P111

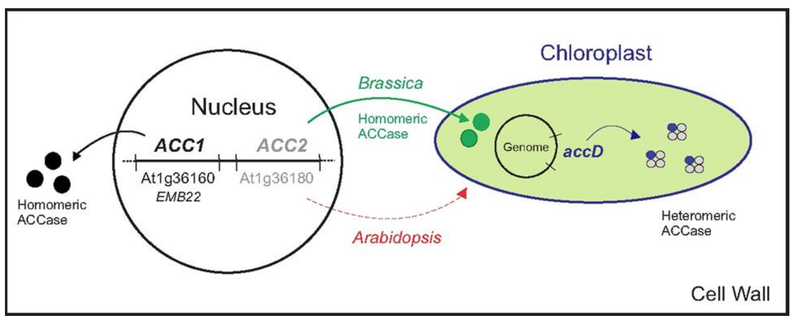
# Natural Variation in the Developmental Consequences of a Loss of Chloroplast Translation in *Arabidopsis thaliana*

**Nicole Bryant, Yixing Wang, David Meinke**

Department of Botany, Oklahoma State University, Stillwater, OK, USA

## Abstract

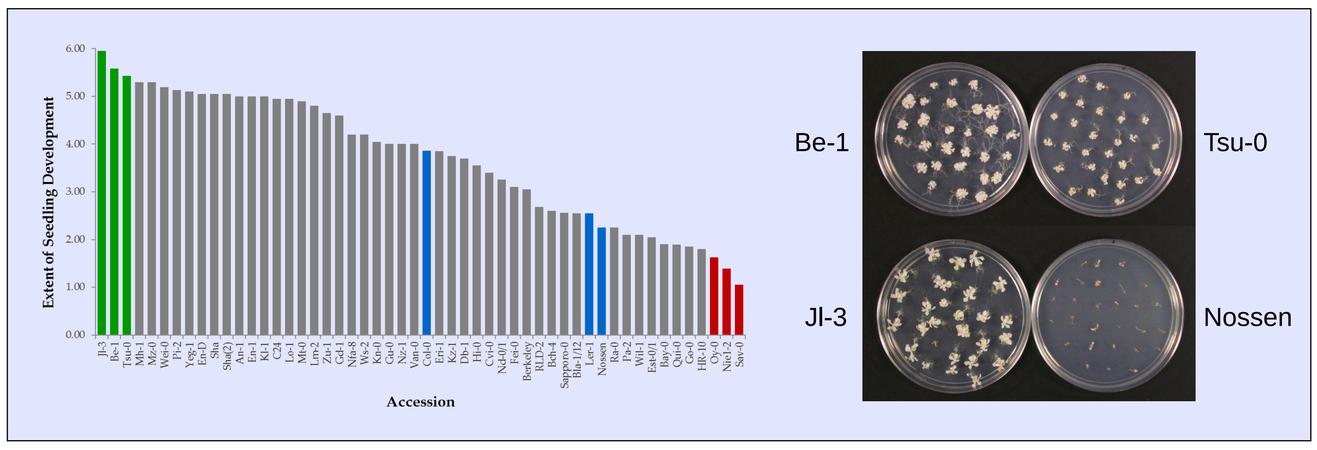
Interfering with chloroplast translation is typically more detrimental to growth and development in *Arabidopsis* than in *Brassica* or maize. This difference appears to reflect, in part, variation in the presence and functionality of a duplicated nuclear gene encoding a plastid-localized acetyl-CoA carboxylase (ACCase) required for fatty acid biosynthesis. In this study, we demonstrate that different accessions of *Arabidopsis thaliana* also vary in their ability to tolerate a loss of chloroplast translation. Two different approaches were pursed to block chloroplast translation: incorporation of spectinomycin into culture media used for seedling growth; and analysis of mutants defective in genes encoding chloroplast-localized ribosomal proteins. From an initial survey of 52 early-flowering accessions germinated on spectinomycin, several were chosen for further analysis: tolerant accessions that produced albino rosettes; sensitive accessions with at most rudimentary leaves; and sensitive/intermediate accessions associated with knockouts of *EMB* genes encoding chloroplast-localized ribosomal proteins. When sensitive and tolerant accessions were crossed and responses of F2 plants analyzed on spectinomycin, results were in some cases consistent with a single locus conferring tolerance. Crosses were then performed between mutants defective in chloroplast translation and representatives of tolerant and sensitive accessions. This resulted in the identification of a suppressor locus (Tsu-0 accession) that partially rescues mutant seeds and maps to the *ACC2* region of chromosome 1. Other genetic modifiers that support further embryo development were also found. Surprisingly, RT-qPCR experiments revealed that *ACC2* expression is not elevated in seedlings of tolerant accessions. Other features that map to the *ACC2* region must therefore be involved. Possibilities include increased translation efficiency, chloroplast protein import, enzyme function, or protein stability. Whether *ACC2* activation in transgenic plants completely rescues mutant embryos remains to be determined. However, *ACC2* knockouts appear to be more sensitive to spectinomycin, consistent with our model. Overall, this work highlights the importance of evaluating accession-specific differences in mutant phenotypes in Arabidopsis.




## Current Model

A single chloroplast gene (*accD*) is critical for embryo development in Arabidopsis. This gene encodes one component of the heteromeric enzyme ACCase, which is required for the initial stages of fatty acid biosynthesis in plastids

## Seedling Responses of *Arabidopsis* Accessions on Spectinomycin




Several tolerant (Tsu-0, Jl-3, Be-1), sensitive (Nossen, Oy-0, Nie1-2), and intermediate (Col-0) accessions were chosen for detailed analysis. $F_2$ seedling responses following crosses between Tsu-0 and Nossen accessions suggested a single locus might be involved. Blue bars represent accessions associated with knockouts of chloroplast-localized ribosomal proteins.

## Embryo Phenotypes of Knockouts of Chloroplast-Localized Ribosomal Proteins Differ According to Parental Accession

| Source | Strong Mutant Alleles | Accession | Embryo Phenotype |
|---|---|---|---|
| Riken | 6 | Nossen | Preglobular |
| Syngenta | 3 | Columbia | Large Globular |
| SALK | 4 | Columbia | Large Globular |
| CSHL | 1 | Ler | Small Globular |
| JIC | 1 | Ler | Small Globular |

### Mutants Chosen for Detailed Analysis

| Gene | Ribosomal Protein | Mutant Allele | Source | Accession | Embryo Phenotype | Embryo Size (µm) |
|---|---|---|---|---|---|---|
| *EMB3126* | L1 | 1 | Riken | Nossen | Preglobular | 25 |
| | | 3 | JIC | Ler | Small Globular | 60 |
| *EMB3137* | S13 | 1 | Riken | Nossen | Preglobular | 25 |
| | | 2 | SALK | Columbia | Large Globular | 90 |

Note the correlation between the stage of embryo arrest and the growth response of wild-type seedlings on spectinomycin.

## Crosses Between Tolerant Accessions and Embryo-Defective Mutants in a Spectinomycin-Sensitive Background

| $F_1$ Parental Plant Genotype: EeST | | | | |
|---|---|---|---|---|
| Gametes | E S | E T | e S | e T |
| E S | WT; S | WT; H | H; S | H; H |
| E T | WT; H | WT; T | H; H | H; T |
| e S | H; S | H; H | X; S | X; H |
| e T | H; H | H; T | X; H | X; T |
| E = *EMB* ; e = *emb* ; S = Sensitive; T = Tolerant | | | | |
| Gametes | Wild-type | Heterozygote | | Aborted |

### Expected Results if Suppressor Identified

$F_1$ Siliques: 25% Mutant ($F_2$) Seeds

$F_2$ Mutant Seeds: 75% are Rescued

Segregating $F_2$ Plants:

25% of Plants: Parental Seed Phenotype
25% of Plants: Rescued Seed Phenotype
50% of Plants: Both Seed Phenotypes

$F_3$ Generation: Plants with later seed phenotypes if other genetic modifiers present

## Partial Embryo Rescue Found in $F_1$ Siliques

| Mutant | Tolerant Accession | Siliques Screened | Seeds Screened | Mutant Seeds | Fraction of Mutant Seeds Exhibiting Embryo Rescue | Avg. Size of Mutant Embryos Identified |
|---|---|---|---|---|---|---|
| *emb3126*-Riken | Tsu-0 | 40 | 1842 | 24.1% | 71.4% | 84 µm |
| *emb3137*-Riken | Tsu-0 | 40 | 1939 | 24.3% | 75.4% | 78 µm |

## Expected Classes of $F_2$ Plants Identified:

| Class | Plants Screened | Total Seeds | Percent Mutant Seeds | Percent Embryo Visible |
|---|---|---|---|---|
| SS | 21 | 3199 | 27.0 | 0.8 |
| ST | 49 | 6103 | 26.3 | 76.7 |
| TT | 31 | 7862 | 25.3 | 99.4 |
| Wild Type | 45 | 1549 | 0.2 | - |
| Total | 146 | 18713 | - | - |

***EMB3126-Riken* x Tsu-0**

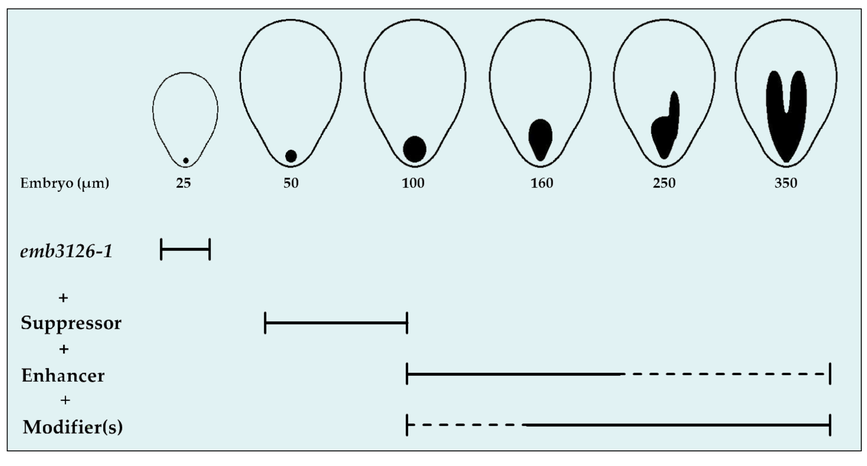
Parental Seed Phenotype (SS)

Both Seed Phenotypes (ST)

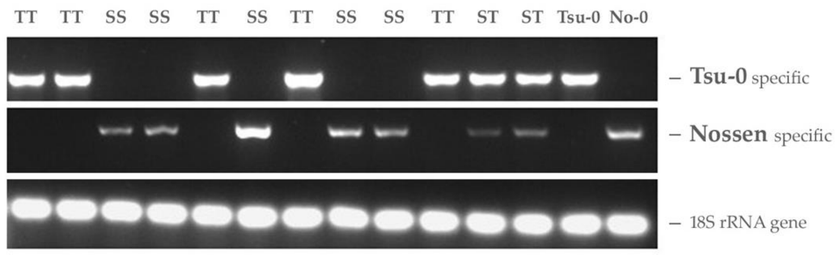
Rescued Seed Phenotype (TT)

Early TT: 16 Plants
Intermediate: 9 Plants
Late TT: 6 Plants

## Genetic Modifiers Identified in Tsu-0 Accession



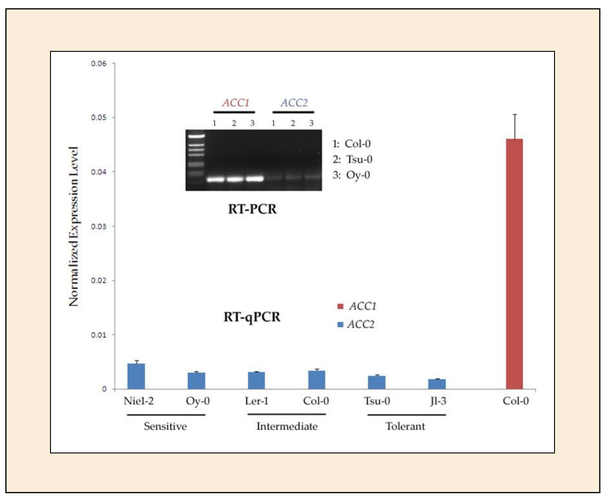

## Suppressor Locus Maps to *ACC2* Region




| $F_2$ Plant Phenotype | Symbol | Plants Genotyped | *ACC2* Genotype Results |
|---|---|---|---|
| Late | TT | 49 | Homozygous Tsu-0 |
| Early | SS | 29 | Homozygous Nossen |
| Both | ST | 2/79 | Heterozygous |
| Wild Type | WT | 0/82 | Not Tested |

## *ACC2* Expression in Different Accessions

Transcript abundance does not explain spectinomycin tolerance




## Accession Differences in ACC2 Protein Sequence Do Not Correlate With Spectinomycin Tolerance

N-Terminal Region Including Chloroplast-Localization Sequence

```
                1        10        20        30        40        50        60        70        80        90       100       106
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+-----|
Jl_3        MEMRALGSSCSTGNGGSAPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLFRTKQQFPLFCFLNPDPISFLDNDVSEAERTVVLPDGSVNG
Tsu-0       MEMRALGSSCSTGNGGSAPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLSRTKQQFPLFCFLNPDPISFLDNDVSEAERTVVLPDGSVNG
Mz_0        MEMRALGSSCSTGNGGSAPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLSRTKQQFPLFCFLNPDPISFLDNDVSEAERTVVLPDGSVNG
Wei_0       MEMRALGSSCSTGNGGSAPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLSRTKQQFPLFCFLNPDPISFLDNDVSEAERTVVLPDGSVNG
Yeg-1       MEMRALGSSCSTGNGGSAPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLSRTKQQFPLFCFLNPDPISFLDNDVSEAERTVVLPDGSVNG
Col-0       MEMRALGSSCSTGNGGSAPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLSRTKQQFPLFCFLNPDPISFLENDVSEAERTVVLPDGSVNG
Ler-1       MEMRALGSSCSTGNGGSTPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLFRTKQQFPLFCFLNPDPISFLDNDVSEAERTVVLPDGSVNG
No-0        MEMRALGSSCSTGNGGSAPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLSRTKQQFPLFCFLNPDPISFLDNDVSEAERTVVLPDGSVNG
Bay-0       MEMRALGSSCSTGNGGSAPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLSRTKQQFPLFCFLNPDPISFLDNDVSEAERTVVLPDGSVNG
Qui-0       MEMRALVSSCSTGNGGSAPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLSRTKQQFPLFCFLNPDPISFLENDVSEAERTVVLPGGSVNG
HR_10       MEMRALGSSCSTGNGGSAPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLSRTKQQFPLFCFLNPDPISFLDNDVSEAERTVVLPDGSVNG
Oy-0        MEMRALGSSCSTGNGGSAPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLSRTKQQFPLFCFLNPDPISFLDNDVSEAERTVVLPDGSVNG
Nie1-2      MEMRALVSSCSTGNGGSAPITLTNISPWITTVFPSTVKLRSSLRTFKGVSSRVRTFKGVSSTRVLSRTKQQFPLFCFLNPDPISFLENDVSEAERTVVLPGGSVNG
```

**Supported by Grants from the National Science Foundation**