1. **Introduction**

There is a need for quality food research more than ever. The pandemic that started in 2019 has made it clear that food security is one of the vital and foremost task people need to take care of (feedingamerica.org). Scientist community needs innovative and creative strategy to secure food for a long run. Carrot, a model plant for my project, is the 7th most consumed crop in the US. Annual crop value of carrots is $864 million (US), and US is the 4th largest producer of carrots i.e., about ~1.4 million tons/year. Carrots are rich in nutrition like carbs, fiber, vitamins and minerals (vitamin A. K1, B6, biotin, potassium), and rich in useful plant compounds like beta-carotene, alpha-carotene, lutein, lycopene, polyacetylenes and anthocyanins. As a plant molecular biologist, one of my projects specifically targets in increasing the shelf -life of carrots by making them disease resistant and healthy using tissue culture technique. I use carrot stable transformation, a tissue culture technique which introduces a gene/s of interest into the plant’s genome permanently. In this method, plant is our host while agrobacteria carrying our gene/s of interest is the carrier. As carrots are a huge use source of nation’s revenue, and are also an important daily food for people, my ultimate goal here is to develop a new variety of carrot with high disease resistance, thus prolonging the shelf-life of carrots.

1. **Problem**

Carrot crop losses amounts to ~ $375 million dollars a year due to plant pathogens. The most common plant pathogen that leads to this big loss is *Sclerotinia sclerotiorum*, a necrotrophic plant pathogenic fungus, also known as white mold, which is capable of infecting wide host range i.e., up to 600 plant species. The problem with this pathogen is that its resilience to grow even in extreme environment and thrives quickly as an opportunistic pathogen in moist and cool conditions.

Chemicals like fungicides are heavily used to control the pathogens like *S. sclerotiorum,* but they are costly, and this pathogen is most likely to develop resistance against the fungicides.

1. **Solution**

Fortunately, carrot has its own chemical defense mechanism to fight pathogens. Carrots innately produce these chemical compounds called ‘polyacetylenes’, which are known to show anti-cancerous, antifungal, antimicrobial properties along with other biological activities. Specifically, falcarinol and falcarindiol-type polyacetylenes are implicated to play a role in plant pathogen defense. The acetylase (FAD-2 like) gene *DCAR-013552* is known to overexpress falcarin and falcarindiol, together as falcarins, in carrots. So, to study polyacetylenes, I use this gene along with other controls- empty vector and PEAQ\_p19\_GFP – and perform carrot stable transformation, which is a technique to permanently introduce the gene of your interest into your host (carrot in my case). Once the gene is introduced, it will continue to express into the generations to come. From the stable transformation, once the transformants (carrot with our genes) are plantlets and then plants, we perform various molecular techniques like PCR (Polymerase Chain Reaction), gel electrophoresis, disease assay, GC-FID (Gas Chromatography- Flame Ionization Detector), and GC-MS (Gas Chromatography-Mass Spectrometry) to confirm the presence of gene, analyze/quantify the polyacetylene content, and its function.

**Programming to solve the problem and speed up the process**

Since the carrot transformation and other analysis procedure take a lot of time, I will be using python to keep track of my experimental progress, to predict and confirm my outcome. I will be using various parameters to automate what I need to know or can know about my experiment. I will be using these 14 parameters:

1. carrot\_variety
2. carrot\_parts
3. agrobacterium\_type
4. conditions
5. time\_in\_MS1D\_dark
6. time\_in\_MS1D\_dl
7. time\_in\_half\_MS1D\_dl
8. time\_in\_quarter\_MS1D\_dl
9. antibiotics\_in\_MS1D\_dl
10. antibiotics\_in\_half\_MS1D\_dl
11. antibiotics\_in\_qaurter\_MS1D\_dl
12. callus\_status
13. PCR\_Gel\_status
14. GC\_MS\_polyacetylene\_percentage

I will set up these parameters in a dictionary format, so I can list and table them as I like. I will set up the code such that I can predict/confirm my outcome even with a combination of parameters. For example: which agrobacterium type and carrot variety will give the highest percentage of callus (undifferentiated cell mass which determines the cell fate for plants), which carrot part, agrobacterium type, and carrot variety gives the highest positive rate for callus formation and PCR, which agrobacterium type and positive callus gives us the lowest GC-MS polyacetylene content etc. This will be an enormous help for me for I can predict and confirm the efficiency of my experiment. This will also help me to implement the carrot stable transformation procedure that is the most effective in our lab in the future.

Notes: MS1D is the media we grow our carrot parts in.

Agrobacterium (bacteria) is used to transfer our genes on interest into carrot parts

**Thank You! ☺**