# Bio-Utilities of Quantum Dots in CRISPR/Cas-9: Functionalization of Q-Dots on Reporter Plasmid for Delivery in Plant Tissue

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## INTRODUCTION

Increasing yield potential thereby rectifying yield gap are important to gain an upper hand in the global food security. Ensuring global food security, while protecting the environment, non-agricultural lands and biodiversity, is the single greatest scientific challenge faced by humankind. It has been predicted that food production needs to increase by about 70% between 2007 and 2050 to feed an estimated >9billion people (FAO 2009; UN, 2017). On the other hand, food security is under threat from ongoing climate change, plateauing of crop yield in many regions and diminishing natural resources (FAO, 2018). Increasing crop yield potential and closing the yield gap are two important aspects of the solutions proposed to achieve global food security in a sustainable manner with minimum environmental footprints.

Conventional *Agrobacterium* mediated gene transfer is rarely 10-20 % efficient in transferring the candidate gene Payloading of reporter gene carrying plasmid on functionalized Quantum dots can be an efficient way to transfer the candidate gene.

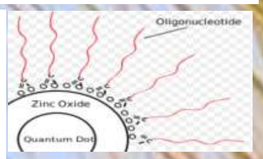


**Figure 1: DNA functionalized quantum dot.**

## MATERIALS AND METHODS

- A proof-of-concept will be identified for the loading of the plasmid DNA on quantum dots. The quantum dots will be treated with compounds to develop designer functional groups that will help in coating or adsorption of plasmid DNA on the surface of the Qdots. The loading of the DNA molecule on Qdots will be identified through gel electrophoresis analysis.
- The plasmid DNA-Qdot nanocomplex will then be incubated with the callus tissue suspension derived from different explants for delivery of the target plasmid DNA or reporter gene. The immature embryos extracted/ obtained from the wheat inflorescence will also be incubated with the plasmid DNA Qdot nano- complex.
- The comparative transformation efficiencies for both the callus suspension and immature embryos will be evaluated. The occurrence of the plasmid DNA will be validated through PCR technique. The unloading of the plasmid DNA payload will also be determined through fluorescence microscopy or flurospectroscopy techniques. The change in the fluorescence emission signal of the Qdots pre and post unloading of the plasmid DNA will be quantified.
- The nano-delivery of the plasmid DNA coding for the Cas9 endonuclease and single guide RNA will be performed in callus tissue/ immature embryo. The transformation and genome editing efficiencies will then be determined by performing the T7 endonuclease assay.

## RESULTS AND DISCUSSION

- 0-dimensional semiconductor colloidal quantum nanocrystals have been most proficiently utilized for tagging of cellular molecules and bio-imaging aspects particularly in biomedicine.
- Utilization of these nanomaterials for gene delivery is rather at an incipient stage.
- No research reports their use for DNA or gene delivery in plants
- Therefore, this research gap is required to be addressed considering the versatility of potential applications of the semiconductor quantum dot.
- Why it has been proposed?
  - Semiconductor QDs exhibit functionalization versatility.
  - These have been used voraciously to tag proteins, peptides and other biomolecules.
  - Its convenient to track the payload due to fluorescence properties of QDs.
- What has been proposed?
  - Loading of plasmid DNA coding for Cas9 endonuclease on QDs and assessment of its successfully delivered into immature embryos through confocal microscopy studies.
  - sg-RNA will be designed for a specific gene to disrupt it.
  - The nicks or mutations caused thereof will cause In-Dels in the requisite gene that will be identified through T7 endonuclease assay
- This putative mutation may be the reason of gene editing in crop plants through CRISPR/Cas9 cassette.
- Probable limitations of the proposed nanomaterials
  - Cytotoxicity due to the heavy metal chemical nature of the semiconductor QDs may lead to killing of the callus cells.
- Future perspectives
  - Advanced role in cell engineering for QDs can be delineated.


**Figure 2: Steps to synthesize quantum dots for biological applications**

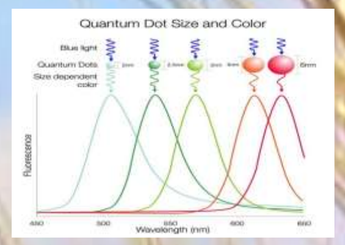


**Figure 3: Multiple Quantum Dot Tunneling in a Semiconductor and Its Wave Function and Probability Density**

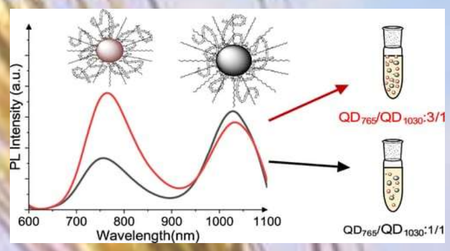


**Figure 4: Emission Spectra of Qdots.**

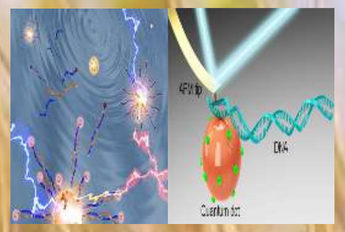


**Figure 5: Payloading of Qdots on DNA**

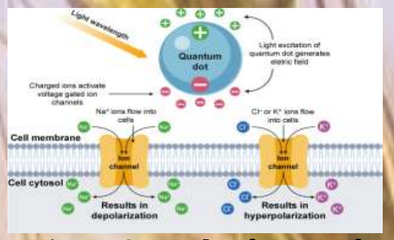


**Figure 6: Mode of entry of qDots into the cells**

## CONCLUSION

- A new protocol for payloading of the components of the genome editing machinery on semiconductor quantum dots will be available.
- Novel method for delivery of CRISPR-Cas 9 system in plant cells will be identified .

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**Acknowledgement**

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