BIOTECHNILOGIES USED IN GENETIC TRANSFORMATION OF *TRITICUM AESTIVUM: A mini overview*

JAMSHAID HUSSAIN*, SEHRISH MANAN, RAZA AHMAD, TOQEER AHMED AND MOHAMMAD MAROOF SHAH

Department of Environmental Sciences, COMSATS Institute of Information Technology, Abbottabad-Pakistan.

Corresponding author e-mail: jamshaidhussain@ciit.net.pk

Abstract

Wheat improvement through genetic transformation has huge potential to meet its ever growing demand. Various methods have been devised and used to achieve this goal. The list includes methods like electroporation, micro-injection, liposome-mediated, biolistic and the *Agrobacterium tumefaciens*-mediated transformation method. Electroporation is effective method of DNA delivery into the target cells however it can be applied to protoplasts only. This has been main disadvantage as protoplast cultures for most of the cereals are strongly genotype dependent and difficult to obtain. Microinjection method, to some extent, is considered targeted gene delivery method. Its use has been limited, so far, for cereal transformation. Moreover it demands highly skilled personals and sophisticated technology. Stable transgene integration has been reported with liposome mediated gene transfer method. Like electroporation, this technique also relies on protoplasts culture which has been its obvious limitation. Genetically modified plants have been developed through particle bombardment method. It has been second most popular method adopted for wheat improvement. However this technique leads to integration of multiple copies of transgene at single locus that ultimately results in gene silencing. *Agrobacterium tumefaciens*-mediated transformation method is most famous method and frequently used for developing transgenic plants, mainly due to its advantages like simple integration pattern, being less laborious, and cost effective. In recent years significant progress has been made to successfully employ this method for transformation of monocotyledonous plants. However, in the case of wheat, involvement of tissue culture has been main hurdle so far, which is mainly because of recalcitrant nature of wheat. In past few years, improved methods have been developed that bypass *in vitro* culturing during transformation method.

Introduction

According to different estimates and analyse, by year 2050, grain production needs to be increased at an annual rate of 2%, to meet human demands (Umer *et al.*, 2008). This increase in production can possibly be increased by two ways; either through increasing the area of crop production or through increasing the yield potential of cultivable wheat varieties. The former option is not feasible as growing population also needs more space for housing and other needs. However, the option is achievable through the use of advance scientific knowledge and techniques (Sramkova *et al.*, 2009). Human beings started wheat improvement right after its domestication. In early days farmers used to select plants for large grains and other desirable characters. After gaining better knowledge they started to cross desirable plants to get hybrids with known properties. Wheat breeding started in nineteenth century, when single line varieties were developed through selection of plants with desired properties. First disease resistant variety, using conventional breeding technique, was developed by Norman Borlaug and his team. Japanese dwarf variety Norin 10 was crossed with disease resistant variety to produce semi dwarf disease resistant and high yield varieties of wheat (Borloug, 1953). This achievement is the base of “Green Revolution”.

A significant increase in yield and development of resistant varieties is unlikely to be attained only through conventional breeding. In recent years, recombinant DNA technology has become a powerful tool for crop improvement. Biotechnology not only increases the efficiency of conventional breeding techniques, but also opens the door for controlling key traits through genetic manipulation. Some uncultivated and closely related varieties of wheat have genes for better quality traits. Although some of these genes have been integrated into wheat genome for genetic improvement, however neither wheat germplasm nor its close relatives are likely to contain the wide variety of genes that would be required to meet future demands. Therefore, novel methods and approaches must be explored to integrate useful genes from any organism (plants, animals or microorganisms) to attain the dramatic yield improvements.

Various DNA delivery methods have been used for integration and expression of foreign gene into wheat genome. In the following sections these methods will be discussed in brief.

Electroporation: Electro-permeabilization or electroporation is used to create small holes in plasma membrane of the cells. These temporary holes permit the cell to uptake foreign substance that may be a piece of DNA, molecular probe or any drug. After a short period of time, pores will reseal automatically. Intensity of electric current is an important factor determining the success of the experiment. An increase in electric voltage may cause cell damage. By applying controlled intensity of electric field and cell's exposure to it, cell damage can be avoided. Electroporation has been proved to be applicable gene transfer method for plants, microbes and animals (Haliloglu *et al*; 2004). Using electroporation as a transformation tool, significant success has been achieved with cereals like maize and rice (Formm *et al*., 1986, Yang *et al*., 1988). It was used to produce herbicide resistance wheat plants, through anther culture-derived embryos as explant (Haliloglu *et al*; 2004).
The electroporation has no effect on regenerable capacity of transformed cells. Being genotype independent it can be used to transform large varieties of plants. Electroporation is simple and effective method with high DNA delivery rate. It can be used to deliver DNA into large number of cells within a very short time (Kathleen *et al*., 1992). However, in plant research main drawback of electroporation-mediated gene transfer is its dependence on protoplasts. Protoplast cultures for most cereals are difficult to obtain and strongly genotype dependent. Thus, success of electroporation technique for wheat transformation is limited (Harris *et al.,* 1988).

Microinjection: In microinjection method the desired gene is injected into the nucleus of the cell (Floras *et al*., 1981). This approach, to some extent, is considered targeted gene delivery method. Cultured tissues, that encourage continued development of immature structures, provide cellular targets for transformation. These immature structures may include immature embryos, meristems, immature pollen, isolated ovules, embryogenic suspension, cultured cells etc (Brown and Thorpe., 1995, Akin-Idowu et al., 2009). The main disadvantage of this technique is the production of chimeric plants with only a part of the plant transformed. Frequencies of somaclonal variations are high with this method of plant transformation (Peschke and Phillips, 1992). When cells or protoplasts are used as targets in the technique of microinjection, glass micropipettes each with 0.5-10µm diameter tip are used for transfer of macromolecules into the cytoplasm or the nucleus of a recipient cell or protoplast (Floras et al., 1981). Recipient cells are artificially bound to a surface (cover slip or slide) for injecting foreign DNA into the cell (Mercer et al., 1982). Although success rate is high but microinjection transformation demands highly skilled personals, more over it is expensive and slow. This technique could not be successfully repeated with cereals (Potrykus, 1991**)**.

Liposome mediated gene transfer: In this technique, large number of plasmids is enclosed in small lipid bags called liposome. Liposome transfer genes on fusion with protoplast; that is mediated using chemicals like PEG (polyethylene glycol). DNA enters the protoplasts due to endocytosis of liposome. As plasmids carrying foreign DNA are enclosed in lipid bags; it provides protection to foreign DNA/RNA from nuclease digestion.

Using liposome-mediated method, npt-II gene, under the control of 35S CaMV promoter, was introduced into protoplast of hexaploid wheat. Chimeric gene was transferred through PEG-mediated DNA uptake. Transformed protoclones show high kanamycin, geneticin and neomycin resistance. Treated protoplast shows transformation frequency between 1 and 2.25×10^{-6} (Mathur *et al.,* 1995).

Besides other advantages, liposome mediated gene transfer method results in stable gene integration. However its big disadvantage has been large number of rearrangements taking place in the host genome (Marsan, 1993).

Particle bombardment: Biolistics approach of transformation involves coating of naked DNA on submicron particles of tungsten or gold. Linear or plasmid both forms of DNA are used in particle bombardment. Gold or tungsten DNA coated particles are bombarded at high velocity into recipient cells (Sanford, 1988; Sanford *et al.,* 1993). DuPont discovered first helium driven particle bombardment system. That was marketed as PDS1000/He by BioRad. Other devices such as the particle inflow gun and the ACCELL electrical discharge technology have also been used successfully for micro-projectile bombardment. Chloroplast and mitochondrion genomes have also been successfully engineered through biolistics approach (Sanford *et al.,* 1993). Effective DNA-transfer has also been reported using *Escherichia coli* or *Agrobacterium* cells as micro-projectiles (Rasmussen *et al.,* 1994).
Particle bombardment effectively distributes DNA over a wide area of the target tissue and is relatively genotype independent. However, several parameters must be optimized for particular explants including the microprojectile type, size and quantity. Helium pressure, propellant force, and target distance of accelerating device also contribute to success of stable DNA integration. Efficiency and stability of integrated DNA can be improved by controlling device parameters (Altpeter *et al.,* 1996; Ingram *et al.,* 1999; Perl *et al.,* 1992; Harwood *et al.,* 2000; Rasco-Gaunt *et al.,* 1999).

Although biolistics transformation method is base for many other effective wheat transformation methods (Sparks and Jones, 2004), but complex transgene integration patterns cause problems in consequent analysis. Particle bombardment integrates various copies of foreign gene at single locus that ultimately results in gene

silencing (Kohli *et al.,* 2003; Sparks and Jones, 2004). Using particle bombardment, transgenic wheat plants, expressing high molecular weight glutenin subunit, were successfully developed (Yao *et al.,* 2006).

Agrobacterium-mediated transformation: Soil borne pathogen *Agrobacterium tumefaciens* is causative agent of crown gall disease. It has ability to transfer a small segment of its DNA (T-DNA) into the host cell's genome. *Agrobacterium tumefaciens-mediated* method is a preferred and method of choice due to unique advantages like the ability to transfer larger DNA segments, minimal probability of rearrangement, insertion of fewer copies of inserted genes, and simple, low cost technology (Dai *et al.,* 2001; Smith *et al.,* 1995). Initially *Agrobacterium tumefaciens*-mediated genetic transformation method was limited to dicotyledonous plant species; in early 1980's first transgenic plants were developed through this method (Gasser and Fraley, 1989). In late 1980's and through 1990s many dicotyledonous plant species had been successfully transformed through *Agrobacterium* method. In spite of having wide range of hosts *Agrobacterium* does not infect monocots in general. After successful demonstration in dicotyledonous plant, efforts began to transform monocotyledonous plants through *Agrobacterium*-mediated transformation method. To date successful transformation events have been reported for monocotyledonous plants with the addition of phenolic compound acetosyringone and with the use of hypervirulent strains (Buck *et al*; 2007). The first breakthrough was achieved in 1994, with successful transformation of rice; the first amongst cereals (Hiei *et al.,* 1997). Now focus was shifted on wheat, one of main staple crop throughout the world. Finally success was achieved in 1997 when first *Agrobacterium*-mediated wheat transformation was reported by Cheng *et al.,* since then many groups have made different attempts to develop an efficient protocol for wheat transformation (Cheng *et al.,* 1997).

Agrobacterium-mediated transformation can be carried out either with or without involvement of tissue culture. In former method, suitable explants are grown on proper medium for callus induction. The calli are infected with *Agrobacterium*, successful transformants are selected and regenerated to obtain transgenic plants. The latter method doesn't involve any *in vitro* culturing and includes techniques like floral dip, seed inoculation method etc.

Agrobacterium mediated transformation via tissue culture: As described above, Transformation through tissue culture firstly involves culture of explant on artificial medium to induce callus formation. The callus is further infected with *Agrobacterium* containing gene of interest. The *Agrobacterium*-mediated transformation of wheat was first reported in 1997 by Cheng *et al*, who used immature embryos and embryogenic calli in a tissue-culture system to produce fertile transgenic wheat plants. Based on Southern blot analysis of 26 events, approximately 35% of the transgenic plants received a single copy of the transgene (Cheng *et al*. 1997).

The major limitation in improvement of wheat transformation through *Agrobacterium* is the successful regeneration of plant from transformed callus (He *et al*; 2010). So, this method is limited to some varieties of plants; that can be regenerated easily. Transformation frequency of wheat can be enhanced by manipulating the factors influencing T-DNA delivery e.g *Agrobacterium* cell density, inoculation period, surfactants and induction chemicals in inoculation media (Zeb *et al.,* 2009).

Agrobacterium-mediated transformation without tissue culture: *Agrobacterium* mediated transformation has not yet become an established and robust method for genetic transformation of wheat because it is strictly genotype dependent. This is mainly due to the large differences in the abilities for callus induction and regeneration among wheat varieties (Supartana *et al*., 2005; Jones 2005). For most of the wheat varieties callus induction and subsequent cell culture has not been possible till to date. Owing to these limitations, in past decade, few techniques have been devised that does not rely on tissue culturing. These techniques include floral dip method and seed inoculation method.

Floral dip method: A floral transformation protocol for wheat was introduced by Zale *et al.,* (2009). It is a simple method that bypasses tissue culture technique. The spikes at different developmental stages were, twice, dipped in suspension of *Agrobacterium tumefaciens* carrying the gene of interest. Transformation was found to be more efficient when spikes were 6-7 cm long and enclosed in sheath. This corresponds to mid to late uni-nucleate microspore stage (4-7 days before anthesis). Few independent transformants of Crocus wheat were created through this method with transformation efficiency of 6.8%. Molecular analysis of transformants showed transgene stability in three to six generations (Zale *et al.,* 2009).

In floral dip method, flower developmental stage is critical factor for successful transformation of wheat (Zale *et al.,* 2009).

In planta method: A new *Agrobacterium*-mediated "*in planta*" transformation protocol has recently been reported by few workers (Razaq *et al.,* 2010; Supartana *et al.,* 2005). This method involves transformation of embryo by making small holes in pre-soaked seeds and introducing the solution of Agrobacterium carrying gene of interest. Different methods have been devised for wounding the seeds e.g. sonication, needle prick and

vacuum infiltration (Chen *et al.,* 2010). In a recent report, embryos of mature seeds were incised with sharp blade, after removing the seed coat. Processed seeds were pricked with a bunch of acupuncture pins dipped in *Agrobacterium* solution harbouring gene of interest (Razaq *et al.,* 2010). The inoculated seeds were allowed to mature into seedlings, transferred to pots and exposed to natural environment (Razaq *et al.,* 2010; Chen *et al.,* 2010). Seeds were collected from transformants and selected on the basis of antibiotic resistance. "*In planta"* transformation method overcomes the disadvantages of the conventional *Agrobacterium*-mediated transformation methods that rely on successful *in vitro* culturing. "*In planta"* transformation method is applicable to wheat varieties that are recalcitrant to regeneration, which is its greatest advantage (Supartana *et al.,* 2005).

References

Akin-Idowu, P.E., Ibitoye, D.O. and Ademoyegun, O.T. (2009). Tissue culture as a plant production technique for horticultural crops. *African Journal of Biotechnology* 8(16): 3782-3788.

Altpeter, F., Vasil, V., Srivastava, V., Stoger, E. and Vasil, I.K. (1996). Accelerated production of transgenic wheat (Triticum aestivum L.) plants. *Plant Cell Reports* 16: 12-17.

Borlaug, N.E. (1953). New approach to the breeding of wheat varieties resistant to *Puccinia graminis tritici. Phytopathology* 43: 467.

Brown, D.C.W. and Thorpe, T.A. (1995). Crop improvement through tissue culture. *World Journal of Microbiology & Biotechnology* 11: 409-415.

Buck, H.T., Nisi, J.E and Salomon, N. (2007). High throughput *Agrobacterium* transformation of wheat: A tool for functional genomics. *Springer* 693-699.

Chen, X., Equi, R., Baxter, H., Berk, K., Han, J., Agarwal, S. and Zale, J. (2010) A high-throughput transient gene expression system for switchgrass (*Panicum virgatum* L.) seedlings. *Biotechnology for Biofuels* 3: 9: available online at: http://www.biotechnologyforbiofuels.com/content/3/1/9.

Cheng, M., Fry, J.E., Pang, S.Z., Zhou, H.P., Hironaka, C.M., Duncan, D.R., Conner, T.W. and Wan, Y.C. (1997). Genetic transformation of wheat mediated by *Agrobacterium tumefacien*ss. *Plant Physiology* 115: 971-980.

D'Halluin, K., Bonne, E., Bossut, M., Beuckeleer, M.D. and Leemans, J. (1992) Transgenic Maize Plants by Tissue Electroporation. *The Plant Cell* 4: 1495-1505.

Dai, S., Zhang, P., Marmey, P., Zhang, S., Tian, W. Chen, S., Beachy, R. and Fauquet, C. (2001). Comparative analysis of transgenic plants obtained by Agrobacterium-mediated transformation and particle bombardment. *Mol Breed* 7: 25-33.

Floros, J., Jonak, G., Galanti, N. and Baserga, R. (1981). Induction of cell DNA replication in GI-specific mutants by microinjection of SV40 DNA. *Exp. Cell Res.* 132: 215-223.

Formm, M.E., Taylor, L.P. and Walbot, V. (1986) Stable transformation of maize after electroporation. *Nature* 319: 791-793.

Gasser, C.S. and Fraley, R.T. (1989). Genetically engineering plants for crop improvement. *Science* 244: 1293-1299.

Haliloglu, K., Stephen Baenziger, P. and Mitra, A. (2004). Genetic transformation of wheat (*Triticum astevium*) anther culture derived embryos by electroporation. *Biotechnol and Biotechnol* 63-68.

Harris, R., Wrght, M., Byrne, M., Varnum, J., Brightwell, B. and Schubert, K. (1988). *Plant Cell Rep.* 7: 337-340.

Harwood, W.A., Ross, S.M., Cilento, P. and Snape, J.W. (2000). The effect of DNA/goldparticle preparation technique and particle bombardment device, on the transformation of barley (Hordeum vulgare). *Euphytica* 111: 67-76.

He, Y., Jones, H.D., Chen, S., Chen, X.M., Wang, D.W., Li, K.X., Wang, D.S. and Xia, L.Q. (2010). *Agrobacterium*-mediated transformation of durum wheat (*Triticum turgidum* L. var. durum cv Stewart) with improved efficiency. *Journal of Experimental Botany* 61: 1567-1581.

Hiei, Y., Komari, T. and Kubo, T., (1997). Transformation of rice mediated by Agrobacterium tumefaciens. *Plant Molecular Biology* 35: 205-218.

Ingram, H.M., Power, J.B., Lowe, K.C. and Davey, M.R. (1999). Optimization of procedures for microprojectile bombardment of microspore-derived embryos in wheat. *Plant Cell Tissue and Organ Culture* 57: 207-210.

Jones, Huw D. (2005). Wheat transformation: Current technology and applications to grain development and composition. *Journal of Cereal Science* 41(2): 137-147.

Kohli, A., Twyman, R.M., Abranches, R., Wegel, E., Stoger, E. and Christou, P. (2003). Transgene integration, organization and interaction in plants. *Plant Molecular Biology* 52: 247-258.

Marsan, P.A., Lupotto, A., Locatelli, F., Qiao, Y. and Cattaneo, M. (1993). Analysis of stable events of transformation in wheat via PEG-mediated DNA uptake into protoplasts. *Plant Science* 93: 85-94.

Mathur, J., Szabados, L. and Koncz, C. (1995) A simple method for isolation, *Arabidopsis thaliana* protoplast liquid culture, transformation and regeneration of *Plant Cell Rep.* 14: 221-226.

Mercer, W.E., Nelson, D., DeLeo, A.B., Old, L.J. and Baserga, R. (1982). Microinjection of monoclonal antibody to proteinp53 inhibits serum-induced DNA synthesis in 3T3 cells. *Proc. Natl. Acad. Sci.* U.S.A. 79: 6309-6312.

Perl, A., Kless, H., Blumenthal, A., Galili, G. and Galun, E., (1992). Improvement of plant-regeneration and gus expression in scutellar wheat calli by optimization of culture conditions and DNA-microprojectile delivery procedures. *Molecular and General Genetics* 235: 279-284.

Peschke, V.M. and Phillips, R.L. (1992). Genetic implications of somaclonal variation in plants. *Adv. Genet.* 30: 41-75.

Potrykus, I. (1991). Gene transfer to plants: Assessment of published approaches and results. *Annual Rev. Plant Physiol Plant Mol. Biol.* 42: 205-255.

Rasco-Gaunt, S., Riley, A., Barcelo, P. and Lazzeri, P.A. (1999). Analysis of particle bombardment parameters to optimize DNA delivery into wheat tissues. *Plant Cell Reports* 19: 118-127.

Rasmussen, J.L., Kikkert, J.R., Roy, M.K. and Sanford, J.C. (1994). Biolistic transformation of tobacco and maize suspension cells using bacterial cells as microprojectiles. *Plant Cell Reports* 13: 212-217.

Razzaq, A., Hafiz, AI., Mahmood, I. and Hussain, A. (2010). Development of *in planta* transformation protocol for wheat. *Afr. J. Biotechnol* 10: 740-750.

Sanford, J.C. (1988). The Biolistic Process. *Trends in Biotechnology* 6: 299-302.

Sanford, J.C., Smith, F.D. and Russell, J.A. (1993). Optimizing the biolistics process for different biological applications. *Method Enzymol* 217: 483-509.

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

Sparks, C.A. and Jones, H.D. (2004). Transformation of wheat by biolistics, in: Curtis, I. (Ed.), Transgenic Crops of the World-Essential Protocols. Kluwer, *Dordrecht*; 19-35.

Sramkova, Z., Gregova, E. and Sturdik, E. (2009).Genetic improvement of wheat- A review. Nova *Biotechnologica* 9: 27-51.

Supartana, P., Shimizu, T., Shioiri, H., Nogawa, M., Nozue, M. and Kojima, M. (2005). Development of simple and efficient *in planta* transformation method for rice (Oryza zativa L.) using *Agrobacterium tumefacien*ss. *J. Biosci. Bioeng.* 100: 391-397.

Umer, J., Ghazala, N. and Ghulam, R. (2008). Correlation and regression of production of wheat leaf rust severity in Bahawalpur and Multan using relevant metrological data. *Mycopath* 6: 23-29.

Yang, H., Zhang, H.M., Davey, M.R., Mulli-gan, B.J. and Cocking, E.C. (1988). Transgenic rice plants produced by electroporation-mediated plasmid uptake into protoplast. *Plant Cell Rep.*7: 421-425.

Yao, Q., Cong, L., Chang, J.L., Li KX, Yang, G.X., Shewery, P. and He GY (2006). Low copy number gene transfer and stable expression in a commercial wheat cultivar via particle bombardment. *J. Exp Bot.* 57: 3737-3746.

Zale, M.J., Agarwal, S., Loar, S. and Steber, C.M. (2009). Evidence for stable transformation of wheat by floral dip in *Agrobacterium tumefacien*ss. *Plant Cell Rep* 28: 903-913.

Zeb, B., Ahmad, I.K., Ali, A., Bacha, S., Mumtaz, S. and Ahmed, Z.S (2009). Study on genetic diversity in Pakistani wheat varieties using simple sequence repeat (SSR) markers. *African Journal of Biotechnology* 8 (17): 4016-4019.