

Introduction to Genetic Screens in Arabidopsis thaliana



Goal: To gain experience in the use of mutant screens to identify developmental and conditional mutants.

Genetic screens: A genetic screen is a procedure or test used to identify and select individuals that possess a phenotype of interest. A genetic screen for new genes is often referred to as forward genetics.

Conditional screen: where plants with mutant phenotypes are identified under a certain environmental condition.

Introduction: Classical (Forward) genetics has provided the raw materials for genetic analysis for over one hundred years. Classical genetics uses genetic variation to dissect the components that determine a certain trait. Although natural variants can be used in genetics (e.g. different varieties of corn), there can be too many differences between unrelated organisms to allow easy analysis. Induced mutations produced through treatment of a genetically similar population, yields genetic variants with relatively few genomic differences. Once a mutation is linked to a particular phenotype, the genetic lesion can be identified through positional cloning or other biotechnology techniques.

Mutagenesis: The chemical mutagen commonly used for *Arabidopsis* is ethylmethane sulfonate (EMS). EMS adds an ethyl group to G and T residues, allowing the modified base to base-pair inappropriately. Mutagenesis is normally performed upon seeds, providing for easy handling of large numbers of individuals. Several problems are encountered in mutating seed. First is the low statistical probability that any one gene will be mutated in any one plant. Thus, many plants need to be screened to detect mutations in that gene. An optimal dose of mutagen will provide as much mutagenic potential as possible, without being so great as to kill the plants. Unfortunately, the kill rate increases exponentially with dose as the mutation rate for any one gene rises linearly. The optimum dose for mutation is that which produces about 37% survival among the mutagenized seeds. The mutation rate can also be measured for a visible known phenotype, such as albinism (lack of chlorophyll). A second problem in mutagenized seed is that there are multiple cells in the embryo of seeds that will give rise to different parts of the plant. Thus, a mosaic plant is formed. Mutations occurring in one cell in the seed may only appear on one side of the plant, if at all. Thus, multiple seeds need to be collected from each plant. Third, recessive mutations are at least 10-fold more likely to occur than dominant mutations. The recessive mutations cannot be observed in the heterozygous plant that grows from the mutagenized seed (called the M1 generation), because only one

homologous chromosome bears the mutation. Therefore, the plant must be crossed with itself to produce a fraction of homozygous progeny. *Arabidopsis* is a good model plant since it is self fertile and this self fertilization in each flower is easy- you just let it alone. As a typical monohybrid cross, only one-quarter of the progeny (called the M2 generation) display the mutant phenotype.

Conditional screen: Surface sterilized seeds will be sown in petri dishes on a growth medium amended with ACC- a precursor for the plant hormone ethylene. ACC effectively stimulates the production of ethylene in the petri dishes and stimulates the triple response: reduction of hypocotyl elongation, a tighter hook forms at the hypocotyls apex, and the hypocotyls becomes thicker. Mutants that are insensitive to ethylene will not respond, and will continue etiolated plant growth. Insensitive mutants will be transferred to ACC-free media until large enough to transfer to soil. Mutant plants will be grown and seed collected.

To Do:

4/28: Sterilize seeds (see handout). Spread wt and mutant seeds on ACC- and ACC-free media (instructions in class). Seal plates with surgical tape. Wrap in foil to exclude light. Chill seeds at 4° C for 2 days.

4/30: Move plates to room temperature. Do not remove foil.

5/5: Score mutant plates for ACC insensitive plants. Transfer mutants to ACC-free plates. Grow in light. Refer to Arabidopsis Diary for further phenotypic analysis.