

## Nanodiagnostic Tools in Plant Breeding

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### Introduction

New plant varieties, with better yield, disease resistance and quality traits, improve agricultural productivity for a growing global population. The traditional plant breeding methods take a long-term research. Different nanodiagnostic methods such as nano fluidics, nanomaterials, bioanalytical nanosensors etc. has the potential for improving plant breeding program to overcome many more problems linked to breeding for resistance, production, and prevention and can possibly be used in living plants in field based assays for transgene expression<sup>[1]</sup>. Nano fluidics such as Open Array or the Fluidigm Dynamic Array technologies supply automated PCR mixes for mega molecular breeding assays. Also, nano genomics-based methods have enabled breeders greater precision breeding have opened up exciting new opportunities for selecting and DNA translocation, which has not only decreased the time consuming required to remove redundant genes, but also allowed the breeder to access useful genes from distant plants.

This is due to the fact that it enables nanoparticles, nanofibers, and nanocapsules to carry foreign DNA and chemicals that change genes<sup>[2]</sup>. However, many plant genomes are complex, de novo sequencing with next generation sequencing technologies is a process fraught with difficulties that then create roadblocks to the employment of these genome sequences for crop enhancement<sup>[3]</sup>. Nanotechnology can specifically target specific plant pathology problems in agriculture such as in plant pathogen interactions and provide new techniques for crop disease control<sup>[4]</sup>. For example using nanotools for gene transfer in plant cells may lead to improvement new resistant cultivars which will minimize expenses on agrochemicals required for plant disease control<sup>[5]</sup>. Nanodiagnostic tools uses include nanoparticle mediated gene or DNA transfer in crops for the improvement of disease resistant cultivars<sup>[6]</sup>.

### Transgene Nanovehicles

The transfer of genes to the target plant cells has been accomplished through the use of a variety of nanotools, including nanoparticles that encapsulate and deliver DNA to target

cells, in addition to nanostructured surfaces that capture and release DNA to cells<sup>[4]</sup>. Nanoparticles are used to transfer DNA and drugs into animal cell and tissues by the process of endocytosis<sup>[7]</sup>, but the use of nanoparticles for DNA delivery in plants was not possible because the plant cell possess a rigid cell wall and plasma membrane. Different metal nanoparticles can be used for gene mediated DNA transfer such as zinc, calcium phosphate, carbon materials, silica, starch, gold, magnetite, strontium phosphate, magnesium phosphate and manganese phosphate<sup>[8]</sup>. ZnS nanoparticles are considered as a desirable gene transporter to deliver DNA into intact plant by using ultrasound mediated technique<sup>[9]</sup>. The honey-comb like mesoporous silica nanoparticles (MSN) system with 3nm particle size that can carrier DNA and chemicals into isolated plant cell and leaves. They loaded the MSN with the gene and the chemical inducer and capped the ends with gold nanoparticles to keep the molecule from leaching out. Uncapped the gold nanoparticles released the chemical and triggers the gene expression in plant under controlled release conditions<sup>[2]</sup>. Carbon coated iron nanoparticles was inserted inside the internal hallow of the leaf petiole of pumpkin. Different type of microscopic methods were used to visualize and follow the transport and deposition of nanoparticles, as well as to verify the possibility of concentrating nanoparticles into targeted specific site of plant cell by small magnet<sup>[10]</sup>. Starch nanoparticles effectively crossed the cell wall, the nanoparticle biomaterial was designed in such a way that it bonded the gene and transported it across the cell wall of plant cells by inducing the formation of transient membrane pores in cell wall, cell membrane and nuclear membrane by using ultrasound method<sup>[11]</sup>.

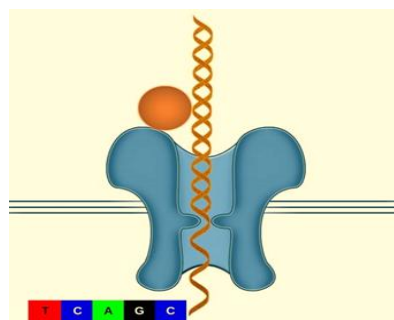
Some challenging reports on Quantum Dots mediated gene delivery have been studied, for example the delivery of plasmid DNA<sup>[12]</sup> into the animal cells, and established genetic transformation and high efficiency transient expression were acquired, which have laid a good foundation for the application of QDs as gene carrier in plant. The ability of carbon nanotubes to penetrate intact plant cell wall and cell membrane has already evaluated. Single-walled carbon nanotubes/fluorescein isothiocyanate and SWNT/DNA conjugates revealed the ability of nanotubes to act as nanotransporters in walled plant cells<sup>[13]</sup>.

Polyamidoamine (PAMAM) dendrimer DNA offered methods for transferring a molecule of interest into a plant cell having a cell wall. Micro-injection with carbon nanofibers (CNFs) containing foreign DNA has been used to genetically modify golden rice enriched with extra vitamin A<sup>[14]</sup>. The current methods are provided for genetically or otherwise modifying plants and for treating or preventing disease<sup>[15]</sup>.

### Nanopore-Based Technologies

Current advances in genomics including DNA sequencing is the most important tools in plant breeding and biotechnology. Quick developments in next generation sequencing (NGS) technologies over the last decade have opened up many new chances to discover the relationship between genotype and phenotype. The 3<sup>rd</sup> Generation systems (TGS) will quickly become common in general plant research and agronomy, and more breeding material are sequenced.

Nanopore-based DNA sequencing protocols allowing single molecule, electrical detection of DNA sequence and have the potential of low sample preparation work, high speed, and low cost<sup>[16]</sup>. These advances are a substantial step forward in improving this inexpensive and potentially more rapid alternative to next-generation sequencing technologies[Figure 1]<sup>[17]</sup>. The knowledge of genetical genomics, diversity and gene function in crop plants needs to analyze the molecular basis of biological systems or phenotypic traits<sup>[18]</sup>.



**Figure 1.** Diagram of a DNA molecule travelling through a protein nanopore (adopted from Khiyami et al<sup>[17]</sup>).

Plant breeders and phytopathologist are needed who can apply nanogenomics and develop nanodiagnostic technologies to accurately advance the improvement process and take advantage of the potential of genomics. A new nano biotechnology method describes new plant gene transfer tools and DNA sequencing systems to improve crop resistance against plant diseases and increase food security.

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