Engineering plants for future: tools and options

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ABSTRACT

The availability of efficient techniques for genetic engineering of plants across taxonomic boundaries is a must to address the challenges posed by the global growth of the human population. This will shorten the time and accelerate the entire process needed for inclusion of novel traits in plants with potential to increase agricultural productivity, improved nutritional quality as well as processing characteristics. This mini-review summarizes current understanding, latest advancements and comparisons of various methods used to date to generate transgenic plants with a special focus on the biological mode of gene delivery into plants. [Physiol. Mol. Biol. Plants 2008; 14(1&2) : 131-135] E-mail : veena@danforthcenter.org

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Global human population is forecasted to reach 7.5 billion by 2020, of whom more than 90% will be in the developing and under-developed countries. The increasing population in developing and under-developed countries puts unforeseeable strain on natural resources, infrastructure, services and governments at all levels as food security becomes the primary concern to avoid widespread famine. The Green revolution that occurred between 1940s and 1970s has been credited with significant agricultural productivity. This increase played an important role in avoiding devastating famine in several countries including India. In addition to increase in human population, new challenges in the recent times are creating an unavoidable impact on the very concept of food security. While the need to improve nutritional value has long been emphasized to reduce malnutrition, a recent challenge comes with increased demand and decreased production of oil. As the discussion broadens on the use and development of alternative energy sources such as bio-fuels (i.e., bio-ethanol and bio-diesel), it will no doubt put further strain on food security. To meet such challenges, there is a constant need to develop new technologies that can offer the cost effective solutions for some of the old problems hindering sustainable achievement of food security. This mini-review outlines basic concepts and technological advances of methods towards improving plant traits that are being utilized to meet grand

challenges for now and future along with a brief discussion about their advantages and disadvantages.

Classical plant breeding to biotechnology

Several food crops cultivated now a days are result of the domestication selection and improvement through classical plant breeding techniques. Many of these crops were originally less productive and at times unsuitable for human consumption. Over the years, traditional plant breeding and selection of these crops have resulted in plants that are more productive and nutritious. Classical plant breeding involves the deliberate interbreeding (crossing) of closely or distantly related individuals to produce new crop varieties or lines with desirable properties. In this approach, plants are crossbred to introduce traits/genes from one variety or line into a new genetic background. It relies largely on homologous recombination between chromosomes to generate genetic diversity. The practice of classical plant breeding is estimated to date back 9,000-11,000 years. Modern plant breeding approaches involves the use of a number of in vitro techniques such as protoplast fusion, embryo rescue or mutagenesis to generate diversity and produce hybrid plants that would otherwise not exist in nature. Even though these approaches of improving crop production has been proven to be very successful over the years, on average it takes 10-15 years of crossing and backcrossing of plants for several generations to produce a new variety. Moreover its use also results in manifestation of undesirable traits in plants as it is difficult to select for only one trait without affecting

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others. Another important limitation is the restricted use of this approach to plants that can mate sexually.

New technologies, such as recombinant DNA technology are being used to overcome the limitations of classical plant breeding and offers new opportunities for fast and efficient methods of crop improvement. Such combinatorial approaches employ molecular techniques to identify, select and modify DNA sequences for a specific genetic trait from a donor organism (microorganism, plant or animal), and transfer the DNA to the recipient organism to complement or suppress a given trait. Incorporation of such technologies enables scientists to genetically modify plants, animals and microorganisms with novel traits beyond the capabilities of traditional breeding and selection technologies. Genetic modification is often faster than conventional breeding techniques, as stable expression of a trait is achieved using far fewer breeding generations. The molecular and genetic principles of plant biotechnology and classical plant breeding and selection are the same, except that it allows precise alteration of an organism than conventional breeding approaches. More importantly it is not restricted by taxonomic boundaries.

Methods of gene delivery in plants – current and future prospects

The basic approach of genetic modification in plants involves the manipulation of its DNA by transformation. Various transformation techniques have been developed to assist the transfer of recombinant DNA into recipient plant species. These transformation techniques involve the insertion of a piece of DNA containing one or more genes from one organism into a chromosome/genome of another organism. In plants, it can be mediated by either a biological vector such as *Agrobacterium tumefaciens* (a common soil bacterium that contains natural ability of inter-kingdom transfer of genetic material) (reviewed in Gelvin, 2003; Tzfira and Citovsky, 2006) or by a direct gene delivery system [such as gene gun, electroporation, microinjection, lasers, polyethylene glycol (PEG), silicon carbide fibers and honeycomb mesoporous silica nanoparticle (MSN) system] that utilizes physical, electrical or chemical means to deliver gene of interest (reviewed in Barcelo and Lazzeri, 1998; Torney *et al.*, 2007). To ensure easy selection of the transgenic plants, gene of interest is often accompanied with an extra segment of DNA known as “selectable marker gene”. These selectable markers can be either a visual marker or an antibiotic resistance gene. However in recent years, several new strategies have been developed to generate “marker free” transgenic plants to address the growing concerns of consumers on the usage of antibiotic

resistance markers in the transgenic plants. These technologies either depend upon promoting the regeneration of transgenic plants after transformation or employ the use of site specific recombination, homologous recombination, transposition and co-transformation for the elimination of marker gene genes soon after transformation (Puchta *et al.*, 2003). Once selected transformed plants are characterized in detail using state of art molecular biology tools. Desired transgenic plants are selected and further reproduced to generate individuals that possess the desired trait. These individuals in turn are raised and utilized with the desired gene actively functioning. Since the first demonstrations of the *Agrobacterium* or direct gene delivery in plant transformation (Barton *et al.*, 1983; Paszowski *et al.* 1984), intensive effort has been devoted to understand the mechanism of the plant transformation, improving the efficiency of procedures and applying them in wide variety of plant species (Gelvin 2003; Herrera-Estrella *et al.*, 2005; Vain, 2007).

Plant transformation mediated by *A. tumefaciens*, a soil plant pathogenic bacterium, is the most popular and economical method for the introduction of foreign genes into plant cells and the subsequent regeneration of transgenic plants. *A. tumefaciens* has the exceptional ability to transfer a particular DNA segment (T-DNA) of the tumor-inducing (Ti) plasmid into the nucleus of infected cells where it is then stably integrated into the host genome and transcribed, causing the crown gall disease (reviewed in Gelvin, 2003; Tzfira *et al.*, 2004). As the keystone of plant transformation, *A. tumefaciens* has been subjected to rigorous studies, resulting in the establishment of several disarmed strain variants (which vary in their host range and transformation efficiencies), along with numerous plasmids and protocols that are uniquely adapted for the genetic transformation of various plant species (see *Agrobacterium* Protocols, 2006). Although *A. tumefaciens* can be used to transform wide range of plants, there are number of plant species of interest in which *A. tumefaciens*-mediated transformation is not reproducible or efficient. In order to overcome this barrier, significant advances have been made in developing direct gene transfer techniques that utilizes a range of vectors including those employed for gene cloning. Just like *A. tumefaciens*-mediated transformation, these methods can be applicable towards both stable and transient gene expression studies. Major advantage of the direct gene delivery methods is that it is species and genotype-independent. However, transformation efficiencies obtained with these methods are generally very low and often influenced by the type of target cell. In addition, these methods are very costly,

require special preparation of the target tissues and specialized equipments thereby limiting their use for routine plant transformation purposes. As a result, *A. tumefaciens*-mediated transformation has remained the preferred system for gene transfer as with a susceptible host. Use of biological mode of DNA delivery by *Agrobacterium* also offers advantages of integrating a fewer copies of foreign DNA into the host plant genome with high efficiency and without the degree of rearrangement or truncation that typically occurs with physical DNA delivery methods (Gelvin, 1998; Palanichelvam *et al.*, 2000; Pawlowski and Somers, 1996), and potentially leads to fewer problems associated with transgene co-suppression and instability (Hansen *et al.*, 1997; Koncz *et al.*, 1994).

Given the advantages of using biological mode of delivering DNA into the plants, most of the previous attempts to improve the genetic transformation of plants and enhancing its host were based on the genetic manipulation of *A. tumefaciens* itself, improvements in plant tissue culture and regeneration conditions. *Agrobacterium*-mediated plant transformation is a highly complex and evolved process involving genetic determinants of both the bacterium and the host plant cell (for details see reviews: Citovsky *et al.*, 2007; Gelvin, 2003; McCullen and Binns, 2006). *Agrobacterium* infection is now known to suppress host defense response and cause alterations in several basic cellular processes of the host cells for genetic modification during the event of successful plant transformation. The complexity of *Agrobacterium*-plant interactions is just beginning to emerge and better understanding of the molecular basis of these interactions would result in significant advancement in extending the utility of *Agrobacterium* in genetic transformation of wide range of agronomically important plants for crop improvement. For example, several plant genes have been identified using the approach of forward and reverse genetics that plays an important role in the process of *A. tumefaciens*-mediated transformation of plants (Zhu *et al.*, 2003). The utilization of functional genomics approach has revealed the comprehensive integrated response by plants during the process of *Agrobacterium*-mediated transformation and further extended the number of plant genes known to be involved in the process of *A. tumefaciens* mediated transformation (Deeken *et al.*, 2006; Ditt *et al.*, 2006; Veena *et al.*, 2003). Some of these examples are genes related to chromatin remodeling, importin- α and genes related in defense responses. Further studies on these genes have conclusively shown that manipulation of the host gene expression can result in enhancement in transformation efficiencies and host range of *A.*

tumefaciens mediated transformation (Bhattacharjee *et al.*, 2007; Crane and Gelvin, 2007; Veena *et al.*, 2007a; Zhu *et al.*, 2003).

Various members of the genus *Agrobacterium* such as *A. rhizogenes*, *A. rubi*, and *A. vitis* are well known for their variable host range and susceptibility. However, there are no serious attempts made to domesticate the use of these related members of *Agrobacterium* strains which can be harnessed to develop new vectors for gene delivery in plants. Of these related genus, *A. rhizogenes* offers significant hope in improving the transformation efficiency in several agronomically important plant species recalcitrant to *A. tumefaciens*. *A. rhizogenes* is a Gram-negative, rod-shaped soil bacterium responsible for the development of the hairy root disease (also called root-mat disease) in plants. More than 450 species of plants (mostly dicots) are known to be susceptible to infection by *A. rhizogenes* (De Cleene and De Ley, 1981; Diouf *et al.*, 1995; Porter, 1991; Yibrah *et al.*, 1996; Tepfer, 1984). The root-inducing plasmid (Ri-plasmid) is similar in structure and composition to the tumor-inducing (Ti) plasmid found in *A. tumefaciens*. In *A. rhizogenes*, the processing of the transferred DNA (T-DNA) from the Ri-plasmid, its transport and integration into the host plant's chromosomal DNA is considered very much similar to that of *A. tumefaciens*. The integration of the Ri encoded T-DNA(s) and subsequent expression of genes encoded within the T-DNA leads to the formation of "hairy roots". Current interest in the *A. rhizogenes* is mainly due to its hairy disease causing characteristics (such as plagiotropic root growth, with a high degree of lateral branching, profusion of root hairs and enhanced growth rates similar to that of undifferentiated callus and their ability to grow in the absence of exogenous plant growth regulators and hormones), that has manifested its use mainly in secondary metabolic production and metabolic engineering (Georgiev *et al.*, 2007; Guillon *et al.*, 2006; Srivastava and Srivastava, 2007). Even though various members of *A. rhizogenes* have been known for their susceptibility to large number of different species of plants including those that are recalcitrant to *A. tumefaciens*, no attempts have been made to generate disarmed variants of *A. rhizogenes*. Our group has been working on understanding the mechanism of hairy root formation and harnessing the wider host range infection capabilities of *A. rhizogenes* for routine plant transformation purposes (Veena *et al.*, 2007a,b). We have recently generated the disarmed variant of *A. rhizogenes* K599 strain, a hyper-virulent strain and demonstrated that it is free of any disease causing abilities and can be successfully used for generating transgenic plants (Veena *et al.*, 2007b) with relatively enhanced

transformation efficiencies in several plant species (such as *Arabidopsis*, tomato, soybean) compared to widely used disarmed strain of *A. tumefaciens* C58C1-pMP90 (similar to GV3101). Disarming of various other strains of *Agrobacterium* may result in addressing some of the issues related to recalcitrance or low transformation efficiencies of agronomically important crop species.

In addition to bacterial genus *Agrobacterium*, other bacterial groups also contain species capable of inter-kingdom genetic exchange. A study by Broothaerts *et al.*, (2005) has generated renewed interest in further exploration of other bacteria such as *Rhizobium* spp. NGR234, *Sinorhizobium melliloti* and *Mesorhizobium loti*, for plant transformation purposes. In their studies, authors have demonstrated that these non-*Agrobacterium* species possess capability for horizontal gene transfer and hence can be potentially used as a gene delivery system for routine plant transformations. These bacterial species, when provided with the machinery needed for the transformation process such as a set of virulence genes encoded by an *Agrobacterium* Ti-plasmid and the T-DNA segment residing on a separate, small binary plasmid, can lead to transient as well as stable transformation of various plant species (Broothaerts *et al.*, 2005). However, transformation frequencies as of now obtained with these bacteria are substantially lower than those routinely obtained through use of strains of *A. tumefaciens*.

CONCLUSION

The mode of DNA/gene delivery in plants has been proven to be an invaluable tool in a wide variety of applications for basic and applied researches in plant sciences. Even though some functional plant transformation tools are available to us, there are several unexplored areas which can be uncovered to develop new cost effective and highly efficient biological systems for plant transformation. The development of such new plant transformation technologies will provide economical and versatile biotechnological tools for the scientific and commercial communities in their constant pursuit of improving plant traits to meet challenges of human needs.

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