**New Techniques of Gene Modification in Plants that Overcome Previous Challenges Using Magnetic Nanoparticles as Gene Carriers**

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**Humans have been genetically enhancing the plants and animals around them for 30,000 years through the practice of selective breeding (Zimmer, 2013). Previous advances in technology have allowed researchers to directly insert genes from one organism into another to produce traits beneficial to the organism through the process of transformation (Cohen et al, 1973). However, it is still difficult and time-consuming to create genetically modified organisms in plants as almost all current methods require regeneration from tissue cultures (Zhao et al, 2017). Recently, a new method, pollen magnetofection, has been proven to be able to be directly inserted into pollen spores via a magnetic nanoparticle, producing transformed seeds and bypassing the need for regeneration. Pollen magnetofection greatly reduces the time needed to create transgenic plants while maintaining pollen/seed viability and can be used to genetically modify almost all flowering plants, including most crops (Zhao et al, 2017).**

**Introduction**

By the year 2050, it is projected that the human population will rise to 9.8 billion individuals, and crop production will need to increase by 70% to meet global demands (Northoff, 2009). Fulfilling these needs is difficult due to limited land availability and growing concerns over environmental degradation associated with current agricultural methods. Genetic engineering has become a vital tool in improving agriculture in recent years, and more research is being conducted to further improve crop yields and efficiency. Genetic engineering can be used to transfer desired characteristics (e.g. enhanced resistance to abiotic and biotic stressors) from one organism to another (Ahmad et al, 2012). However, it is still challenging to quickly and easily generate genetically modified crops as most modern methods require regeneration from tissue cultures. Regeneration, or the process of inducing totipotency (i.e. the ability of a cell to divide and produce all cell types present in an organism) in cultured plants cells to grow a new plant, is a long, labor intensive process which ultimately has a low rate of success (Hansen & Wright, 1999). In addition, many crop species are especially difficult to modify through traditional techniques and thus new methods need to be employed to widen the diversity of crops that can be genetically enhanced (Zhao et al, 2017). Specifically, cotton is notorious as being an extremely difficult plant to induce growth in a genotype independent manner after genetic modification (i.e. cotton cells will revert genetic modifications made during the regeneration process; Kumria et al, 2003)

Transformation, or the genetic alteration of a cell by introduction of extraneous DNA, especially by a plasmid, is a process commonly used to create genetically modified organisms. Transforming pollen is a promising alternative to traditional plant transformations, as pollen releases active DNA during pollination and fertilization. Exogenous DNA (i.e. DNA originating from outside the organism of study) can be inserted into pollen, and the resulting transformed pollen can create transgenic seeds with the desired characteristics. Previous attempts to create DNA-transformed pollen through traditional methods have not been very successful, and “low frequency transformations, non-reliable transformation efficacy and requirement of sophisticated equipment have limited their wide application” (Zhao et al, 2017).

Nanobiotechnology is currently being applied to provide new techniques in creating DNA-transformed pollen. One such application, magnetofection, uses magnetism to force exogenous DNA into target cells. These magnetic nanoparticles (MNPs), such as Fe3O4, can be coated in a positively charged substance that attracts and binds DNA, converting the nanoparticles into mobile carriers for transformative DNA. A magnetic field can then be induced to force the DNA-bound nanoparticles into small apertures within pollen wall structure. The surface apertures are areas on pollen where the wall is thinner, allowing for greater permeability into the cell (Zhao et al, 2017).

**Recent Progress**

MNP-DNA complexes were successfully created and characterized by electron microscopy, dynamic light scattering, zeta potential analysis, and agarose gel electrophoresis. Electron microscopy confirmed that MNPs have a spherical shape and that large amounts of DNA was bound to and condensed by MNPs. Zeta potential, which measures the stability of emulsions (i.e. how well DNA binds to MNPs), also confirmed that the positively charged MNPs were complexing with the electronegative DNA. Furthermore, band analysis of gel electrophoresis proved that DNA was completely bound to the MNPs as they migrated together, and that the MNP-DNA complex inhibited the digestion of DNA through ligases (Zhao et al, 2017).

A key factor that plagued previous pollen-transforming techniques was pollen viability. Cotton was chosen as a model because it has been notoriously hard to genetically modify through transformation due to its difficult regeneration. Pollen magnetofection proved to not cause damage to pollen as germination rates did not differ. This is thought to be due to the fact that MNPs are extremely small and can easily pass through the apertures found in pollen walls, reducing the perturbation common in other methods (Zhao et al, 2017).

Fluorescent microscopy confirmed that MNPs were internalized by cotton pollen, transferred to the pollen tube, and migrated within the tube as it elongated. Additionally, transmission electron microscopy was performed to further confirm that MNPs were located within the pollen and pollen tubes. The fact that the MNPs migrated along the pollen tube suggests that the MNPs are involved during fertilization (Zhao et al, 2017).

Expression of exogenous DNA through magnetofection confirmed that MNPs could successfully transform cotton pollen. Plasmids containing the GUS gene, which enzymatically stains blue in the presence of an X-gluc solution, were transformed into cotton pollen and a resulting blue coloration confirmed that the pollen was expressing the GUS enzyme. Similar transformations were prepared with a plasmid encoding kanamycin resistance and insect resistance and transgenic seeds were produced via artificial pollination. T1, T2, and T3 generations were created after initial kanamycin screening and self-pollination. Gene expression was characterized with various PCR techniques, dot blot, Southern blot, and ELISA. These assays revealed that the plasmids were incorporated into the genome at multiple sites, that the genes of interest were being transcribed, and that the proteins were effectively expressed in the transgenic cotton plants. Additionally, bioassays were performed to show the insecticidal effects of the transgenic cotton plants. The results showed that the cotton was effectively transformed and became resistant to pests. Due to the multiple integrations of the plasmids into the genome, transgenic pure lines could only be achieved after three generations. However, pure lines will not have segregation of the desired genes and will pass on the genes to the next generation (Zhao et al, 2017).

**Discussion**

Pollen grains are ideal targets for transformation techniques as they are numerous, can be easily separated, and provide direct entry of foreign DNA into the germline through artificial pollenation. Various methods have been proposed to target pollen grains, but none have been able to ensure reproducibility, efficiency, and stability. Pollen magnetofection has been proven to successfully integrate exogenous DNA into pollen and create stable, transgenic plants with superior traits (Zhao et al, 2017).

The MNPs proved to be excellent carriers for transporting desired genes into pollen as they stabilized the plasmids and did not interfere with DNA function. MNPs were effectively taken into pollen tubes and delivered exogenous DNA for integration into the genome during fertilization. The overall process was simple compared to previous methods and did not require intensive equipment. The process of pollen magnetofection did not perturb pollen grains and therefore did not affect pollen viability. Experimental conditions can be improved upon to increase transgenic events as various handling and environmental factors affect germination rates (Zhao et al, 2017).

Since pollen magnification specifically targets pollen, any flowering plant could effectively be genetically modified through this technique. Transgenic pepper and pumpkin crops were also successfully transformed in this experiment and shows that the methods used in this experiment can be useful in transforming a wide variety of crop species with various beneficial traits. The limitation, however, is that the structure of pollen may vary from species to species, so different plants will still need to be studied to examine if they could be modified in this way (Zhao et al, 2017).

Overall, pollen magnetofection decreases the time it takes to successfully transform and genetically modify new crop species. It has also shown great success in areas that traditional transformation methods failed in. This system shows great promise for the eventual modification of many more crop species to adapt to changing environments and/or meet the demands of a growing populace (Zhao et al, 2017). As the global population continues to grow towards nearly 10 billion persons, drastic changes to current agriculture methods need to be made. Sustainability, environmental protection, and increased yields are all characteristics that future crops will need to have, and pollen magnetofection will allow for the easier transformation of current crops to yield these characteristics.

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