Plants Modifications – **Introduction**
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★ Introduction: Start with a question (ex. Why should plants be genetically modified?)
  - Mentioned tech (CRISPR and Agrobacterium DNA-transferase)

Agrobacterium-mediated transformation (AMT). Agrobacterium is a genus of a gram-negative bacteria that can transfer a gene into a plant nucleus. Agrobacterium sense an injured plant cell then attach its body to the plant membrane to transfer a specific DNA sequence. Naturally, the transferred DNA segment (T-DNA) is a part of a tumor induced plasmid, Figure 1. This natural occurring method of transferring genetic material is used to produce different types genetically modified plants (Song, 2019).

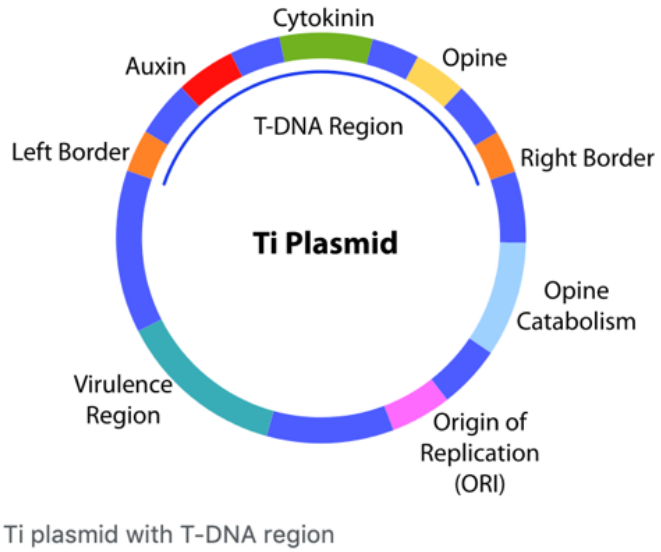

Figure 1) T-DNA as a part of Ti Plasmid. T-DNA is bounded by the left and right borders where further cutting of this segment. https://en.m.wikipedia.org/wiki/Transfer_DNA

Gene transfer via AMT requires a plasmid construction where the desired sequence is inserted in the T-DNA region. Plasmid includes different regions that encodes different proteins that aid in the transfer of the T-DNA to the plant cell. The T-DNA region has a length of around 24,000 nucleotides (Barker, 1983). inside the cell, the transferred T-DNA will carry on to the nucleus and get integrated in the cell genome (Tzfira, 2006), figure 2.

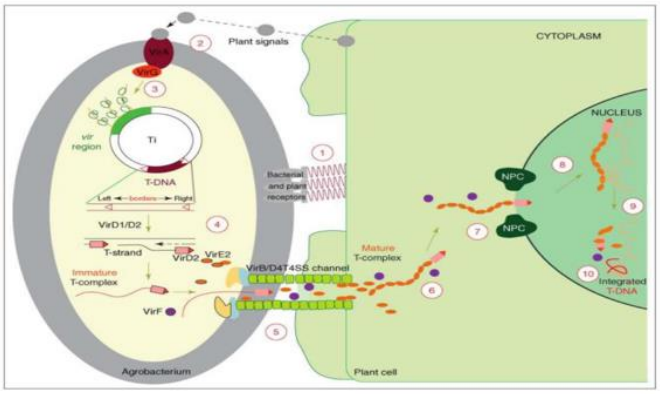

Figure 2.5: Process of *Agrobacterium*-mediated transformation in a plant cell. (1) Recognition and attachment of the *Agrobacterium* to the host cell; (2) The sensing of specific plant signals by the *Agrobacterium* VirA/VirG; (3) The *vir* gene region is activated; (4) A mobile copy of the T-DNA is generated by the VirD/D2 protein complex, and delivered as a VirD2-DNA complex (immature T-complex); (5) The immature T-complex and several other Vir proteins are delivered into the host cell's cytoplasm; (6) The mature T-complex formed when VirE2 is associated with the T-strand, then travels through the host-cell cytoplasm; (7) T-complex is actively imported into the host-cell nucleus; (8) inside the nucleus, the T-DNA is recruited to the point of integration; (9) T-DNA stripped of its escorting proteins; (10) Finally, the DNA was integrated into the host genome. (Adapted from Tzfira *et al.*, 2004)

Figure 2) T-DNA tansferance between Agrobacterium and plant cell.

- Stress experienced by plants:
  - Summary of what ROS are and what are Nate has to say about ROS and their
  - Modifications
- Thesis: The genetic modification of plants is/isn't an ethical practice. This is because of X,Y,and Z.

★ Body:

- Moving forward to herbicides, as human made stress:

  - Glyphosate and gene modification:
    - A- Glyphosate and rice:
      - What gene was added/ removed
      - Where did the gene come from?
      - How did they edit the genome?
        - CRISPR
      - What were their results?
      - What were their concerns?
    - B-Glyphosate and Corn:

In addition, Weeds growing in maize via a competitive relationship lower maize yield by 40 %. (Chikoye, 2004). Treating maize and weeds with glyphosate provides a solution to weeds. Glyphosate is commonly used herbicide that interfere with the Shikimate Pathways. Shikimate Pathway is important in the biosynthesis of aromatic amino acids and folic acid in a seven step

pathway (figure 3). The pathway is present in different organisms such as plants, fungi and bacteria. One of the steps is the synthesis of 5-Enolpyruvylshikimate-3-Phosphate (EPSP) via the enzyme *5-Enolpyruvylshikimate-3-Phosphate synthase* (EPSPS). Glyphosate inhibits EPSPS which leads to, in case of a plant, death. (Herrmann, 1999).

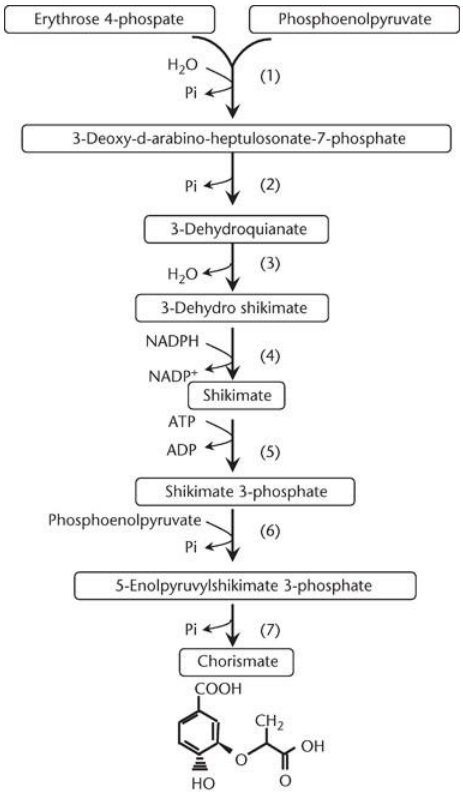

Figure 3) Shikimate Pathways seven steps.

Growing maize that is tolerant to glyphosate provide possible solution to weeds competitive relation with maize. Trans-infecting Tobacco plants with bacterium E Coli
EPSPS that encodes AM79 aroA showed high tolerance to glyphosate but (Cao et al. 2012)
What gene was added/ removed?

- Where did the gene come from?
- How did they edit the genome?
  - CRISPR
- What were their results?
- What were their concerns?

- C- Jada's Imida and wheat:
  - What gene was added/ removed?
  - Where did the gene come from?
  - How did they edit the genome? (crispr)
  - What were their results?
  - What were their concerns?
- Moving forward to Kevin's topic (increase iron content in rice)

- What gene was added/ removed?
  - (A) lactoferrin cDNA subcloned into the plant binary expression vector pCB 302-3 (Xiang et al. 1999) using Xba I restriction sites
  - (B) Ferritin gene
- Where did the gene come from?
  - (A) The original plasmid pBI-LF containing human Lf cDNA was kindly provided by Dr. Hiroyuki Anzai
  - (B) From *Phaseolus vulgaris* into rice grains, increasing their iron content up to twofold
- How did they edit the genome?
  - (A & B)Agrobacterium
- What were their results?
  - (A) Lf can be absorbed my fetus' and infant but not adult
  - (B) Increasing their iron content up to twofold
- What were their concerns?
  - (A) No application after infancy and allergenicity of "Golden rice"
  - (B) Phytase enzyme that facilitates iron absorption is modified into plant but once the rice is cook it becomes inactive

★ Conclusion:
- Restate thesis: Gene editing plant is/isn't an ethical practice.
- What are the benefits resulted from gene editing ?
- What are the negatives found from gene editing?
- What regulations do we suggest?

Citation

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