In developing countries, they have increased more than one-third (increase from 27%) of the global crop area. And from last nine-year period from 1996 to 2015, global area of biotech crops has increased more than 47-fold from 1.7 million ha to 81.0 million ha with an increasing proportion grown in developing countries. Among them, transgenic crops in the global area in 2011 were with *Bt* corn at 9.1 million hectares. The increased area and impact of the five developing countries (China, India, Argentina, Brazil and South Africa) growing biotech crops are an important and with implications for the future adoption and acceptance of biotech crops worldwide (ISAAA, 2015).

Transgenic Crops Area Country-wise

In 2011, there are around 10 countries in the world. Later, it has been increased to 14

[2]Department of Biotechnology, Sri Padmavati Mahila Visva Vidyalayam, Tirupati-517 502 (Andhra Pradesh), India.
[3]Department of Food Technology, Vikrama Simhapuri University, SPSR, Nellore-524 003 (Andhra Pradesh), India.
[4]Department of Life Sciences, Universidad de las Fuerzas Armadas-ESPE,Sangolquí- P. O. Box : 171-5-231B, Ecuador, South America.
[5]Department of Chemistry, P. E. S. College of Engineering, Mandya-571 502 (Karnataka), India.

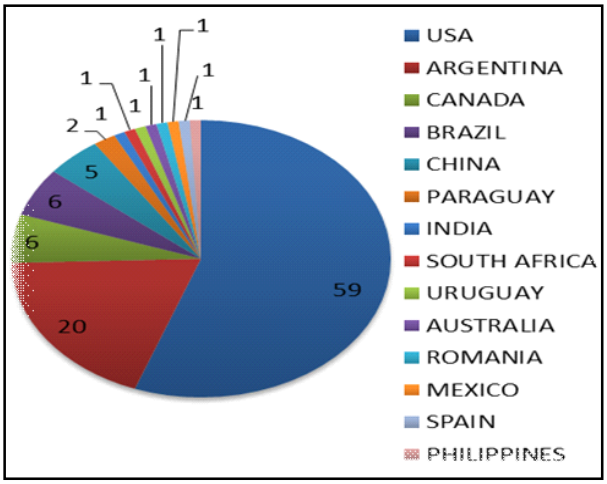

Fig. 1. Share (%) of transgenic crops in different countries during 1913.

countries by 2013 (Fig. 1). More than 59% increase of transgenic crops across the world by balancing, stabilizing and adapting biotech crops, USA with 47.6 million ha (59% of global total), followed by Argentina with 16.2 million ha (20%), Canada 5.4 million ha (6%), Brazil 5.0 million ha (6%), China 3.7 million ha (5%), Paraguay with 1.2 million ha (2%), India 0.5 million ha (1%), South Africa 0.5 million ha (1%), Uruguay 0.3 million ha (<1%), Australia 0.2 million ha (<1%), Romania 0.1 million ha (<1%), Mexico 0.1 million ha (<1 %), Spain 0.1 million ha (l %) and the Philippines 0.1 million ha (~1%). Based on annual percentage growth in area, of the eight leading biotech crop countries, India had the highest percentage year-on-year growth in 2014 with 400% in *Bt* cotton area over 2013, followed by Uruguay (200%), Australia (l00%), Brazil (66%), China (32%), South Africa (25%) and Canada (23%). Molecular tools of biotechnology made drastic change in crop improvement. It has solved the problems of traditional breeding and germplasm. By manipulating the genes, it changes gene pool diversity in related and unrelated species for useful needs to the farmers by desired character in single event. With this, transgenic crops were introduced to global countries in 1990 and became landmark to history.

Tissue Culture and Regenerations

The plant cell grows aseptically in nutrient medium, has an inherent capacity to give whole plant. Later the observations made into technique were called tissue culture. After enormous efforts and research, this technique was developed into crop improvement programme in agriculture, forestry and horticulture. In this investigation, it has been implemented to the agriculture. The capability of cells which develop into tissue and organ under suitable conditions, giving rise to whole plant, is called as totipotent or cellular totipotency.

Cellular Competence

Several factors such as morphological, cytological, biochemical and molecular aspects might play a vital role in distinction between totipotent and non-totipotent cells. The mixture of cells comprising the tissue explants varies physiologically, biochemically and developmentally (Lindsey and Yeoman, 1985), that leads to the cellular heterogeneity in which certain cells in an explant are "competent" enough to respond to the *in vitro* culture conditions (Potrykus, 1990). The capability of a cell or group of cells responding to an induced stimulus for a developmental process refers to the term "competence", which further represents a series of each of the genetic, epigenetic and physiological processes of the responding cell. The ability to induce differentiation in tissue culture is recognized as morphogenesis and the culture should comprise a population of cells that are physiologically and biochemically uniform and express synchronized pattern of cell division, enlargement and differentiation (Meinns and Binns, 1979). The totipotent cells develop into somatic embryos, shoot and root by altering developmental pathways with growth hormones. The competent cells/ explants play a key role in regeneration and gene transfer methods. For efficient regeneration system, factors affecting competent cell are genotype, source of explant, culture condition and growth regulators.

Effect of Genotype

In order to regenerate a plant through *in vitro* culture, the choice of the genotype forms the most important factor (Ritcbie and Hodges, 1993). The response of particular cultivars within a species responds differently to in vitro culture (Brown and Thorpe, 1986), which further illustrates that the genetic component

is highly influential on success of *in vitro* culture and plant regeneration (Hodges *et al.,* 1986). However, Vasil and Vasil (1986) stated that the genotypic effect could be solved by the use of correct combination of explant and culture condition used. Further the development of genotype-independent methods can provide efficient transformation procedures (Sharma and Anjaiah, 2000).

Source of Explant

The type of explant largely affects plant regeneration from immature organs, meristematic and undifferentiated tissues and young tissues form the most responsive and reliable explant sources (Vasil and Vasil, 1986). Genetic, epigenetic or physiological changes that occur in mature cells might be the reason for stage specific response (Halperin, 1986). Factors of the explant to be considered include size, orientation in culture, pre-treatment and inoculation density (Brown and Thorpe, 1986). Seedling explants form the most feasible explants for *in vitro* regeneration studies for their yearlong availability, uniformity and availability in large numbers (Geetha *et al.,* 1999).

Culture Conditions

Regeneration of complete plant from a single cell in controlled environment or *in vitro* was first attempted by Vasil and Hildebrandt (1965). Components of a medium include inorganic macro- and micro-nutrients, reduced nitrogen, carbon source, vitamins and growth regulators (Gamborg and Eveleign, 1968). Of these components, the concentration and ratios of the growth regulators have proven to be most culture initiation and morphogenesis (Vasil, 1988). In addition to the nutritional value, reduced nitrogen (Halperin, 1966) and sugar component (Brown and Thorpe, 1986) further affect morphogenesis. Many researchers have put forward various compositions of a nutrient medium for the growth of plant tissue (Gamborg and Eveleign, 1968). Inorganic salts are supplied in two groups as macro salts or nutrients and as micronutrients. The salts needed in higher amounts are called macronutrients, which include nitrogen, phosphorus, sulphur, magnesium, calcium and potassium. Nitrogen is mostly provided in two forms as nitrates and ammonium compounds. The salts needed in trace amounts are called as micro salts, which include boron, zinc, molybdenum, manganese, copper and iron, etc. Carbohydrate is supplied usually as sucrose. The most commonly used ammonium acid is glycine. Apart from nitrogen sources present in the inorganic salts, amino acids and amides are used in plant tissue culture media (Hu *et al.,* 1990). The most widely used amino acids L-aspartic acid, asparagine, L-glutamic acid, glutamine and L-arginine. Vitamins are required in trace amounts as they catalyse the enzyme system of the cells. Vitamins B1 (thiamine) are the most commonly used vitamins for all plant tissue cultures. Other groups of vitamins, which stimulate growth, are nicotinic acid, vitamin B2 (riboflavin), vitamin B_6-pyridoxine, vitamin C (ascorbic acid) and vitamin 12-cyanocobalamine (Hu *et al.,* 1990). Murashige and Skoog (1962) first reported the use of MS medium. It is the most preferred medium for tissue culture based studies on wide plant species. The medium was formulated with the entire essential macro and micro mineral elements and hence had become the first complete medium designed for *in vitro* culturing of diverse plant species. To date, MS medium is the most widely used culture medium for various tissue culture practices such as micro propagation, callus induction regeneration, etc. However, specific media for specific plant groups are available; for example, B_5 and L_2 media have been used for legumes (Thu *et al.,* 2003).

Growth Regulators

Several growth regulators are known to stimulate the biological activity in cultured plants. Shoot bud differentiate was first reported by Skoog (1944), *in vitro* cultured tobacco path. Cyto hormones promote cell divisions and regulate growth and development that include kinetin, a cytohormone that was discovered by Miller *et al.* (1955). Root-shoot differentiation in the above system was proposed by Skoog and Murashige. Miller (Murashige Skoog, 1962) and Hither stated that it was regulated by auxin-cytokinin ratio. The most widely used cytokinins are adenine, kinetin, zeatin, benzyl adenine and auxins are IAA (indole-3-acetic acid), NAA (a-naphthalene acetic acid), IBA (indole-3-butyric acid), and 2,

4-D (2, 4-dichlorophenoxyacetic acid), etc. The hormones are physiologically active and also determine the acidity and alkalinity of the culture media to a small extent. Based on the constituents, culture media are of two types, chemically defined and chemically undefined. In a chemically defined medium, the composition and concentration of all the constituents is known and it is prepared from inorganic and organic chemicals. Whereas in a chemically undefined medium, the concentration of the constituents is unknown due to the addition of natural products such as coconut milk, casein hydrosalate, etc. A medium can be solid, semi-solid or liquid. Further, culture media is influenced by physical factors such as light, pH and temperature (Evan *et al.,* 1981). The idea of culture of plant tissue was conceived in 1902 by Gottlieb Haberland of Berlin. He proposed that plant cells were totipotent i. e. isolated cells; if given the proper environment and nutrition they had the capacity to regenerate into entire plants. Actually, the concept of totipotent is implications in the statement of cell theory as Scheiden and Schwann expressed the view that each living cells of a multi cellular organism would be capable of developing independently if provided with the proper external conditions.

Regeneration Pathways

The regenerations differ from species to species. Various cells, tissues and organs from different species develop into whole plant. Regeneration pathways are : (a) organogenesis to callus, (b) organogenesis to whole plant without callus, (c) direct embryogenesis and (d) indirect embryogenesis. Majorly two pathways are put forward for regeneration i. e. 1. Organogenesis and 2. Embryogenesis.

Organogenesis

Organogenesis means the development of adventitious organs or primordial from undifferentiated cell mass in tissue culture by the process of differentiation. Alterations of the auxin and cytokinin ratios can control morphogenesis in tobacco was reported by Skoog and Miller (1957). The developmental stage and physiological state of the explant at the time of culture would affect the ability to induce direct organogenesis and differentiation without an intervening callus stage (Thomas and Davey, 1975). The hypothesis of organogenesis was advanced by Torrey (1966), who suggested that organogenesis via callus started with the development of a group of meristematic cells i. e. meristemoids that could respond to the factors within the system to initiate a primordial depending on the kinds of factors inducing either root, shoot or embroids. For the induction of organogenesis via callus on a particular medium, the medium should cause dedifferentiation (callus induction), attainment of competence, induction for the organogenic pathway and determination for the pathway and should not interfere with the morphogenic expression of the developmental pathway (Christianson and Warnick, 1985). Therefore, organogenesis was found to be the most reliable pathway for the regeneration of transgenic plants. *In vitro* organogenesis is controlled by a number of factors which are given below :

Size of explant
Source of explant
Age of the explants
Age of culture

Somatic Embryogenesis

In plant tissue culture, the developmental pathway of numerous well-organized, small embroids resemble the zygotic embryos from the embryogenic potential. Somatic plant cell of the callus tissue or cells of suspension culture is known as somatic embryogenesis.

Types of Somatic Embryogenesis

1. Direct embryogenesis

Cells of explant undergo direct embryogenesis from pro-embryonic determined cells in absence of callus proliferation. Direct embryogenesis was initially reported in *Glycine max* (Finer, 1988) and *Zea mays* (Vain *et al.,* 1989).

2. Indirect embryogenesis

Cells of explant first undergo callus proliferation and embroids develop within the callus tissue from induced embryogenic cells.

Indirect embryogenesis is a common pathway in which somatic embryos get induced and develop from proliferated callus (McWilliam *et al.,* 1974; Williams and Maheswaran, 1986). The explant most often used in indirect embryogenesis is the immature zygotic embryo (Finer, 1994). Adventitious embryonic has been reported in increasing number of plants, including cereals (Vasil, 1988), woody plants (Von Arnold, and Wallin, 1988) and recalcitrant tropical crops such as banana (Novak *et al.,* 1989).

Gene Cloning

Biotechnology plays an important tool in genetic engineering. It has made great success by introducing the foreign DNA and its gene expression. In genetic transformation, transfer gene is called as transgene and whole process is called transgenesis. In these, recombinant DNA plays a role in gene cloning and apart of genomic DNA (or) cDNA segment (or) specific gene linked to a vector which forms an rDNA molecule. Later the gene transfers to host cell. There are two types of vectors which carry a gene, (1) Binary vector (Hoekema *et al.,* 1983) and (2) A co-integrative vector (Fraley *et al.,* 1985). All these plant genetic vectors have essential features like multiple unique restriction sites, which facilitate to gene of interest, bacterial origin of replication and selectable markers. Initially co-integrated vectors are used for transfer of genes and are placed in host cells. In these vectors, the genes encoding growth regulators have been deleted to avoid interference with the regeneration of normal plants and are placed with dominant scorable and selectable markers (Bevan *et al.,* 1983). Most of the plasmid vectors in current carry a replicon derived from the plasmid P^{CAMBIA} (Ausubel, 1990). Plasmid vectors used for cloning have been specially developed by adding certain features like (a) Reduction in size of vector to a minimum; (b) Introduction of selectable markers and synthetic polycloning sites and (c) Incorporation of axillary sequences, etc. The process of gene cloning has following four essential components that include :

1. Cloning vehicles or vectors
2. Enzymes for cutting and joining the DNA fragment into vector molecules
3. DNA fragments i. e. gene libraries
4. Selection of a clone of transformed cells that has acquired the recombinant chimeric DNA molecule

Genetic Transformation Methods

Transformation means transfer of gene to nuclear genome of plant cell which is capable of giving rise to whole plant is called as genetic transformation. The major components for the development of transgenic plants are as under :

- The development of tissue culture regeneration systems
- Preparation of gene constructs and transformation with suitable vectors
- Efficient techniques of transformation for the introduction of genes into the crop plants
- Recovery and multiplication of transgenic plants
- Molecular and genetic characterization of transgenic plants for stable and efficient gene expression
- Transfer of genes to elite cultivars by conventional breeding methods
- Evaluation of transgenic plants for their effectiveness in alleviating the biotic and abiotic stresses in the field condition
- Biosafety assessment including health, food and environmental safety
- Growth of genetically modified plants

Types of Genetic Transformation

(i) *Agrobacterium* transformation
(ii) Biolistic or micro-projectile

***Agrobacterium*-mediated Gene Transfer**

The plant molecular biology and genetic engineering was basically the consequence of the discovery and study of the plant pathogen *Agrobacterium tumefaciens* (Gheysen *et al.,* 1987). *A. tumefaciens,* a gram-negative soil bacterium, causes crown gall tumors at the wound site of many dicotyledonous plants (Smith and Townsend, 1907). The crown gall formation is due to the transfer of a specific DNA fragment called the T-DNA (transfer DNA) from tumor inducing (Ti) plasmid of the bacterium to the plant cell. The transfer of T-DNA and its integration into the plant nuclear genome leads to crown gall phenotype (Schell

et al., 1979). T-DNA contains genes, which encode for the production of auxins (Akiyoshi *et al.,* 1984), and cytokinins (Barry *et al.,* 1984). Further, these genes encode for the production of enzymes involved in the synthesis of opines (Dessaux *et al.,* 1992). *Agrobacterium* strains were classified based on opines encoded by Ti plasmids as octopine, nopaline, agropine, succinmopine or chrysopine strains (Dessaux *et al.,* 1992; Vandequin-Dranrart *et al.,* 1995). The transfer of T-DNA into plants requires three bacterial genetic elements, which constitute (1) 25 bp imperfect repeats flanking T-DNA (Van Haaren *et al.,* 1988) acting in cis-orientation (Zambryski *et al.,* 1983); (2) Virulence genes *(vir* genes) encoded by Ti plasmid that are involved in processing of T-DNA and their transfer from the bacterium to the plant cell (Hooykaas *et al.,* 1994); and (3) Bacterial elements consisting of a number of bacterial chromosomal genes that are necessary for T-DNA transfer and for the attachment of bacterium to the plant cell.

Biolistics

Among the physical methods for artificial transfer of exogenous DNA, biolistic transformation is relatively novel method. The term 'biolistic' (biological ballistics) was coined to describe the transfer of foreign DNA into living cells or tissues through bombardments with particle gun. The method was developed to overcome the limitation of DNA delivery in other methods. It avoids the need of protoplasts and is better in efficiency. The method can be used for any plant cells, leaves, root sections, embryos, seeds and pollen. The method involves bombardment of panicles carrying DNA of interest onto target cells using high velocity transfer mechanism. Sanford *et al.* (1987) first developed the methods in 1987 and in their model system onion epidermal cells were used. Transferred genomic RNA of tobacco mosaic virus (TMV) using this method and 30-40% of the epidermal cells of onion showed the signs of virus replication. Boynton *et al.* (1988) carried out particle bombardment on *Chlamydomonas* that could stably integrate transgenes in the chloroplast genome. Subsequently, Svab *et al.* (1990) produced tobacco plants with stably transformed chloroplasts. The technique is finding universal application in the direct transformation of whole cells in culture, in tissue and in sub-cellular organelles such as mitochondria and chloroplasts.

Biolistics have been proven to be the most effective means of transformation of plant organelles (Daniell *et al.,* 1991). The particle gun has also been used with pollen, early-stage embroids, somatic embryos and meristems. Some target tissues such as embryogenic suspension cultures and meristematic tissue (McCabe *et al.,* 1988) have proven to be transformable and able to give rise to transgenic plants. Transgenic plants through bombardment were produced from wide range of crops including both in dicots and monocots important of crop species. This includes from shoots apices of maize (Zhang *et al.,* 1996), immature zygotic embryos of wheat (Becker *et al.,* 1994), immature inflorescence of *Tritordeum* (Barcelo *et al.,* 1994) leaves and pollen grains of tobacco and embryonic cell suspension of soybean (Finer and McMullen, 1991). Stable transformation was reported in maize and soybean (Kamm *et al.,* 1990), *Glycine max* % (Hadi *et al.,* 1996), *Gossypium hirsutum* (McCabe and Martinell, 1993), *Phaseolus vulgaris* (Zhang *et al.,* 1997), *Arachis hypogaea* (Brar *et al.,* 1994), *Oryza sativa* (Toriyama *et al.,* 1988), *Zea mays* (Lowe *et al.,* 1995), *Hordeum vulgare* (Ritala *et al.,* 1994), sorghum (Devi and Sticklen, 2001) and pearl millet (Devi and Sticklen, 2002). Although, the biolistic-mediated transformation technique has been shown to be highly successful with broad efficacy, due to its less precise in its transgene integration pattern, it may lead to multiple copy number and emergence of chimera flank limiting their further usage.

When the biolistic process is applied to plant tissues, plants regenerated from such tissue are usually chimeric in terms of introduced foreign gene due to random bombardment of small number of cells in a multiple system. In this method, DNA of interest is coated on heavy micro particles of tungsten or gold DNA used for coating the particles is first precipitated with $CaCl_2$, spermidine and polyethylene glycol. They are carried by nylon macro-projectile and are accelerated into living target cell at a very high initial velocity. Nylon macro-projectile is withheld in the barrel after a short while by the stopping plate, while only DNA coated macro-projectile enters the aperture of stopping

plate and continues to accelerate towards target cell and strikes it. This causes penetration of exogenous DNA through cell wall. For stable transformation to occur, the amount of DNA reaching the cells, thickness for the tissue being penetrated and potential of the target tissue or cell to the regenerate into plant are the important factors. Biolistic transformation technique has been shown to be successful with papaya, sugarcane, soybean, tobacco, maize, etc. Genomes of sub-cellular organelles have been made accessible to genetic manipulations by this method. This method is broader than that of *Agrobacterium* but less precise in its transgenic integration pattern.

Application of Biolistic/Micro-projectile Bombardment for the Production of Transgenic Plants

Following micro-projectile bombardment, foreign genes have been delivered and expressed in both dicots and monocots including economically important crop species such as soybean, wheat, corn and rice (Cao *et al.,* 1990). Biolistics have proved to be the most effective means of transformation of plant organelles (Dmiell *et al.,* 1991). Expression of the introduced genes was quantified by studies based on transgene regulation in specific plant cells or tissues (Seki *et al.,* 1991a). Transgenic plants were produced from wide range of crops through micro-projectile bombardment, that include the transgenic plants produced from bombardment of shoot apices of maize (Zhong *et al.,* 1996); immature zygotic embryos of wheat (Becker *et al.,* 1994) and immature inflorescence of *Tritordeum* (Barceló *et al.,* 1994). Leaves of tobacco (Tomes *et al.,* 1990) roots *of Arabidopsis* anthers of rubber tree (Arokiaraj *et al.,* 1994), pollen grains of tobacco (Stoeger *et al.,* 1995) and embryogenic cell suspension of soybean (Finer and McMullen, 1991).

The first bombardment experiment was carried out on *Chlamydomonas* that could stably integrate transgenes in the chloroplast genome (Boynton *et al.,* 1988) and the same approach was subsequently used by Svab *et al.* (1990) to produce tobacco plants with stably transformed chloroplasts. Varsha *et al.* (1996) emphasized the potentiality of micro-projectile bombardment in transforming plant cells to produce transgenic plants. Christou *et al.* (1989) reported first stable transformation of soybean, subsequently in maize (Kamm *et al.,* 1990). *Glycine max* and *Gossypium hirsutum* (McCabe and Martinell, 1993), *Phaseolus vulguris* (Zhang *et al.,* 1996), *Arachis hypogaea* (Brar *et al.,* 1994), *Zea mays* and *Hordeum vulgare* (Ritala *et al.,* 1994), sorghum (Devi and Sticklen, 2001) and pearl millet (Devi and Sticklen, 2002).

Comparative Studies of *Agrobacterium*-mediated Gene Transfer and Biolistic/Micro-projectile Bombardment

For micro-projectile bombardment, the most important is to first examine the following available factors :

- Tissue culture systems
- Determine the modes of regeneration
- Identification of potential explants
- Location of the cells involved
- Optimize tissue culture conditions to increase the number or accessibility of cells
- Develop conditions for non-lethal transfer of DNA into large number of such cells per bombardment.

Physical or micro-projectile bombardment methods for gene transfer do not involve any intermediate vector organism. No need of *Agrobacterium* strain. The size of the plasmid genetic sequence should be small, otherwise more than 10 Kb it gets into fragmentation (Pietnak *et al.,* 1986). There is no *Agrobacterium* and cell interaction during transformation. No need of antibiotic treatment for transgenic plants. No need of *vir* gene sequence. The absence of *Agrobacterium* also eliminates the possibility of accidental release of recombinant bacteria into the environment, reducing the possibility of unintentional movement of alien genes to non-target plants.

In *Agrobacterium* transformation, genetic sequences are introduced into disarmed Ti plasmids, which carry essential genetic elements required for the DNA transfer. The gene transfer occurs with modified pathogen process by *Agrobacterium*. DNA transfer takes place between plasmid carrying *Agrobacterium* and recipient host cell. A define DNA segment is cut from the Ti plasmid molecule transformed to recipient cell and integrated to plant chromosome. Whereas in physical method, gene transfer will not involve any intermediate vector.

REFERENCES

Akiyoshi, D. E., Klee, H., Amasino, R. M., Nester, E. W. and Gordon, M. P. (1984). T-DNA of *Agrobacterium tumefaciens* encodes an enzyme of cytokinin biosynthesis. *Proc. Natl. Acad. Sci., USA* **81** : 5994-98.

Arokiaraj, P., Jones, H., Cheong, K F., Coomber, S. and Charlwood, B.V. (1994). Gene insertion into *Hevea brasiliensis. Plant Cell Rep.* **13** : 42543.

Ausubel, F. M. (1990). Construction of recombinant DNA libraries, chapter 5 (b). Current protocols in molecular Biology. 2. John Wiley and Sons, NY.

Barcelo, P., Hagel, C., Becker, D., Martin, A. and Lonz, H. (1994). Transgenic cereal *(Tritordeum)* obtained at high efficiency by micro-projectile bombardment of inflorescence tissue. *Plant J.* **5** : 583-92.

Barry, G. F., Rogers, S. G., Fraley, R. T. and Brand, L. (1984). Identification of a cloned cytokinin biosynthetic gene. *Pro Acad. Sci., USA* **81** : 4776-80.

Becker, D., Brettscheider, R. and Lon, H. (1994). Fertile transgenic wheat from micro-projectile bombardment of scutellar tissue. *Plant J.* **5** : 299-300.

Bevan, M., Barnes, W. M. and Chilton, M. D. (1983). A chimeric antibiotic substance gene as a selectable marker for plant cell transformation. *Nature* **304** : 184-87.

Boynton, J. E., Gillham, N. W., Harris, E. H., Hosler, J. P., Johnson, A. M. and Jones, A. R. (1988). Chloroplast transformation in Chlamydomonas with high velocity micro-projectile. *Science* **240** : 1534-38.

Brar, G. S., Cohen, B. A., Vick, X. X. and Johnson, G. W. (1994). Recovery of transgenic peanut (*Arachis hypogaea* L.) plants from elite cultivars using ACCELL technology. *Plant J.* **5** : 745-53.

Brown, D. C. W. and Thorpe, T. A. (1986). Plant generations by organogenesis. In : *Cell Culture and Somatic Cell Genetics in Plants, Vol. 3,* I. K. Vasil (ed.). Academic Press, New York. pp. 49-66.

Cao, J., Wang, Y. C., Klein, T. M., Sanford, J. C. and Wu, R. (1990). Transformation of rice and maize using the biolistic process. In : *Plant Gene Transfer,* C. J. Lamb and R. N. Beachy (eds.), Wiley-Liss, New York. pp. 21-23.

Christianson, M. L. and Warnick, D. A. (1985). Temporary requirement for phytohormones balance in the control of organogenesis. In *Vitro Cell Dev. Biol. Pl.* **1** : 494-97.

Christou, P., Swain, W. F., Yang, N. S. and McCabe, D. E. (1989). Inheritance and expression of foreign genes in transgenic soybean plants. *Proc. Natl. Acad. Sci., USA* **86** : 7500-04.

Daniell, H., Krishnan, M. and McFadden, B. A. (1991). Transient expression of beta-glucuronidase in different cellular components following biolistic delivery of foreign DNA into wheat leaves and calli. *Plant Cell Report* **8** : 615-21.

Dessaux, Y., Petit, A. and Tempe, J. (1992). Opines in *Agrobacterium* biology in molecular signals in plant-microbe communications. CRC Press, Boca Raton. pp. 109-36.

Devi, P. and Sticklen, M. B. (2001). Culturing shoot-tip clups of *Sorghum bicolor* (L.) Moench and optimal micro-projectile bombardment parameters for transient expression. *J. Cytol. Genet.* **2** : 89-96.

Devi, P. and Sticklen, M. B. (2002). Culturing shoot-tip clumps of pearl millet [*Pennisetum glaucum* (L.) R. Br.] and optimal micro-projectile bombardment parameters for transient expression. *Euphytica* **125** : 45-50.

Dmiell, H., Krishnan, M. and McFadden, B. A. (1991). Transient expression of beta-glucourinidase in different cellular components following biolostic delivery of foreign DNA into wheat leaves and calli. *Plant Cell Rep.* **9** : 615-21.

Evan, D. A., Sharp, W. R. and Flick, C. E. (1981). Growth and behaviour of cell cultures embryogenesis and organogenesis. Methods and applications in agriculture. In : *Plant Tissue Culture,* T. A. Thorpe (ed.). McGraw Hill Publishers, New York & London. pp. 45-113.

Finer, J. J. (1988). Apical proliferation of embryogenic tissue of soybean [*Glycine max* (L.) Merrill]. *Plant Ccll Rep.* **7** : 238-41.

Finer, J. J. (1994). Plant regeneration via embryogenic suspension cultures. In : *Plant Cell Culture–A Practical Approach,* R. A. Dexon and R. A. Gonzales (eds.). Oxford University Press, Oxford. pp. 67-102.

Finer, J. J. and McMullen, M. D. (1991). Transformation of soybean via particle bombardment of embryogenic suspension culture tissue. *In Vitro Cell Dev. Biol. Pl.* **27** : 175-82.

Fraley, R. T., Rogers, S. G., Horsch, R. B., Eichholtz, D. A., Flick, J. S., Fink, C. L., Hoffman, N. L. and Sanders, P. R. (1985). The SEV system–a new disarmed Ti plasmid vector system for plant transformation. *Biotechnology* **3** : 629-35.

Gamborg, O. L. and Eveleign, D. E. (1968). Cultured methods and a detection of glucanases in suspension cultures of wheat and barley. *Can. J. Biochem.* **46** : 417-21.

Geetha, N., Venkatachalam, P. and Lakshmi, S. G. (1999). *Agrobacterium*-mediated genetic

transformation of pigeonpea (*Cajanus cajan* L.) and development of transgenic plants via direct organogenesis. *Plant Biotechnol.* **16** : 213-18.

Gheysen, G., Van Montagu, M. and Zambryski, P. (1987). Integration of *Agrobacterium tumefaciens* transfer DNA (T-DNA) involves rearrangement of target plant DNA sequences. *Proc. Natl. Acad. Sci., USA* **84** : 6169-73.

Hadi, M. Z., McMullen, M. D. and Finer, J. J. (1996). Transformation of 12 different plasmids into soybean via particle bombardment. *Plant Cell Rep.* **15** : 500-05.

Halperin, W. (1966). Alternative morphogenetic events in cell suspensions. *Amer. J. Bot.* **53** : 443-53.

Halperin, W. (1986). *Cell Culture and Somatic Cell Genetics of Plants, Vol. 3.* Academic Press, New York. pp. 3-47.

Hodges, T. K., Kamo, K. K., Imbrie, C. W. and Beware, M. R. (1986). Genotype specificity of somatic embryogenesis and regeneration in maize. *Biotechnology* **4** : 219-23.

Hoekema, A., Hirsch, P. R., Hooykaas, P. J. and Schilperoort, R. A. (1983). A binary plant vector strategy based on separation of vir and T-region of the *A. tumefaciens* Ti plasmid. *Nature* **303** : 179-81.

Hooykaas, P. J. J. and Beijersbergen, A. G. M. (1994). The virulence system of *Agrobacterium tumefaciens. Annu. Rev. Phytopathol.* **83** : 529-34.

Hu, C. Y., Chee, P. P., Chesney, R. H., Zhou, J. M., Miller, P. D. and O'Brien, W. T. (1990). Intrinsic gus like activities in seed plants. *Plant Cell Rep.* **9** : 1-6.

Kamm, G. W., Spencer, T., Mangano, M., Adams, T. R., Daines, R. J., Start, W. G., O'Brien, J. V., Chambers, S. A., Adams, W. R. and Willetts, N. G. (1990). Transformation of maize cells and regeneration of fertile transgenic plants. *Plant Cell* **2** : 603-18.

Lindsey, K. and Yeoman, M. M. (1985). Dynamics of plant cell cultures. In : *Cell Culture and Somatic Cell Genetics of Plants, Vol. 2,* I. K. Vasil (ed.). Academic Press, New York. pp. 61-101.

Lowe, K., Bowen, B., Hoerster, G., Ross, M., Bond, D., Pierce, D. and Gordon-Kamm, B. (1995). Germ line transformation of maize following manipulation of chimeric shoot meristem. *Biotechnology* **13** : 679-82.

McCabe, D. E. and Martinell, B. J. (1993). Transformation of elite cotton cultivars via particle bombardment of meristems. *Biotechnology* **11** : 596-98.

McCabe, D. E., Swain, W. F., Martinell, B. J. and Christou, P. (1988). Stable transformation of soybean (Glycine *max)* by particle acceleration. *Biotechnology* **6** : 923-26.

McWilliam, A. A., Smith, S. M. and Street, H. E. (1974). The origin and development of embryoids in suspension culture of carrot (*Daucus carota*). *Am. Bot.* **38** : 243-50.

Meinns, F. and Jr. Binns, A. N. (1979). Cell differentiation in plant development. *Bioscience* **29** : 221-25.

Miller, C. O., Skoog, F., von Salbn, M. H. and Strong, F. M. (1955). Kinetin, a cell division factor from deoxyribonucleic acids. *J. Amer. Chem. Soc.* **77** : 1392.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay mith tobacco tissue culture. *Physiol. Plant.* **15** : 473-97.

Novak, F. J., Afza, R., Van Duren, M., Perea-Kallos, M., Longer, B.V. and Xiolang, T. (1989). Somatic embryogenesis and plant regeneration in suspension cultures of desert (AA and AAA) and cooking (ABB) banana (*Musa* spp.). *Biotechnology* **7** : 154-59.

Pietnak, M., Shillito, R. D., Hohn, T. and Potrykus, L. (1986). Expression in plants of two bacterial antibiotic substance genes after protoplast transformation with a new plant expression vector. *Nucleic Acids Res.* **14** : 5857-68.

Potrykus, I. (1990). Gene transfer to cereals–An assessment. *Biotechnology* **8** : 535-42.

Ritala, A., Aspergren, K., Kurten, U., Salmenkallio-Marttila, M., Mannonen, L., Hannus, R., Kanppinen, V., Teeri, T. H. and Enari, T. M. (1994). Fertile transgenic barley by particle bombardment of immature embryos. *Plant Mol. Biol.* **24** : 317-25.

Ritcbie, S. W. and Hodges, T. K. (1993). Cell culture and regeneration of transgenic plants. In : *Transgenic Plants, Engineering and Utilization, Vol. 1,* S. D. Kung and R. Wu (eds.). Academic Press. pp. 147-78.

Sanford, J. C., Klein, T. M., Wolf, E. D. and Allen, N. (1987). Delivery of substances into cells and tissues using a particle bombardment process. *Particulate SCI Tech.* **5** : 27-37.

Schell, J., Van Montagu, M., DeBeuckeleer, M., DeBlock, M., Depicker, A., De Wilde, M., Engler, G., Genetello, C., Hernalsteens, J. P., Holsters, M., Seurinck, J., Silva, B. S., Van Vliet, F. and Villarroel, R. (1979). lnteractions and DNA transfer between *Agrobacterium tumefaciens,* the Ti-plasmid and the plant host. *Proc. Royal Soc., London* **204** : 251-66.

Seki, M., Komeda, V., Lida, A. F., Yamada, Y. and Morikawa, H. (1991a). Transient expression of beta-glucuronidase in *Arabidopsis thaliana* leaves and roots and *Brassica napus* stems using a pneumatic particle

gun. *Plant Mol. Biol.* **17** : 259-63.

Sharma, K. K. and Anjaiah, V. (2000). An efficient method for the production of transgenic plants of peanut (*Arachis hypogaea* L.) through *Agrobacterium tumefaciens* genetic transformation. *Plant Sci.* **159** : 7-19.

Skoog, F. (1944). Growth and organ formation in tobacco cultures. *Amer. J. Bot.* **31** : 19-24.

Skoog, F. and Miller, S. (1957). Chemical regulation of growth and organ formation in plant tissue cultured *in vitro* sympsoc. *Exp Biol.* **11** : 118-40.

Smith, E. F. and Townsend, C. O. (1907). A plant tumor of bacterial origin. *Science* **25** : 671-73.

Stoeger, E., Fink, C., Pfosser, M. and Heberle-Bore, E. (1995). Plant transformation by particle bombardment of embryogenic pollen. *Plant Cell Rep.* **14** : 273-78.

Sva'b, Z., Hajdukiewicz, P. and Malign, P. (1990). Stable transformation of plastids in higher plants. *Proc. Natl. Acad. Sci., USA* **87** : 8526-30.

Thomas, E. and Davey, M. R. (1975). From single cell to plants. Wykeham, London.

Thu, T. T., Mai, T. T. X., Dewaele, E., Farsi, S., Tadesse, Y., Angenon, G. and Jacobs, M. (2003). *In vitro* regeneration and transformation of pigeonpea [*Cajanus cajan* (L.) Millsp.] *Mol. Breed.* **11** : 159-68.

Tomes, D. T., Weissinger, A. K., Ross, M., Higgins, R., Drummond, B. J. and Schaff, S. (1990). Transgenic tobacco plants and their progeny derived by micro-projectile bombardment of tobacco leaves. *Plant Mol. Biol.* **14** : 261-68.

Toriyama, K., Arimoto, Y., Uchimiya, H. and Hinata, K. (1988). Transgenic rice plants after direct gene transfer in to protoplasts. *Biotechnology* **6** : 1072-74.

Torrey, J. G. (1966). The initiation of organized development in plants. *Adv. Morphogen.* **5** : 39-91.

Vain, P., Yean, H. and Flament, P. (1989) Enhancement of production and regeneration of embryogenic type II class in *Zea mays* L. by $AgNO_3$. *Plant Cell Tissue Organ Cult.* **18** : 143-51.

Van Haaren, M. J. J., Sedee, N. J. A., Krul, M., Sehilperoot, R. A. and Hooykaas, P. J. J. (1988). Function of heterologous and pseudo border repeats in T region transfer *vir* the octopine virulence system of *Agrobacterium tumifaciens. Plant Mol. Biol.* **11** : 773-81.

Vandequin-Dranrart, V., Petit, A., Poneet, C., Ponsonnet, C., Nesme, X., Jones, J. B., Bouzar, H., Chilton, W. S. and Dessaux, Y. (1995). Novel Ti plasmids in *Agrobacterium* strains isolated from fig tree and chrysanthemum tumors and their opine like molecules. *Mole. Plant Microbe Interactions* **8** : 311.

Varsha, R. K. D., Luthra, R. and Kumar, S. (1996). Micro-projectile mediated plant transformation–A bibilographic search. *Euphytica* **95** : 269-94.

Vasil, I. K. and Hildebrandt, A. C. (1965). Differentiation of tobacco plants from single isolated cells, microcultures. *Science* **150** : 889-90.

Vasil, I. K. (1988). Progress of the regeneration and genetic manipulation of cereal crops. *Biotechnology* **6** : 397-402.

Vasil, I. K. and Vasil, V. (1986). Regeneration in cereal and other grass species. In : *Cell Culture and Somatic Cell Genetics of Plants, Vol. 3,* I. K. Vasil (ed.). Academic Press, New York.

Von Arnold, S. and Wallin, A. (1988). Tissue culture methods for clonal propagation of forest trees. *IAPTC Newsl.* **56** : 2-13.

Williams, E. G. and Maheswaran, G. (1986). Somatic embryogenesis factors influencing coordinated behaviour of cells as an embryogenesis group. *Ann. Bot.* **57** : 443-62.

Zambryski, P., Joos, H., Genetello, C., Leemans, J., Van Montagu, M. and Schell, J. (1983). Ti-plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity. *EMBO J.* **2** : 2143-50.

Zhang *et al.* (1996). Regeneration of fertile transgenic *indica* rice plants following micro-projectile transformation of embryogenic suspension culture cells. *Plant Cell Rep.* **15** : 465-69.

Zhang, Z., Coyne, D. P. and Mitra, M. (1997). Optimization of parameters influencing biolistic DNA transformation of common bean. *Improvement Cooperative, USA* **40** : 7-8.

Zhong, H., Sun, B., Warkentin, D., Zhang, S., Wu, R., Wu, T. and Sticklen, M. B. (1996). The competence of maize shoot meristems for integrative transformation and inherted expression of transgenes. *Plant Physiol.* **110** : 1097-1107.