

Mendel's *Le* gene and stem elongation

Christine Beveridge and Kerry Condon

Background

The genetic variation among individuals of the same species is usually due to slightly different sequences of a subset of genes in the genome. In order to study a particular gene, we often use naturally occurring or induced genetic variations. In some cases, a gene with 3000 bases of DNA will have a different function, simply because of a single base deletion or substitution. This has turned out to be the case for genes studied in garden pea by the monk, Gregor Mendel, the man often regarded as the father of genetics. The tall gene *LE* and the dwarf gene *le* are named alleles; they are the same gene, except they differ in a single base-pair of DNA. The tall gene or plant with that carries *LE* is termed the wild-type (WT) and the dwarf gene, *le*, the mutant. The main reason for this is that the WT gene is more common and, more importantly, plants that carry *Le* and *le* have a WT appearance (phenotype).

The green revolution that led to massive improvements in crop yield was led largely caused by the *le* mutation in pea because dwarf plants allocate less energy to stems, leaving more to the seeds and pods and are less prone to lodging (falling over). Stem elongation is controlled by several factors including plant developmental stage or state, light quality, nutrient and genotype. Many of these components are thought to mediate changes in elongation through modifications relating to plant hormones, particularly gibberellins (GA)s. These modifications may be through affecting the level, transport or perception of hormones. Much of our understanding of plant hormones comes from experiments with mutants affected in one of these processes.

Hypotheses

Our main hypotheses tested in this experiment are that:

- (1) GAs promote stem elongation in pea;
- (2) The Massey cultivar is dwarf because it is deficient in GA;
- (3) Plants in the dark can elongate efficiently, even if they may be deficient in GA.

You will be supplied with WT and two different mutant lines to test responses to exogenous GA. If the dwarf plant is deficient in GA, adding extra GA to it should make it tall. If Massey is GA deficient, based on a response to GA that matches plant height in WT, we can test if this affects its ability to elongate in the dark.

Materials and Methods

There will be two commercially available cultivars of pea (*Pisum sativum* L.); Telephone (tall) and Massey (dwarf).

3 treatments; dark grown plants and control and +GA₃ for light grown plants. GA₃ is a synthetic bioactive GA. There will be 15-20 plants per treatment.

Treatments Table

Cultivar or Line	Genotype	Light grown		Dark grown	
		Control	+GA	Control	
Tall	WT				
Dwarf	<i>le</i>				

Procedure

- 1) Carefully place a nick in the testa (seed coat) of each seedling, without disturbing the embryo. Use a single sided razor blade and pay attention to your thumbs and fingers! This nick should be 2-8 mm² and will enable access of the GA solution to the seed.
- 2) Treat the surface of the exposed seed with the GA solution. Use 2μL of a 10μg/μL solution of GA₃ in 50 or 100% ethanol or methanol in water. If this is not possible, then wash the seeds in a water solution containing GA.
- 3) After these seeds are dry, plant them at 2 cm depth in pots containing potting mix that has been thoroughly saturated with water.
- 4) In 10-14 days, measure total plant height and internode length for each plant in each treatment (a total of 24 plants). Select an internode (or internodes) that are representative of the GA-response you see and measure the same internode(s) for all treatments. You may choose to measure the length of internodes 3 to 6. This accounts for the delay in GA-uptake at the first nodes and that different treatments may lead to a different number of nodes. Count node numbers from the base of the plant upwards. There are small scale leaves at the first two nodes; the first true leaf is node 3.

Data Recording

For each plant, measure the following traits and record on the scoring sheets on the next two pages.

- the number of leaves expanded, including the two scale leaves at the base of the plant (**LE**);
- the total length of the stem from node 1 to the apex (**TL**) in cm +/- 1 mm; and
- the length between the basal internodes (**Int.**) in cm +/- 1 mm, as decided for this experiment.

Add your data to the scoring sheet on the board to share with the rest of the class.

Data Analysis

Calculate the mean and standard error (SE) for each treatment group and genotype (see Appendix 1).

Plot the data in a graph showing mean +/- SE.

Compare the effects of the different treatments and the responses of the two mutant genotypes. Use a student T-test to test for significant differences (see Appendix 1).

What conclusions can you draw from these responses?

Prepare your report in the Practical Report format given in this Learning Guide.

Here are some questions to think about for this prac:

- Why were the seeds planted into already wet pots, rather than water afterwards?
- What is the effect of continuing plant growth and development on the response to a single hormone application.
- Can you use your data to explain the action of *le*?

Reading:

<http://www.plantphys.net/article.php?ch=e&id=276>.

Appendix 1 (Statistics)

Calculation of Means, Standard Errors in Microsoft Excel and the Students t-test

The chart below directly from Excel shows how to calculate means and standard errors.

Gen.	Treatment	TL	Treatment	TL
WT	-GA	1	+GA	17
WT	-GA	2	+GA	18
WT	-GA	3	+GA	19
WT	-GA	4	+GA	20
WT	-GA	5	+GA	21
WT	-GA	6	+GA	22
WT	-GA	7	+GA	23
WT	-GA	8	+GA	24
WT	-GA	9	+GA	25
WT	-GA	10	+GA	26
WT	-GA	11	+GA	27
WT	-GA	12	+GA	28
WT	-GA	13	+GA	29
WT	-GA	14	+GA	30
WT	-GA	15	+GA	31
WT	-GA	16	+GA	32
	Mean	21.1	Mean	22.6
	SE	0.46	SE	0.46

mean=AVERAGE(D3:D18)
 standard error (SE)=standard deviation/ \sqrt{n} , where n is the number of plants
 =STDEV(D3:D18)/SQRT(COUNT(D3:D18))

On-1 from your calculator is standard deviation for a small population.

The t-test to consider if the control (-GA) and +GA treatments are significantly different is as follows:

The **t-value** =
$$\frac{\text{mean}_1 - \text{mean}_2}{\sqrt{(\text{SE}_1^2 + \text{SE}_2^2)}}$$

or the differences of the means divided by the square root of the addition of each SE squared. In this example, t=2.31.

T-Table

df	0.1	0.05	0.01	0.001
1	3.078	6.314	31.82	318.3
2	1.886	2.92	6.965	22.33
3	1.638	2.353	4.541	10.21
4	1.533	2.132	3.747	7.173
5	1.476	2.015	3.365	5.893
6	1.44	1.943	3.143	5.208
7	1.415	1.895	2.998	4.785
8	1.397	1.86	2.896	4.501
9	1.383	1.833	2.821	4.297
10	1.372	1.812	2.764	4.144
11	1.363	1.796	2.718	4.025
12	1.356	1.782	2.681	3.93
13	1.35	1.771	2.65	3.852
14	1.345	1.761	2.624	3.787
15	1.341	1.753	2.602	3.733
16	1.337	1.746	2.583	3.686
17	1.333	1.74	2.567	3.646
18	1.33	1.734	2.552	3.611
19	1.328	1.729	2.539	3.579
20	1.325	1.725	2.528	3.552
21	1.323	1.721	2.518	3.527
22	1.321	1.717	2.508	3.505
23	1.319	1.714	2.5	3.485
24	1.318	1.711	2.492	3.467
25	1.316	1.708	2.485	3.45
26	1.315	1.706	2.479	3.435
27	1.314	1.703	2.473	3.421
28	1.313	1.701	2.467	3.408
29	1.311	1.699	2.462	3.396
30	1.31	1.697	2.457	3.385
40	1.303	1.684	2.423	3.307
50	1.295	1.676	2.403	3.261
100	1.29	1.66	2.364	3.174
1000	1.282	1.646	2.33	3.098
inf.	1.282	1.64	2.326	3.091

The t-value is then compared to a t-Table using the **degrees of freedom (df) = $n_1+n_2 - 2$** , where n is the population size of each population. Our example has a *df* of 30. As $t=2.31$, or t-value falls between the 0.05 and 0.01 values.

A difference between the two means is considered significant at the 0.05, 0.01 or 0.001 level if the t-value is GREATER than the value in the t-Table at the appropriate degree of freedom. Significance at the 0.05 level, which is the arbitrary cut-off value for significance means that there is a 5% chance that the difference occurred by chance alone.

In this case, we would write that the total length (TL) of GA treated plants is significantly greater ($P<0.05$) than control plants.

/