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Chapter 10

Integration of Genetic Engineering and Nanoscale Technologies for Innovative Phytobiological Modulation

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Abstract: From ancient times to today, humans have rapidly advanced technology to improve their lifestyles. However, food demand has become a major challenge due to limited agricultural land and a growing population. To address this, advancements in genetic engineering and nanotechnology have emerged. Genetic engineering modifies genes using techniques like CRISPR-Cas9 to develop stress-resistant crops, enhancing production. At the nanoscale, nanotechnology plays an important role in plant biotechnology by remediating gene delivery, biocompatibility, and plant regeneration. While widely applied in microbial and material sciences, its use in plants is still developing. Combining genetic engineering and nanotechnology can revolutionize agriculture, improving crop systems and production. More research is needed on nanomaterials, their interaction with genes, their efficiency, and their potential risks. Advancing these technologies will help combat global hunger and ensure food security by enhancing agricultural sustainability and efficiency.

Keywords: Genetic engineering, food, genes, improvement, nanotechnology, plant production.

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1 Introduction

Plants are the basis of the food cycle on Earth. They have been an essential component of each ecosystem, whether land or marine. They are the vital resources of food, medicine, timber, and raw materials for industry. Plants are also an important component of the biological cycle. Socially, plants have always been a vital part of human existence. However, changes like the increase in population, climate change, pollution, global warming, diseases, and urbanization have been a threat to the population and diversity of plants (Singh *et al.,* 2021; Squire *et al.,* 2023). To subdue these problems, scientists have been developing various technologies that can enhance the populations of plants even in unfavourable circumstances.

Plants have been primary source of food. From the era of the green revolution to modern times, many advanced technologies have been introduced to sustain the population and diversity of plants, but the rising population has raised the problem of food security and nutritional security of the global population. Therefore, it is important to continue researching and implementing innovative ideas in agriscience technologies to ensure sustainable food production for future generations. These problems have concerned many researchers to develop various genetic manipulation techniques, and one of them is plant genetic engineering. The growing food requirement with a worldwide growing population calls for revolutionizing agricultural technologies that can enhance the grain yield under erratic weather conditions and reduce the losses caused by insect pests and plant pathogens (Singh *et al.,* 2021). For fulfilling such demands, genetic engineering has been utilized in different plant species. Currently, multiple generations of genetic engineering have been developed by the researchers (Wu *et al.,* 2023).

Genetic engineering is a sub-branch of plant biotechnology that combines the use of science and technology to edit and manipulate the genetic base of plant cells. Through plant genetic engineering, scientists can enhance crop yields, resistance to biotic/abiotic factors, and can even increase the nutritional value of food crops through biofortification. The widely used methods of plant genetic manipulation include biolistic approaches such as *Agrobacterium tumefaciens-mediated* transformation (AMT), biolistic gene gun, and protoplast transfection (Squire *et al.,* 2023). The *Agrobacterium-mediated transformation* (AMT) is a commonly utilized method in which a targeted DNA is added to the nuclear genome of cell but this method is applied in a limited number of plant species. This approach has resulted in addition of random DNA, altering the plant genes and causing variance in gene expression at edited sites (Niazian *et al.,* 2017). On the other hand, the biolistic DNA delivery, uses a high-pressure gene gun to aim plant tissues which randomly adds up DNA into the chromosomal region beyond cell walls and membranes, which results in tissue damage and numerous insertions into random regions of plant genome (Toda *et al.,* 2019). Although popular, due to these limitations, these methods need some reformations (Sanzari *et al.,* 2019). Therefore, a new branch of science and technology (nanotechnology) has been used in combination with genetic engineering to tackle these problems and provide ways for precise gene editing without any damage to the plant cells.

Nano-technology is a sub-branch of science and technology which deals with the particle size ranged from 1-100 nm. It has been successfully tested in various branches of science and technology, such as microbiology, zoology, and engineering. In field of plant science, its use is still under research. Nanotechnology can be highly advantageous in agricultural sciences to develop elite varieties for stress resistance and higher nutrient concentration. Nanobiotechnology approaches have increased the accuracy of plant breeding in creating exciting novel by decreasing the time needed to remove unwanted genes and giving the breeder access to crucial genes from vast germplasm, with the possibilities for efficient gene selection and transformation (Pérez-de-Luque, 2017). Utilization of nanotechnology in agriculture have been seen for nano-sensors, nano-pesticides, and nano-fertilizers, which has generated significant interest in recent research. Nanomaterials also provide a versatile function with biomolecules. Nanoparticle-mediated delivery systems have been widely utilized for gene transition in plants. As a result, nano-particles (1-100 nm) having adjustable physical and chemical properties can be used efficiently as a vector for transporting genetic cargo across the cell organelles especially, the cell wall. More precisely, delivery of biomolecule cargo consisting of DNA, RNA, and proteins to plant cells is becoming important. As a result, nanoparticle-mediated molecular delivery of biomolecules delivery could be an intriguing way to overcome the limitations of traditional delivery methods that also enhances the transformation processes (Squire *et al.*, 2023; Wu *et al.*, 2023). Nanomaterials can act as important nanocarriers that help to achieve precise delivery of genetic material to plant genetic base. Different types of nanomaterials have been implemented in plant science but the most popular nanomaterials used as nanocarriers are mentioned in Table 1.

Table 1. Types of nanocarriers used in plant science.

Type of carriers		**Site of delivery**
Carbon carriers	Carbon dots	Cytoplasm, chloroplast
	Single Wall Carbon Nanotubes (SWCNTs)	Cytoplast, chloroplast, mitochondria, nucleus
	Multi Wall Carbon Nanotubes (MWCNTs)	Nucleus
Metallic carriers	Gold Nanospheres	Cytoplasm
	Gold Nanorods	
	Gold Nanoclusters	
	Iron Oxide Clusters	Nucleus
Silicon carriers	Mesoporous Silicon	Nucleus
Bio-inspired carriers	Liposomes	Nucleus
	Vesicles	

2 Traditional Plant Genetic Engineering Approaches and Their Limitations

Plant genetic engineering is an important strategy for sustainable crop development and breeding, and it is expected to be the key in future breeding programmes. However, the traditional approaches have been limited by various factors that need reformation to advance the research technology.

Table 2. Traditional genome editing methods & their limitations

S.No.	**Traditional Delivery Methods**	**Merits**	**Limitations**
1	*Agrobacterium*	Well-established protocols, low cost, and widely used technique.	Genotype-dependent, limited cargo type, Antagonism between Agrobacterium and plants, not applicable in several plant species.
2	Particle bombardment	Promising in the genome engineering of mitochondria and chloroplasts, suitable for all cargos.	Random insertions, tissue type-dependent, host genome damages often happen and expensive equipment and materials.
3	PEG	Highly efficient in genome engineering of protoplast, suitable for all cargos.	Time-consuming, cell limitations, regeneration inefficient, polyploid formation.
4	Plant virus	Genotype-independent, high level of transient expression.	Cargo size limitations, plant species restrictions, and safety concerns related to crop yield.

3 Need for nanotechnology in genetic engineering

Traditional gene delivery methods include delivering genes into plant cells using Agrobacteria or external pressures (such as a gene gun or electroporation). However, these approaches usually have disadvantages, such as plant cell injury, low transformation efficiency, and DNA integration at random locations in the genome. To solve these problems, researchers have invented new strategies for transferring exogenous genes, such as the nanoparticle-mediated gene delivery method, which can deliver biomolecules to healthy plant cells without the need for external forces and co-deliver the CRISPR/Cas or ZFNs machinery into plant tissues, which mediates targeted DNA insertion at suitable genomic sites (Su *et al.*, 2023). Nanotechnology offers numerous advantages, yet its application in genetic engineering remains relatively underexplored. As global populations continue to grow, traditional breeding and genetic manipulation techniques alone may not be sufficient to meet the increasing food demands. It has the potential to overcome the limitations of conventional genetic engineering by offering precise manipulation techniques with higher success rates and fewer constraints. It opens new avenues of research, including plant-nanomaterial interactions, phytotoxicity studies, and the sustainability of nanomaterials. Additionally, it enables the efficient delivery of large molecular cargos, such as transcription factors and site-specific nucleases, enhancing the effectiveness of genetic modifications in plants. By combining nanotechnology with genetic engineering, the development of elite plant varieties can be accelerated compared to traditional breeding methods, making it a promising and advanced field of research in plant science.

4 Applications of nanomaterials in plant genetic engineering

Nanotechnology has had significant consequences on many research sectors, including medicine, energy, and industry. In plant science, the researcher has proven a successful record of nanomaterials and their wide use in plant character enhancement. Nanoparticles (NPs) also act as a delivery system for nutrients and nucleic acids and cause stress priming in crops. Nano-particles promote organogenesis and shoot growth in tissue culture by lowering contamination and accelerating growth. Some of the plant developmental regulators are delivered by nano-mediated methods to improve embryogenesis and regeneration in species like sorghum and indica rice. Nanoparticles also make it easier to transport genetic materials for genome modification and direct germline editing (Squire *et al.*, 2023). To increase both temporary and stable transformation efficiency, nanoparticle-mediated gene transformation systems had been effectively implemented utilizing model plant and crop protoplasts, leaves, or roots. Nanomaterials are fascinating biomolecular carriers in plants because of their small dimensions, biological compatibility, adjustable characteristics, and variety. Controlling the transport of goods to various plant species and tissues is the most significant function of nanotechnology in plant genetic engineering (Wu *et al.*, 2023).

5 Types of Nanoparticle-Mediated Gene Delivery

Nano-mediated gene delivery system provides the precision of work as an assurance of lesser damage to the plant cell. Nanoparticle-mediated gene transfer can carry biomolecules into intact plant cells without using any kind of external force. Nanoparticle-mediated gene delivery can be transferred through various methods. Magnetic nanoparticles, peptide nanoparticles, layered double hydroxide nanosheets (LDH), DNA nanostructures, and carbon nanotubes are commonly used for the nano-mediated delivery of genetic materials to plants (Su *et al.*, 2023).

5.1 Magnetic Nanoparticle-Mediated Gene Delivery

It includes wrapping the magnetic nanoparticle (MNP) with plasmid DNA to form an MNP-DNA complex, which is then introduced into pollen under the action of a magnetic field, the MNP-DNA-pollen complex then enters the plant via pollination and integrates into the offspring's genome of subsequent generations, resulting in transgenic seeds that regenerate into transgenic plants. The advantage of Pollen magnetization is that it improves genetic transformation efficiency, reduces species dependence, minimizes the regeneration process, lessens breeding times, and accomplishes high-quality screening and multi-gene co-transformation, all of which are essential to accelerate the breeding of new transgenic plant varieties (Yan *et al.,* 2022; Su *et al.,* 2023).

5.2 Peptide-Mediated Gene Delivery

Peptides have a low molecular weight and degradable amino acid repeats, such as cell-penetrating peptides (CPPs) and protein transduction domains (PTDs), which are both synthetic or naturally generated low molecular weight cationic and/or amphiphilic. In peptide-mediated gene delivery, negatively charged DNA attaches to CPPs (which consist of polycations at the N-terminus), and the peptide-DNA complex enters plant cells via vacuum or compression (Su *et al.,* 2023).

5.3 Layered-Double-Hydroxide-Mediated Gene Delivery

LDHs are a type of ionic layered compound that consists of positively charged sublayers, including charge-compensating anions and solvates, as well as an interlayer containing charge-balancing anions and water. LDHs' cationic nature allows them to attach strongly to negatively charged DNA (Zhang *et al.,* 2021; Yan *et al.,* 2022; Su *et al.,* 2023).

5.4 DNA-Nanostructure-Mediated Gene Delivery

DNA nanotechnology uses DNA's base-pairing precision to assemble artificial ssDNA sequences into nanostructures and supramolecular structures with well-defined sizes, shapes, and geometries (including tetrahedrons) by attaching various biomolecules to the cargo attachment site, such as DNA, siRNA, or protein. Nanostructures of DNA of various sizes and shapes have now been made and they are crucial for DNA, RNA and protein drug delivery in animal models (Wang *et al.,* 2021; Su *et al.,* 2023).

5.5 Carbon nanotube gene delivery

In this method, carbon nanotubes (CNTs) are used for gene delivery. CNTs are of two types: (i) Single Walled Carbon Nanotubes (SWCNTs) and (ii) Multi Walled Carbon

Nanotubes (MWCNTs). SWCNTs are made of graphene layers with cylindrical nanostructures of 0.7-3.0 nm in diameter, whereas MWCNTs are composed of multiple SWCNTs with a diameter of 220 nm. The DNA-CNTs complex, generated by DNA and carbon nanotubes, can enter the plant nucleus via the plant cell wall (Mohanta *et al.*, 2019; Su *et al.*, 2023).

5.6 CRISPR/Cas/ZFN-mediated targeted DNA insertion

Conventional gene transfer methods are frequently combined with DNA at random places in the genome, resulting in the loss or silencing of several vital functional genes and altering plant agronomic properties. It is an excellent method for delivering DNA into appropriate genomic locations in plants. Researchers are looking at various ways of targeted DNA insertion in plants to achieve high efficiency and selection of numerous targeted genomic regions, for example CRISPR/Cas and ZFNs (Zinc finger nuclease) (Su *et al.*, 2023).

5.6.1 CRISPR/Cas: This system consists of a Cas nuclease and a guiding RNA molecule, which direct Cas to generate DSB (DNA double-stranded breaks) with a specific nucleotide sequence on the genomic target. End joining or homologous recombination (gene knock-in) of foreign donor DNA may alter the genome, resulting in allele substitution or targeted transgene insertion. CRISPR/Cas is a highly adaptable technology because a specific region of the guide RNA may readily alter to vary the recognition specificity (Singh *et al.*, 2023; Su *et al.*, 2023).

5.6.2 ZFN: Zinc finger nuclease (ZFN) is a chimeric nuclease containing two domains, i.e., the zinc finger protein DNA (a binding domain) and a non-specific DNA (a cleavage domain). Wright *et al*. 2009 first demonstrated the use of ZFNs in targeted DNA insertion in plants, verifying the idea that ZFNs can induce homologous recombination and target DNA implantation in plants. Shukla *et al.* 2005 employed ZFNs to introduce an herbicide-resistance gene into inositol-1,3,4,5,6-pentaphosphate 2-kinase (IPK1), deactivating it (Shukla *et al.*, 2005; Wright *et al.*, 2009; Carroll, 2011; Su *et al.*, 2023).

6 Nanotechnology in gene transformation

Nanomaterials have various applications. The inclusion of nanomaterials in plant genetic engineering for stress endurance, biofortification, and the development of elite varieties are the new practical achievements of this technology. But the main problem that occurs during the genetic transformation of plants is their cell wall, which acts as a barricade in the delivery of external biomolecules to the plant cell. Though, different methods of gene transformation (*Agrobacterium* transformation and biolistic methods) are being used worldwide for DNA delivery in plant cells which exclude the problem of biomolecule delivery in plant cells but these methods have also been limited by their narrow host range and extensive damage to plant cells, which often affects plant development. These constraints can be subdued by the using nanomaterial-based plant genetic transformation as these particles are easy to move through the plant cell wall and also protect the cell from further damage, inducing growth of the cells. But due to the limitations of research, most of the leading studies for nanomaterial mediated delivery system in plant genetic engineering successful results have only been achieved in plant cell cultures (Sanzari *et al.*, 2019). Recent examples of practical plant genetic delivery using nanomaterials are listed in Table 2.

Further, Carbon nanotubes (CNTs) have been also utilized in delivering the plasmid DNA into rice tissues, including leaves and embryos. This method achieved the transient expression of reporter genes (GFP, YFP, GUS) and showed potential for CRISPR-Cas vector delivery. This approach bypasses the need for tissue culture and regeneration, providing a direct route for plant transformation (Dunbar *et al.*, 2022). Gene silencing is another utilization of nano-based transformation. Small interfering RNA (siRNA) are used to silence plant genes, which are delivered by nanoparticles. This method can avoid the need for bacterial or viral vectors and enables transient and species-independent gene knockdown (Demirer & Landry, 2021).

Table 3. Nano-materials & their uses in plant genetic engineering (Sanzari *et al.*, 2019).

S.No.	Nanomaterial used	Plant species	Impact	References
1	CuO (20-30nm)	*Cucumis sativus*	Improved plant growth.	Alawadhi *et al.,* 2018
2	Mesoporous silica nanoparticles (MSNs)	*Zea mays*	Successful genome editing.	Valenstein *et al.,* 2013
3	Layered double hydroxide clay nanosheets (LDH) or Bio Clay.	Tobacco	Guard against the Cauliflower Mosaic Virus (CMV).	Mitter *et al.,* 2017
4	Mesoporous silica nanoparticles (MSNs)	*Allium cepa*	Administrative release of chemicals and nucleic acids.	Torney *et al.,* 2007
5	Multi-wall carbon nanotubes (MWCNs)	*Arabidopsis thaliana*	Enhanced seedling germination, growth, and flowering.	Shen *et al.,* 2010

A study conducted by Chang *et al.* 2013, showed a simple co-culture manner in which mesoporous silica nanoparticles (MSNs) penetrated the cell wall and drove a transient gene expression in intact *Arabidopsis thaliana* roots. MSNs that were coated with DNA were exposed to nanoparticles for 48 hours to change the plant and roots of *Arabidopsis* seedlings of 2-3 weeks. The result showed that MSNs can carry DNA to cell tissues such as the cortex, endoderm, and numerous organelles, allowing for a targeted delivery. According to the study, MSNs can easily transport cells via vascular bundles and to cells containing genes and other chemicals of interest (Mujtaba *et al.*, 2021).

6.1 Nanotechnology for DNA Delivery

Research conducted by Torney *et al.* 2007, also founded that the MSNs could carry DNA and substances in cell organelles. MSNs containing gene and its chemical inducer, were coated with gold nanoparticles to cover the pores, which were subsequently used as a genetic weapon system, that can release the chemicals and activating gene expression in plants when opened. In contrast to other systems that carry genetic material via gold nanoparticles, the MSNs allows for the delivery of vast amounts of genetic substances and chemicals which are impermeable to cell membrane of targeted cells alongside with DNA (Mujtaba *et al.*, 2021).

An experiment conducted by Demirer *et al.* 2019, utilized a single-wall carbon nanotubes (SWNTs) to transfer GFP (green fluorescent protein)-encoding dicot plasmid DNA to arugula, wheat, and cotton leaves. The rise in GFP expression and fluorescent protein range demonstrated successful system that may allow the movement of biomolecules through biological barriers, protect the genetic material from destruction and deliver it into nucleus, thereby increasing the chances of gene expression (Mujtaba *et al.*, 2021).

6.2 Nanotechnology for RNA Delivery

A study by Mitter *et al.* 2017, revealed that after loading double stranded ribo nucleic acid (dsRNA) into LDH (layered double hydroxide clays), they remain resistant to nuclease activity and remain accessible for up to 30 days after spraying. The examination was done by using Fluorescence microscopy to examine the intake of fluorophore-labelled dsRNA in plant cells following topical administration to *Arabidopsis thaliana* leaves for 48 hours. In terms of crop protection, loading dsRNA in LDH nanofibers has shown effective generation of resistance against viruses of a specific sequence compared to those challenged in the assays. Also, the results have shown that it can reduce the number of necrotic lesions in leaves of *Vigna unguiculata* and *Nicotiana tabacum* (Mujtaba *et al.,* 2021).

6.3 Nanotechnology Against Biotic Stress

Biotic stress, especially chewing insect pests, has had a significant influence on food crops in recent years. In contrast, rising number of pesticides creates severe threat to our ecological system. Therefore, using nanoparticles as a carrier can enhance the efficacy of industrial pesticides. Numerous nanoparticles also act as biocidal agents. For example, to reduce the harmful effects of *Xanthomonas perforins* in tomatoes DNA-directed silver (Ag) nanoparticles have proven to be effective (Ocsoy *et al.*, 2013; Mujtaba *et al.,* 2021).

6.4 Nanotechnology Against Abiotic Stresses

Drought, heat, cold, salt, heavy metals, and other stresses continue to cause huge losses in plant populations. Plants produce a large number of free radicals when exposed to abiotic stressors, most likely as a result of mitochondria rupture. As a result, antioxidant enzyme levels decrease drastically, resulting in plant cell damage or, in some circumstances, death. In keeping with this, the use of Fe_3O_4 NPs, a form of nano-enzyme, resulted in activity similar to antioxidant enzymes. Nowadays, different nano-enzymes such as fullerene C_{60}, Au, CeO_2, Mn_3O_4 and platinum (Pt), were utilized as NPs to overcome abiotic constraints in plants (Mujtaba *et al.*, 2021).

6.5 Nanotechnology for CRISPR-dCas9 Delivery

Recently Alallam *et al*. 2020, used electrospray to generate, optimize, and characterize alginate nanoparticles containing two CRISPR plasmids. The nanoparticles had a 99.0% encapsulation efficiency, maintaining the integrity of the charged substance. In addition, the nanoparticles effectively introduced the Cas9 transgene into HepG2 cells (Wu *et al.*, 2023).

7 Challenges and future scope

Certain challenges, which can be summarized as follows, prevent the full potential of using Nanomaterial in agriculture: (i) To create and synthesize safe nano-medicines for plant have no negative impact on plant development (Sabo-Attwood *et al.*, 2012); (ii) Research about precise mechanisms of Nano-material uptake and mobilization in plants

(Ranjan *et al.,* 2017; Sanzari *et al.,* 2019); and (iii) Lack of multidisciplinary approaches, which are necessary for development of nanotechnology in plants (Sanzari *et al.,* 2019). Nanoparticles are key vectors capable of bypassing tissue and plant cell wall barriers, accelerating transformation processes by transporting exogenous chemicals beyond the plasma membrane. Transfer of miRNA, RNA, and CRISPR into plants via nano-carriers is an emerging idea that requires additional research. Currently, there has been a limited amount of study into the use of nano-carriers as transport agents for genetic alteration in plants, which provides an intriguing method for managing pest and pathogen incidence around the world. The transmission of genes through nano-carriers requires understanding of plant-pathogen interactions. The adoption of these techniques of control can reduce pesticides usage in huge quantities. However, evaluations of side effects for environmental, human and animal health must be conducted thoroughly with substances that have been treated with nanoparticulate systems and are genetically engineered (Mujtaba *et al.,* 2021). It is also important to constantly study the concerns related to the nanomaterials employed in agriculture. Natural or manmade nanoparticles are found in significant quantities, and there is a need to study their adverse effects (Wu *et al.,* 2023). Although NPs have the power to attain remarkable levels of precision at the subcellular level, the approach still has significant drawbacks. Many NPs, still require transformation tools (gene guns or electromagnetic fields). Also, nanomaterial-mediated transport is still less effective than biological delivery methods. Therefore, increasing the delivery efficiency of CRISPR reagents is critical to enable the genome editing for in hand applications of nanomaterials (Demirer *et al.*, 2019). Future improvements in nanomaterials as biomolecular delivery carriers with fewer negative consequences could significantly enhance plant biotechnological approaches, solving many issues faced during food grain production and selective breeding. A novel strategy combining nanotechnology and plant genetic engineering by genomic selection has been successfully utilized in the gene bank of wheat landraces for genomic prediction, which might allow the utilization of nanotechnology in the future cultivated gene pools (Mujtaba *et al.,* 2021).

Conclusions

Nanotechnology is an advanced technology that has the power to drive the future of agricultural systems. Nanotechnology integrated with genetic engineering opens a new way for research to create elite varieties in less time. Though it has some disadvantages but in contrast, considering their advantages they are very few. The application of nanomaterials in genetic engineering has been hindered by many factors, but repeated experiments have mended ways for researchers to fully utilise these in the enhancement or improvement of plants. This field requires more and more research work to fully utilize the scope of nanomaterials in crops and can become a solution for plant genetic engineering. The integration of this technology will surely become an aid to the upcoming problem of food security and will create a food system that could become a solution for global hunger and food production.

References

Ahmar, S., Mahmood, T., Fiaz, S., Mora-Poblete, F., Shafique, M.S., Chattha, M.S and Jung, K-H. (2021). Advantages of nanotechnology-based genome editing system and its application in crop improvement. Frontiers in Plant Science, 12:663849.

Ahmed, M. (2017). Peptides, polypeptides and peptide–polymer hybrids as nucleic acid carriers. Biomaterials Science, 5(11), 2188-2211.

Alallam, B., Altahhan, S., Taher, M., Mohd Nasir, M. H. and Doolaanea, A. A. (2020). Electrosprayed alginate nanoparticles as crispr plasmid dna delivery carrier: preparation, optimization and characterization. Pharmaceuticals, 13 (8), 158, DOI: 10.3390/ph13080158

Alawadhi, H., Mosa, K. A., El-Naggar, M., Ramamoorthy, K., Elnaggar, A., Wartanian, S., Ibrahim, E., & Hani, H. (2018). Copper Nanoparticles Induced Genotoxicty, Oxidative Stress and Changes in Superoxide Dismutase (SOD) Gene Expression in Cucumber (Cucumis sativus) Plants. Frontiers in Plant Science, 9. https://doi.org/10.3389/fpls.2018.00872

C. X., Zhang, Q. F., Li, J., Bi, F. C. and Yao, N. (2010). Induction of programmed cell death in Arabidopsis and rice by single-wall carbon nanotubes. American Journal of Botany, 97: 1602–1609.

Carroll, D. (2011). Genome engineering with zinc-finger nucleases. Genetics, 188(4), 773-782.

Chang, F.-P., Kuang, L.-Y., Huang, C.-A., Jane, W.-N., Hung, Y., Hsing, Y. C. and Mou, C.-Y. (2013). A simple plant gene delivery system using mesoporous silica nanoparticles as carriers. Journal of Materials Chemistry B: Materials for Biology and Medicine, 1 (39), 5279– 5287,

Crossa, J., Jarquín, D., Franco, J., Pérez-Rodríguez, P., Burgueño, J., Saint-Pierre, C., Vikram, P., Sansaloni, C., Petroli, C., Akdemir, D. and Sneller, C. (2016). Genomic prediction of gene bank wheat landraces. G3: Genes, Genomes, Genetics, 6(7), pp.1819-1834.

Demirer, G. S. and Landry, M. P. (2021). Efficient transient gene knockdown in tobacco plants using carbon nanocarriers. Bio-protocol, 11(1). https://doi.org/10.21769/BIOPROTOC.3897.

Demirer, G. S.; Zhang, H.; Matos, J. L.; Goh, N. S.; Cunningham, F. J.; Sung, Y.; Chang, R.; Aditham, A. J.; Chio, L.; Cho, M.-J., Staskawicz, B. and Landry, M. P. (2019). High aspect ratio nanomaterials enable the delivery of functional genetic material without DNA integration in mature plants. Nature nanotechnology, 14 (5), 456– 464, DOI: 10.1038/s41565-019-0382-5.

Dong, O. X., & Ronald, P. C. (2021). Targeted DNA insertion in plants. Proceedings of the National Academy of Sciences, 118(22), e2004834117.

Dunbar, T., Tsakirpaloglou, N., Septiningsih, E. M. and Thomas, M. J. (2022). Carbon Nanotube-Mediated Plasmid DNA Delivery in Rice Leaves and Seeds. International Journal of Molecular Sciences, 23(8), 4081. https://doi.org/10.3390/ijms23084081.

Fraceto, L. F., Grillo, R., De Medeiros, G. A., Scognamiglio, V., Rea, G., & Bartolucci, C. (2016). Nanotechnology in agriculture: Which innovation potential does it have? Frontiers in Environmental Science, 4. https://doi.org/10.3389/fenvs.2016.00020

Mishra, S., Keswani, C., Abhilash, P. C., Fraceto, L. F. and Singh, H. B. (2017). Integrated Approach of Agri-Nanotechnology: challenges and future trends. Frontiers in Plant Science, 8.

Mitter, N., Worrall, E. A., Robinson, K. E., Li, P., Jain, R. G., Taochy, C., Fletcher, S. J., Carroll, B. J., Lu, G. Q. and Xu, Z. P. (2017). Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. Nature Plants, 3(2).

Mohanta, D., Patnaik, S., Sood, S. and Das, N. (2019). Carbon nanotubes: Evaluation of toxicity at biointerfaces. Journal of pharmaceutical analysis, 9(5), 293-300.

Mujtaba, M., Wang, D., Carvalho, L. B., Oliveira, J. L., Espirito Santo Pereira, A. D., Sharif, R. and Fraceto, L. F. (2021). Nanocarrier-mediated delivery of miRNA, RNAi and CRISPR-Cas for plant protection: current trends and future directions. ACS Agricultural Science & Technology, 1(5), 417-435.

Niazian, M., Noori, S. S., Galuszka, P. and Mortazavian, S. M. M. (2017). Tissue culture-based Agrobacterium-mediated and in Planta transformation methods. Czech Journal of Genetics and Plant Breeding, 53(4): 133–143.

Ocsoy, I., Paret, M. L., Ocsoy, M. A., Kunwar, S., Chen, T., You, M. and Tan, W. (2013). Nanotechnology in plant disease management: DNA-directed silver nanoparticles on graphene oxide as an antibacterial against Xanthomonas perforans. ACS Nano, 7 (10), 8972–8980, DOI: 10.1021/nn4034794 107.

Pérez-de-Luque, A. (2017). Interaction of nanomaterials with plants: what do we need for real applications in agriculture? Frontiers in Environment Science, 5:12.

Sabo-Attwood, T., Unrine, J.M., Stone, J.W., Murphy, C.J., Ghoshroy, S., Blom, D., Bertsch, P.M. and Newman, L.A. (2012). Uptake, distribution and toxicity of gold nanoparticles in tobacco (Nicotiana xanthi) seedlings. Nanotoxicology, 6(4), pp.353-360.

Sanzari, I., Leone, A. and Ambrosone, A. (2019). Nanotechnology in plant science: To make a long story short. Frontiers in Bioengineering and Biotechnology, 7:120.

Shukla, V.K., Doyon, Y., Miller, J.C., DeKelver, R.C., Moehle, E.A., Worden, S.E., Mitchell, J.C., Arnold, N.L., Gopalan, S., Meng, X. and Choi, V.M. (2009). Precise genome modification in the crop species Zea mays using zinc-finger nucleases. Nature, 459(7245), pp.437-441.

Singh, S., Chaudhary, R., Deshmukh, R., and Tiwari, S. (2023). Opportunities and challenges with CRISPR-Cas mediated homologous recombination-based precise editing in plants and animals. Plant molecular biology, 111(1), pp.1-20.

Singh, K. K., Meena, R. K., Singh, S.P. and Chauhan, J.S. (2021). Approaches to Relieve a Worldwide Global Warming through Agriculture, In: Akshat Uniyal & Isha Sharma, (eds) Trends in Agriculture: Traditional and Modern Approaches. pp. 279-289.

Singh, S.P. Singh, K.K. and Kumari, B. (2021), Assessment of Biodiversity Loss and Sustainability Due to Urbanization, In: Akshat Uniyal & Isha Sharma, (eds) *Trends in Agriculture: Traditional and Modern Approaches*. pp. 325-334

Squire, H. J., Tomatz, S., Voke, E., González-Grandío, E. and Landry, M. (2023). The emerging role of nanotechnology in plant genetic engineering. Nature rev. bioeng., 1, (314-328).

Su, W., Xu, M., Radani, Y. and Yang, L. (2023). Technological Development and Application of Plant Genetic Transformation. International Journal of Molecular Sciences, 24(13), 10646.

Toda, E., Koiso, N., Takebayashi, A., Ichikawa, M., Kiba, T., Osakabe, K., Osakabe, Y., Sakakibara, H., Kato, N. and Okamoto, T. (2019). An efficient DNA- and selectable-marker-free genome-editing system using zygotes in rice. Nature Plants, 5(4), 363–368.

Torney, F., Trewyn, B. G., Lin, V. S. Y. and Wang, K. (2007). Mesoporous silica nanoparticles deliver DNA and chemicals into plants. Nature Nanotechnology, 2: 295–300.

Valenstein, J. S., Martin-Ortigosa, S., Peterson, D. J., Lin, V. S., Trewyn, B. G., Lyznik, L. A. and Wang, K. (2013). Mesoporous silica Nanoparticle-Mediated Intracellular CRE protein delivery for maize genome editing via LOXP site excision. PLANT PHYSIOLOGY, 164(2), 537–547. https://doi.org/10.1104/pp.113.233650Shen.

Wang, Y., Benson, E., Fördős, F., Lolaico, M., Baars, I., Fang, T., Teixeira, A.I. and Högberg, B. (2021). DNA origami penetration in cell spheroid tissue models is enhanced by wireframe design. Advanced Materials, 33(29), p.2008457.

Wright, D.A., Townsend, J.A., Winfrey Jr, R.J., Irwin, P.A., Rajagopal, J., Lonosky, P.M., Hall, B.D., Jondle, M.D. and Voytas, D.F. (2005). High-frequency homologous recombination in plants mediated by zinc-finger nucleases. The Plant Journal, 44(4), pp.693-705.

Wu, K., Xu, C., Li, T., Ma, H., Gong, J., Li, X., Sun, X. and Hu, X. (2023). Application of Nanotechnology in Plant Genetic Engineering. International journal of molecular sciences, 24(19), 14836. https://doi.org/10.3390/ijms241914836.

Xu, Z. P., Zeng, Q. H., Lu, G. Q. and Yu, A. B. (2006). Inorganic nanoparticles as carriers for efficient cellular delivery. Chemical Engineering Science, 61(3), 1027-1040.

Yan, Y., Zhu, X., Yu, Y., Li, C., Zhang, Z. and Wang, F. (2022). Nanotechnology strategies for plant genetic engineering. Advanced Materials, 34(7), 2106945.

Zhang, Y., Xu, H. and Lu, S. (2021). Preparation and application of layered double hydroxide nanosheets. RSC advances, 11(39), 24254-24281.

Zhi, H., Zhou, S., Pan, W., Shang, Y., Zeng, Z., & Zhang, H. (2022). The promising nanovectors for gene delivery in plant genome engineering. International Journal of Molecular Sciences, 23(15), 8501. https:// doi.org/10.3390/ijms23158501