# A genetic screen for ethylene-response mutants in *Arabidopsis*

## Objective

To carry out a "genetic screen" for two different types of mutants in the ethylene hormone response pathway: 1) mutants that are "ethylene-insensitive", which cannot sense the ethylene hormone at all, and 2) "constitutive ethylene-response" mutants that behave as if they are perceiving ethylene even when they are not!

## Introduction

What is a mutant screen?

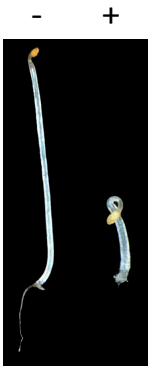
In order to manipulate plant genes for genetic engineering, it is essential to first identify and have an understanding of the genes that we wish to alter. In fact, a major goal in plant research is to identify all of the genes that comprise a plant and to identify which biological process each gene functions in. Some examples of important processes are seed germination, flower development, and responses to diverse environmental signals (e.g., light, gravity, temperature, water availability, air quality, pathogens, soil conditions) crucial for survival. Every process typically requires the action of many genes, and together these genes comprise pathway(s) that lead to the normal phenotype or the appropriate response. Once we identify which genes act in which pathways, we will have the capability to manipulate those genes to genetically engineer plants that can, for instance, grow under harsh conditions.

How do scientists uncover the genes that are involved in such processes? One powerful way is to carry out a "genetic screen" to identify mutants that are defective in the process of interest, as revealed by an altered phenotype in the mutant but not in the normal (wild-type) plant. Each mutant will have a defect in a gene that has a role in the process of interest. For example, if you carry out a genetic screen for flowers that lack petals, you can expect to identify plants carrying mutations in genes that are involved in petal formation. Subsequent steps (not performed in this lab) can lead you to cloning of the gene, obtaining its DNA sequence and determining its protein sequence, which can give an even better understanding of the gene's function.

For this lab, we will be using the tiny model plant *Arabidopsis thaliana.* Much of our knowledge about plant genes has come from a wide variety of mutant screens in *Arabidopsis thaliana*.

Ethylene-response mutants

The gaseous molecule ethylene ($C_2H_4$) is a plant hormone that has many diverse effects on plant growth and development. Ethylene plays an important role in fruit ripening, abscission, senescence and responses to wounding, flooding and other stresses. The ability to manipulate ethylene responses will have a substantial impact on agriculture and horticulture by optimizing fruit ripening, delaying senescence, and producing plants with increased stress tolerance.




The "seedling triple response" phenotype provides a rapid and easy way to screen for ethylene-response mutants. When germinating seedlings are grown in the dark and exposed to ethylene, they exhibit a triple response phenotype, which consists of a shortened and thickened hypocotyl, a short root and an exaggerated apical hook, as displayed by the seedling on the right treated with (+) ethylene.

In this lab, you will be given seeds produced by plants that have been treated with a mutagenic chemical to induce random mutations in the genomic DNA. (*NOTE*: The seeds are not carrying the mutagenic chemical and are harmless to humans.) The mutagen may or may not mutate a gene of interest in the ethylene-response pathway. You will germinate the seeds in the dark and search among the germinated seedlings for mutants that fail to respond to exogenous ethylene as well as mutants that show a "constitutive response" even in the absence of ethylene.

- What should an insensitive mutant seedling look like and under what conditions should we be able to identify it?

- What would a constitutive response mutant look like and under what conditions should we be able to identify it?

In place of treating seedlings with ethylene gas (which is difficult to work with!), we will germinate the seedlings on media containing a compound called 1-aminocyclopropane-1-carboxylic acid (or ACC), as described in the lecture. The seedlings will take up the ACC through their roots and convert the ACC into ethylene gas.

**Materials** (per student pair)

- a marker pen for labelling the plates
- 1 tube of 40 mg *Arabidopsis thaliana* seeds from plants that have been treated with a chemical mutagen
- 1 filter paper circle
- 1 petri dish containing plant growth media with 20 uM ACC (ACC is an ethylene precursor that the seedling takes up through the root and converts into ethylene gas)
- 1 petri dish containing plant growth media
- aluminum foil

**Procedure**

1. Label each petri dish with your name on the bottom of the dish (not on the lid).

2. Fold a filter paper circle in half and then in half again. Open the paper to create a "bowl".

3. Carefully tap out about half of the seeds from the tube into your filter paper bowl. Half of the tube contains about 1000 seeds (~20 mg). *How much does a single seed weigh?*

4. Gently tap the filter paper so that the seeds fall onto the agar surface of one of the petri dishes. *MAKE SURE TO DISTRIBUTE THE SEEDS **EVENLY** ON THE AGAR. Hint: let the seeds fall off a rounded edge of the filter paper, rather than pouring off from the crease.

5. Repeat steps 2 and 3, using the other petri dish.

6. Wrap each plate in two sheets of aluminium foil to ensure complete darkness. MAKE SURE THAT THE PLATES ARE RIGHT SIDE UP WITH THE LIDS ON TOP (SO THAT THE AGAR IS ON THE BOTTOM AND THE SEEDS ARE ON TOP) – *Why?*

7. Incubate the foil-wrapped plates at 4C for 3 days to help synchronize seed germination. This is called "stratification". The plates should be kept flat, not at an angle. *Why?*

8. Incubate the foil-wrapped plates at 20C for 4 days to allow them to germinate. Again, the plates should be kept flat, not at an angle.

9. After the 4-day incubation, unwrap the plates and closely examine the seedlings for any ethylene-insensitive mutants and any constitutive ethylene-response mutants.

10. If we had more time, we could grow the seedlings into plants. We would use forceps to carefully lift candidate mutants from the petri dish and transfer them to soil. The seedlings are very delicate so you would take care not to squeeze them with the forceps or you could kill them! The seedlings would be gently placed onto pre-moistened soil and then the pots would be covered with plastic wrap to help maintain high humidity until the seedlings take root in the soil. Water every 3 days and record the plants' phenotypes as they grow.

**Questions to think about**

1. Which agar plate is for which mutant screen?

2. Which of the two screens is easier and why?

3. Why are the plates incubated in the dark?

4. Why is it important to have the plates in a certain orientation (flat, not at an angle)?

5. Did you find any candidate mutants in your genetic screen? How many did you find?

6. The *Arabidopsis* genome has ~25,000 genes, and the mutagen will hit each gene randomly. Why do you need to screen many seeds to find a mutant of interest?

7. Why do you think *Arabidopsis* has been an excellent plant to use for genetic screens? How would the ethylene mutant screen be different if you were using soybean or corn?

8. What does a constitutive ethylene-response phenotype suggest about the wild-type gene's function?

9. Can you come up with an idea for a mutant screen for a different biological process (one that does not involve ethylene) and describe how you would carry out the screen?

10. (optional) Do the candidate mutant plants show any mutant phenotypes as they are growing in the soil? If yes, are the adult plant phenotypes consistent with your assessment of ethylene insensitivity or constitutive ethylene response?