Texas Tech University, Plant and Soil Science Spring Seminar 2024

Title: **Understanding single cell regeneration pathway and development of tissue-culture free regeneration and gene-editing platform in crops**

Speaker: **Arjun Ojha, Ph.D. Candidate**

Location: **MCOM (153)**

Date/Time: **Thursday, March 21st / 12:00 – 1:00 PM**

Abstract

The concept of plant cell totipotency, which traces back over a century, is the foundation of modern plant biotechnology. The plant cells have a remarkable developmental plasticity that has been profoundly employed in both basic and applied research. Yet, the intricate molecular mechanisms governing somatic cell regeneration in plants remain largely unknown. With the development of groundbreaking technologies like single cell transcriptomics, it is possible to unravel the mysteries of plant cell regeneration and shed light on the hidden molecular cues involved in the developmental processes. The major objective of this study is to decode the genetic basis of somatic cell regeneration in plants by using single cell transcriptomics approach in Nicotiana benthamiana, a model organism with shorter lifespan and relatively easy to isolate somatic cells (protoplasts) with higher regenerative capacity. Examining six different developmental phases of tobacco-based somatic cell regeneration, it is envisioned to uncover molecular markers and cell types required for plant regeneration at single-cell resolution. The molecular information uncovered in this study could provide an unprecedented view of tissue regeneration in plants that can be applied in other plant species "recalcitrant" to regeneration. This could be a game changer in efficient regeneration of the progeny plants which is one of the major bottlenecks in crop improvement via the application of precise genome editing techniques like CRISPR/Cas9.

CRISPR/Cas9 have revolutionized both basic and applied biological research within a decade of its development. It has been profoundly used in crop engineering research thereby transforming the pace of plant breeding and trait discovery. However, the delivery of gene editing reagents and regeneration of the edited progeny are the biggest hurdles in efficient genetic engineering and crop improvement. Current delivery methods and regeneration are largely dependent on tedious, lengthy, and costly in vitro (tissue culture) processes. Moreover, plant regeneration and genetic transformation are highly genotype-dependent and therefore, only very few of all plant species can be genetically modified. Therefore, the development of efficient genotype-independent plant transformation and gene-editing methods in several recalcitrant crops including cotton, soybean, sorghum, common bean, etc. is important in applying this technology for crop improvement. To overcome these challenges, we have created a synthetic cascade to express genes called developmental regulators that are involved in stem cell activity, rapid tissue differentiation, and the regeneration process. This system is successfully tested in vivo, without a need for tissue culture in the model plant Nicotiana benthamiana (tobacco) and applied in tomato with successful results. Our data suggested the successful development of robust technology for gene-editing and regeneration of gene-edited plants as we envisioned.