Screening for NERDs in *Arabidopsis thaliana*

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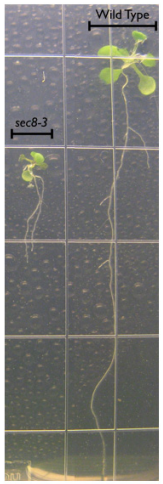
Abstract:

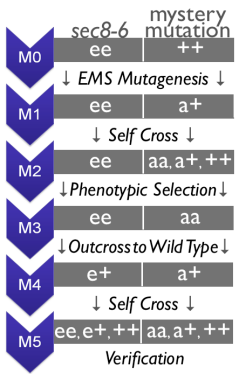
Plant survival depends upon the directed growth of plant roots into the soil, where essential water and nutrients can be obtained. The molecular basis of this root growth depends upon a protein complex called the exocyst. Mutations affecting exocyst proteins result in slow root growth and dwarf roots (see figure to right). In non-plant species the exocyst has been shown to be involved in exocytosis and secretion, but exactly how the exocyst functions to affect root growth is unknown. To better understand the exocyst's role in root growth, a screen was developed to identify proteins that interact with the exocyst. These interacting proteins are named **N**ew **E**nhancers of **R**oot **D**warfism, or NERDs. I worked on the last step of the screen for NERDs, searching the phenotypes and genotypes of over 6,000 Arabidopsis plants in 21 families for signs of an interaction. Two families yielded interesting results, potentially identifying two new NERDs involved with the exocyst in determining the rate and direction of root growth, as well as the formation and morphology of root hairs. My results indicate the genetics involved in identifying these NERDs is more complicated than anticipated, and provide a direction for future investigations.



Above: A severe exocyst mutation (sec8-3) affects root development.

The Nerd Screen:

The goal of the NERD Screen is the identification of novel exocyst interactors. Through EMS exposure, random mystery mutations were introduced into many lines homozygous for the *sec8-6* mutation. These lines were manipulated to isolate those new mutations that interacted synergistically with *sec8-6*. The last step of the screen, and the step that I carried out this summer, is called M5 verification. If an exocyst interactor was mutated through EMS exposure, a severe mutant phenotype would occur in one in sixteen generation M5 plants, which would be homozygous for the mystery mutation and *sec8-6*.



M5 Verification

M4 Pollen Parent

M4 Female Parent	++	e+	+a	ea
++	++ ++	e+ ++	++ a+	e+ a+
e+	e+ ++	ee ++	e+ a+	ee a+
+a	++ a+	e+ a+	++ aa	e+ aa
ea	e+ a+	ee a+	e+ aa	ee aa

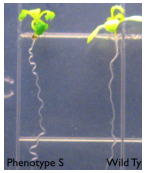
No Phenotype (3/4)
Mutant Phenotype (3/16)
Severe Mutant Phenotype (1/16)

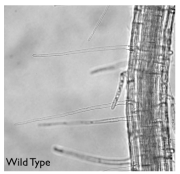
e: *sec8-6* (exocyst mutation) allele, **a**: mutant allele from EMS mutagenesis, **+**: wild type allele

Family 9 Phenotype S:

Plants with phenotype S do not have any root hairs, and have squiggly roots. Plants with this phenotype are 90% homozygous for *sec8-6*. This is significantly greater than the 25% expected for a non-interactor. Alternative hypotheses are discussed below.

Phenotype S Observed: 56/332 or **~2.7/16**



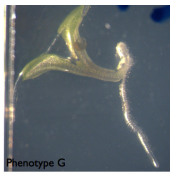


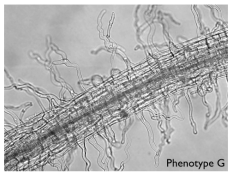
		Family 9 Genotypes					
		ee		e+		++	
Phenotype	Total	#	%	#	%	#	%
Negative Result			25%		50%		25%
WT	9	1	11%	5	56%	3	33%
S	20	18	90%	2	10%	0	0%

Family 10 Phenotype G:

Plants with phenotype G have shorter roots with short, dense, abnormally shaped root hairs. Phenotype G plants are 87% homozygous for *sec8-6*. This is significantly greater than the 25% expected for a non-interactor. Alternative hypotheses are discussed below.

Phenotype G Observed: 50/299 or **~2.7/16**

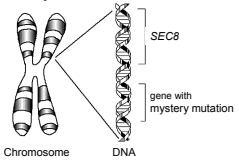




		Family 10 Genotypes					
		ee		e+		++	
Phenotype	Total	#	%	#	%	#	%
Negative Result			25%		50%		25%
WT	13	1	8%	8	62%	4	31%
G	15	13	87%	2	13%	0	0%

Hypothesis A:

sec8-6 and the mystery mutations that cause phenotypes S and G may be linked genes. This means that they are located near each other on the same chromosome, increasing their chances of being inherited together. Crossing over may allow for some independent assortment. If this hypothesis is confirmed, the mutated proteins that cause these phenotypes may not be exocyst interactors.



Hypothesis B:

The mystery mutations that cause phenotypes S and G may act as dominant alleles, meaning that they may be expressed with only one copy of the allele present. The phenotypic expression ratio would be 3/16, similar to my observations. The mutated proteins that cause these phenotypes may be exocyst interactors.

M4 Pollen Parent

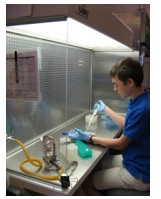
M4 Seed Parent	++	e+	+a	ea
++	++ ++	e+ ++	++ a+	e+ a+
e+	e+ ++	ee ++	e+ a+	ee a+
+a	++ a+	e+ a+	++ aa	e+ aa
ea	e+ a+	ee a+	e+ aa	ee aa

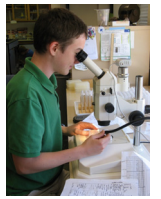
No Phenotype (13/16)
Mutant Phenotype (3/16)

e: *sec8-6* (exocyst mutation) allele, **a**: mutant allele from EMS mutagenesis, **+**: wild type allele

Procedure:

- Prepared plates with growth medium under a laminar flow hood
- Put seeds onto plates with a micropipettor
- Grew plates upright in a climate-controlled growth chamber
- Made visual observations with the aid of a dissecting microscope on days three, five, and seven
- Performed DNA extraction with the aid of a drill and special bit
- Amplified DNA by PCR on a RoboCycler
- Imaged DNA following gel electrophoresis





Results:

Family	Outcome	Family	Outcome
1	appears to be 1/4	12	1/16 category not valid
2	genotyping negative	13	appears to be 1/4
3	genotyping negative	14	appears to be 1/4
4	too many phenotypes, dead	15	*sec8-6* not present
5	genotyping negative	16	*sec8-6* not present
6	genotyping negative	17	*sec8-6* not present
7	chlorosis	18	*sec8-6* not present
8	genotyping negative	19	*sec8-6* not present
9	putative NERD	20	*sec8-6* not present
10	putative NERD	21	*sec8-6* not present
11	no 1/16 category		

Conclusion:

Families 9 and 10 are putative NERDs, however additional research is required to confirm and investigate this finding.

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