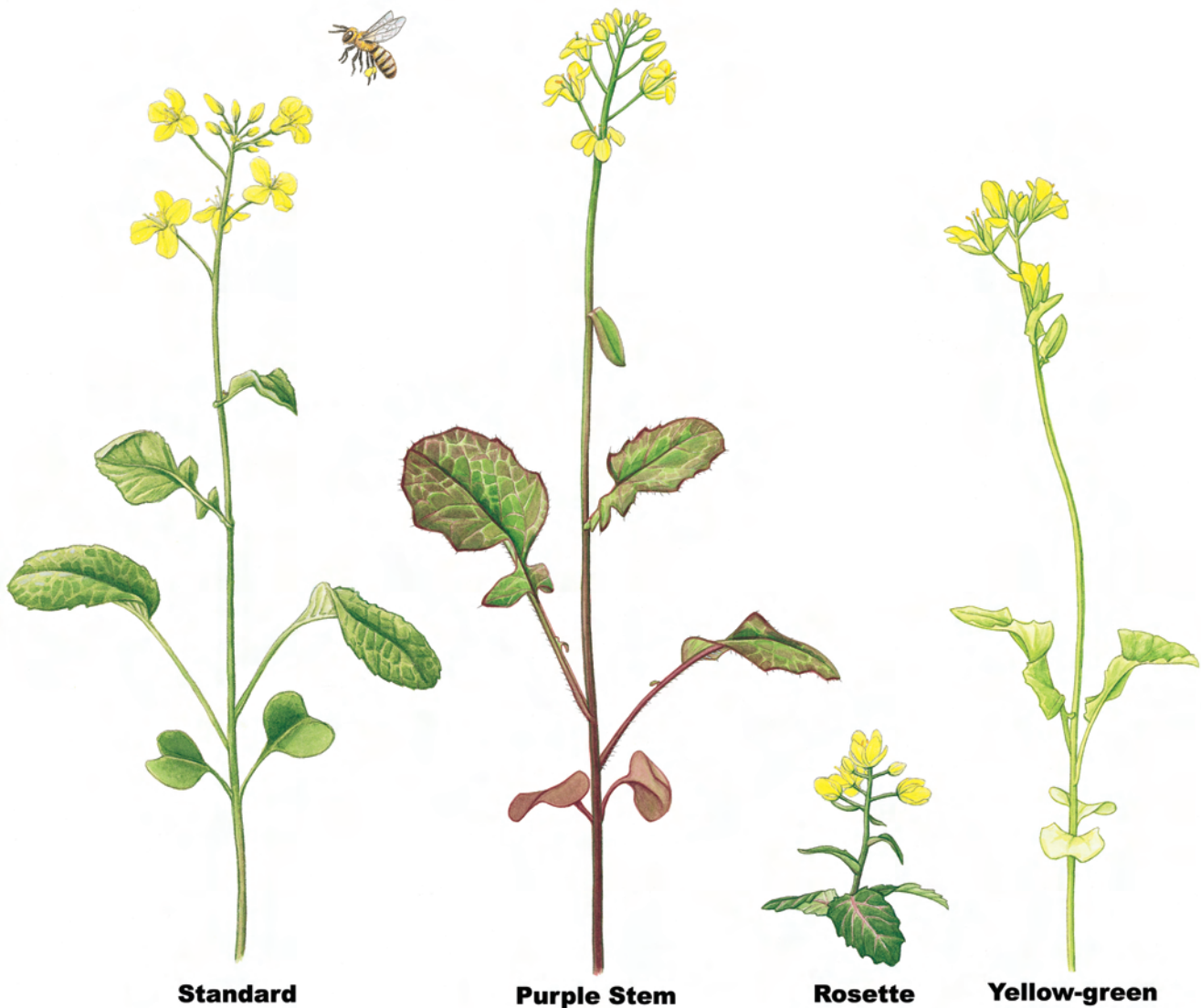




Investigating Mendelian Genetics with Wisconsin Fast Plants™



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For additional activities,
student pages and related
resources, please visit
the Wisconsin Fast
Plants' website at
www.fastplants.org



Investigating Mendelian Genetics with Wisconsin Fast Plants™

Activity Materials

Wisconsin Fast Plants™ Monohybrid Genetics Kit Components

- 1 pack of 25 Purple Stem Wisconsin Fast Plants™ Seeds (parent)
- 1 pack of 25 Non-Purple Stem Seeds (parent)
- 1 pack of 200 F1 Wisconsin Fast Plants™ Seeds (from a cross of the parent strains [anl/ANL])
- 1oz pelleted fertilizer
- 2 watering trays
- 2 watering mats
- wicks (package of 70)
- 1 packet anti-algal square (2 squares per packet)
- 8 watering pipettes
- 1 L potting soil
- 1 package of dried bees
- four 4-cell quads
- 16 support stakes
- 16 support rings
- Growing Instructions

Wisconsin Fast Plants™ Dihybrid Genetics Kit Components

- 1 pack of Non-Purple Stem Wisconsin Fast Plants™ Seeds (parent)
- 1 pack of Yellow-Green Leaf Wisconsin Fast Plants™ Seeds (parent)
- 1 pack of F1 Wisconsin Fast Plants™ Seeds (from a cross of the parent strains [anl/ANL, ygr/YGR])
- 1oz pelleted fertilizer
- 2 watering trays
- 2 watering mats
- wicks (package of 70)
- 1 packet anti-algal square (2 squares per packet)
- 8 watering pipettes
- 1 L potting soil
- 1 package of dried bees
- four 4-cell quads
- 16 support stakes
- 16 support rings
- Growing Instructions



Investigating Mendelian Genetics with Wisconsin Fast Plants™



Welcome to the wonderful world of Wisconsin Fast Plants™ and investigations to engage students in studying Mendelian genetics with hands-on experimentation. Originating from decades of innovative research by Professor Paul Williams at the University of Wisconsin-Madison, Wisconsin Fast Plants™, *Brassica rapa*, are now used by millions of students worldwide.

The investigations in this booklet were designed for students to develop understanding of the following biological concepts and skills:

- Mendel's law of segregation and law of independent assortment
- Inheritance of 2 traits
- How genotypes influence phenotypes
- Scientific inquiry, including interpretation of evidence

Wisconsin Fast Plants™ Genetics Background Information

Phenotypes and Genotypes

Rapid-cycling *Brassica rapa* has been designated the cultivar name "RCBr." **Anthocyanin** is a purple pigment found in many plants, including Wisconsin Fast Plants™. Anthocyanin is best observed when the plants are 4-7 days old. Look on the stems and hypocotyls, under the cotyledons, and at the leaf tips. A single gene, the anthocyaninless gene (*anl*), in Wisconsin Fast Plants™ regulates whether or not anthocyanin will be expressed. In the homozygous, recessive form (*anl/anl*), anthocyanin expression is completely suppressed, and the plants appear a bright green color (which is the "non-purple stem "

phenotype). If the genotype is *anl/ANL* or *ANL/ANL*, then anthocyanin is expressed at varying levels and the plants are the "purple stem" phenotype. The genotype of the wild type is *ANL/ANL*.

The yellow-green gene (*ygr*) in Wisconsin Fast Plants™ determines whether the leaves will be yellow-green or green in color. In the homozygous, recessive form (*ygr/ygr*), the leaves appear a pale, yellow-green color (which is the "yellow-green leaf" phenotype). If the genotype is *ygr/YGR* or *YGR/YGR*, then the leaves appear green (which is the "green leaf" phenotype). The genotype of the wild type is *YGR/YGR*.

The rosette mutant stock is Wisconsin Fast Plants™ stock that is homozygous for the recessive mutant gene, *ros*, a conditioning deficiency in gibberellin that results in a short, rosette plant form. In the homozygous, recessive form (*ros/ros*), the plants have the rosette form. If the genotype is *ros/ROS* or *ROS/ROS*, then the plants have the standard form. The genotype of the wild type is *ROS/ROS*.

(Note: The rosette phenotype can be induced to grow normally by applying exogenous gibberellin in the form of gibberellic acid.)

Terminology

P_1 = maternal parent

P_2 = paternal parent

F_1 = first-generation offspring, the result of crossing P_1 and P_2 plants

F_2 = second-generation offspring, the result of intermating the F_1 plants

Growing Tips

(See *Growing Instructions* for complete guide)



- To ensure high seed yields and to optimize expression of the genetic traits used in this investigation, follow the Growing Instructions carefully.
- It is easiest to observe the purple color on the hypocotyls (stems) when the plants are 4-7 days old.
- The intensity of purple color is affected by the environment. More light yields a deeper purple color, as does reduced fertilizer.
- Do not germinate Yellow-Green Leaf plants in Petri plates. The yellow-green color is virtually indistinguishable from the green color when seeds are germinated in Petri plates. Use a soil medium instead.
- Expect an approximate 9:3:3:1 ratio of plants in the F_2 generation. Due to the random nature of gamete segregation, an exact 9:3:3:1 ratio is unlikely to be observed. Use the ratio as a foundation for understanding the Law of Segregation.

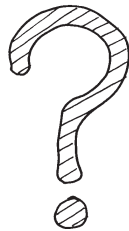
GENETICS ACTIVITIES OVERVIEW

Since the dawn of agriculture, humans have been trying to improve and increase their food supply. Historically, this was done by selecting the best plants and animals, then breeding them to get the tastiest, hardiest, most nutritious, or most appealing offspring. But no one really understood how these traits were inherited. Many people believed that traits “blended” in the offspring, or that only one parent passed a trait on to its offspring.

In the mid-1800’s, a monk named Gregor Mendel gathered evidence that began explaining how inheritance works. Mendel studied discrete and contrasting traits. For example, the traits that he explored in pea plants included tall vs. short plants and purple vs. white flowers. His careful, repetitious work yielded two principles of genetics: the Law of Segregation and the Law of Independent Assortment. This activity is designed for students to engage in a hands-on experiment to see evidence for these two principles.

QUESTIONS

- How can you distinguish whether the mutant trait you are using is inheritable or is the result of environmental effects?
- If the mutant type is under genetic control, is it conditioned by a single gene, a few genes, or many genes? What clues will help you determine how many genes are involved?
- If a single gene controls the mutant type, how will you determine which is the dominant and which is the recessive?
- What is your hypothesis about the inheritance of the mutant trait?



Key Concepts

- Law of Segregation: Paired alleles separate during the formation of gametes.
- Law of Independent Assortment: Each allele segregates independently of the other allele in gamete formation.
- Alleles retain their identity during gamete production and pollination/ mating.
- Two alleles (one from each parent) are paired in the offspring; this constitutes the genotype of a trait.
- The offspring’s phenotype is based on the expression of both alleles; one allele may “mask” the expression of another in a dominant/recessive relationship, or both alleles may contribute to the apparent phenotype.

Monohybrid Activity Overview

In this activity, students will observe three generations of Wisconsin Fast Plants™; students will investigate paternity by observing phenotype and inferring genotype.

This investigation spans the entire life cycle of the mother and father (parent) generation (P_1 and P_2) and the first-generation offspring (F_1) of plants; and an additional nine days allow students to observe the second generation offspring (F_2). This activity is designed for students to investigate the inheritance of a single gene in Wisconsin Fast Plants™.

Note: *To ensure success of the plants — and to optimize conditions for expression of the genetic traits used in this investigation — it is important that you carefully follow the enclosed Wisconsin Fast Plants™ Growing Instructions flyer.*

Materials

Wisconsin Fast Plants™ seeds - Seeds of two generations are included:

wild-type RCB_r seeds (P_1) (non-purple stem, green, standard)

mutant-type seeds (P_2) (purple stem [anl/anl], yellow-green [ygr/ygr], or rosette [ros/ros])

F_1 non-purple stem, yellow-green leaf growing systems

Each growing system includes:

- 1 circular watermat
- 1 long watermat wick
- 1 small container
- 1 large container (reservoir)
- 4 pots
- 4 small blue watermat wicks
- potting mix
- fertilizer
- stakes and ties
- labels

Three Possible Mutants

1. Anthocyaninless - mutant gene anl in the homozygous condition, anl/anl, blocks the expression of anthocyanin (results in completely green seedlings).
2. Yellow-green - mutant gene ygr in the homozygous condition, ygr/ygr, produces yellow-green cotyledons and leaves.
3. Rosette - mutant gene ros in the homozygous condition, ros/ros, results in gibberellin deficiency and leads to lack of stem elongation.

Monohybrid Activity

Genetics Investigation Procedure

In this investigation, you and your classmates will be given the seeds from one generation of Wisconsin Fast Plants™: the first-generation offspring (F_1). Four days after planting, you will be able to make observations about the phenotype of the young plants. Your teacher will plant seeds from the previous generation, the mother (P_1) and father (P_2) generation. How will you distinguish whether the mutant trait you are using is inheritable?

First Generation (F_1 Hybrid)

Day 1

Follow the “Growing Instructions” and plant F_1 seeds in all four cells of your quad. Be sure to label your quad.

Your teacher will plant a quad of each of the parental seed types (wild type and mutant type) for comparison.

Day 4 to 5

Compare the F_1 plants and both parental types. Record the number of wild-type and mutant plants that you observe among the F_1 plants in a data table. Thin to the most vigorous plant in each cell.

Day 14 to 18

Pollinate plants. Gather pollen on a bee stick from one of your F_1 plants and use it to pollinate another F_1 plant (there should be two or three flowers open on most plants when you start pollinating). Pollen may be exchanged among as many of the F_1 plants as you wish (within your quad or among all plants in the class; the more, the better at this stage). Do not worry about removing the anthers from a plant being pollinated; because the wild-type RCBrs are **self-incompatible**, little **self-pollination** will occur. Be sure that pollen from the parental-type demonstration plants is not used for these pollinations.

After pollinating, insert the bee stick in the potting mix at the base of one of your plants.

Pollinate plants about 2 and 4 days later. It is best to pollinate six to eight flowers. After your last pollination, pinch or cut off **all** un-opened buds and shoots so that uncontrolled pollinations cannot occur.

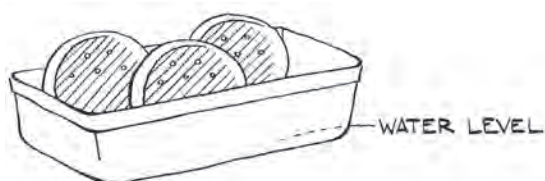
Day 20 to 45

Continue to remove new buds and shoots for the next 2 weeks. Keep plants watered and growing for 3 weeks after you finish pollinating to allow the seeds to ripen. Dry plants and harvest seeds as described in the “Growing Instructions.” Seeds from your plants may be combined.

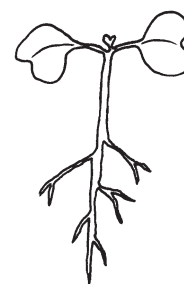
Second Generation (F₂)

Day 1

The mutant traits anl, ygr, and ros, are all recognizable at the seedling stage. However, **ygr (yellow-green leaf) need to be grown in soil** because the yellow-green color is nearly indistinguishable when seeds are germinated in Petri dishes. If using ygr, repeat the planting procedure outlined for the First Generation to grow second generation seedlings (see the growing instructions for detailed information about planting and growing Wisconsin Fast Plants™ in soil).



If using the Petri dish method, place one filter paper disc in the lid of a Petri dish. Write your name and the symbol of the mutant in pencil on the filter paper. Add water until the paper is wet; pour off excess water. Place 25 seeds in four neat rows on the upper two-thirds of the filter paper. Place the cover on the dish and stand the dish in about 2 cm of water in a reservoir. Tilt the dish slightly so that water collects at the bottom and place the dish under the light bank. Seeds should all be above the water line.



3-5 Day Seedling

Day 3 to 5

Observe seedlings and record numbers of mutant and wild-type plants in a data table.

Discussion Questions

1. Describe the plants in the F₁. What does this suggest about the inheritance pattern of the mutant trait?
2. What do the combined class data for the F₂ suggest about the inheritance of the mutant trait? (Remember to consider the number of genes involved as well as dominance or recessiveness.)
3. What can you conclude from the χ^2 and probability values?
4. Are the deviations from a 3:1 ratio in the F₂ significant?
5. Do the results agree with your original hypothesis? If not, what is your modified hypothesis?
6. What additional cross(es) would you do to verify your hypothesis?
7. If the mutant trait you used was rosette, when counting the number of rosette plants germinating in the F₂ generation, the assumption is made that seeds not germinating are rosettes. Would this assumption be valid if you were using F₂ seeds that had been produced by a previous year's class instead of your own freshly harvested seeds? Explain.

Dihybrid Genetics Activity

This activity follows the same basic procedure as the monohybrid activity; however, the P_1 and P_2 plants are homozygous for two traits, one recessive and one dominant.

There are four phenotypes used for this dihybrid genetics investigation:

- **Non-Purple** - (green) no purple anthocyanin pigment is expressed in the plant (anthocyaninless). The genotype for this single gene recessive mutant is anl/anl
- **Purple** - a pigment known as purple anthocyanin is visible on the hypocotyl. This trait is wild-type (dominant) for anthocyaninless and is referred to as ANL/ANL
Note: Anthocyanin is best observed when the plants are 4–7 days old. Look for anthocyanin expression on the stems and hypocotyls, under the cotyledons, and at the leaf tips.
- **Yellow Green** - this genotype, ygr , when homozygous (ygr/ygr) produces a plant that is lighter green than the normal green Fast Plant
- **Normal Green** - this genotype is wild-type (dominant) for yellow green and is referred to as YGR

<p>MOTHER GENERATION (P_1)</p> <p>Seed Packet Name: Non-Purple Stem Phenotype: non-purple stem, green leaf Genotype: $anl/anl, YGR/YGR$</p> <p>The P_1 plants are homozygous with the recessive alleles for anthocyanin expression (anl) and the dominant allele for yellow green leaf color (YGR). Anthocyanin expression is suppressed, so the stems appear bright green. The leaves and cotyledons also appear green.</p>	<p>FATHER GENERATION (P_2)</p> <p>Seed Packet Name: Yellow-Green Leaf Phenotype: purple stem, yellow-green leaf Genotype: $ANL/ANL, ygr/ygr$</p> <p>The P_2 plants are homozygous with the dominant allele for anthocyanin expression (ANL) and the recessive allele for yellow-green leaf color (ygr). Anthocyanin is expressed, so the stems show some purple color. The leaves and cotyledons are a pale, yellow-green color.</p>
<p>FIRST-GENERATION OFFSPRING (F_1)</p> <p>Seed Packet Name: F_1 Non-Purple Stem, Yellow-Green Leaf Phenotype: purple stem, green leaf Genotype: $anl/ANL, YGR/ygr$</p> <p>The F_1 plants are the result of crossing the P_1 and P_2 plants. They are heterozygous with both a dominant and a recessive allele for anthocyanin expression. Anthocyanin is expressed, so the stems show some purple color. They are also heterozygous with both a dominant and a recessive allele for yellow-green color. The leaves and cotyledons appear green. These plants are phenotypically different from both parents.</p>	<p>SECOND-GENERATION OFFSPRING (F_2)</p> <p>There is no seed packet for this generation. See page 16 for a list of genotypes.</p> <p>The F_2 plants are a result of intermating the F_1 plants. They represent all the genotypes listed in the P_1, P_2, and F_1 generations. The phenotypic ratio is approximately* 9:3:3:1 as follows:</p> <ul style="list-style-type: none"> 9 purple stem, green leaf 3 purple stem, yellow-green leaf 3 non-purple stem, green leaf 1 non-purple stem, yellow-green leaf <p>* Ratio is approximate, due to the random nature of independent assortment and segregation of gametes.</p>



Test Cross

The test cross is the essential cross in genetic analyses that provides the most informative indications of independent assortment or linkage between two traits being tested. In the test cross, F_1 progeny are crossed to a stock that is homozygous for the mutant alleles being examined for linkage.

The power of the test cross in linkage mapping is that the combined recombinant frequency (recombinant class count/total) is equal to genetic map units separating the two traits.

m.u. = recombinant class count/total

In testing two unlinked recessive mutants the frequency of each parental class and each of the two expected recombinant classes is 0.25. Consider recovering the recombinant mutant class from your F_2 to be used as a tester.



Chi-Square Test

A test that is often applied to determine how well observed ratios fit expected statistical ratios is known as the chi-square (χ^2) test. This test calculates the deviations of observed numbers from expected numbers into a single numerical value called χ^2 . The difference between the number observed and the number expected for a phenotype is squared and divided by the number expected. This is repeated for each phenotype class. The χ^2 value consists of the summation of these values for all classes. The formula for χ^2 (Suzuki, et al., 1986) is:

$$\chi^2 = \text{total of } \frac{(\text{observed} - \text{expected})^2}{\text{expected}} \text{ over all cases}$$

Associated with each χ^2 value is a probability that indicates the chance that, in repeated experiments, deviations from the expected would be as large or larger than the ones observed in this experiment.

In the following table, the column “Degrees of Freedom” is one less than the number of different phenotypes possible. In this experiment we have two possible phenotypes (the normal and the mutant) so there are $2 - 1 = 1$ degree of freedom. If the probability is greater than 5% (0.05), we accept the observed data.

Possibility of Chance Occurrence in Percentage

Degree Freedom	90%	80%	70%	50%	30%	20%	10%	5% (sig.)	1%
1	0.016	0.064	0.148	0.455	1.074	1.642	2.706	3.841	6.635
2	0.211	0.446	0.713	1.386	2.408	3.219	4.605	5.991	9.210
3	0.584	1.005	1.424	2.366	3.665	4.642	6.251	7.815	11.34
4	1.064	1.649	2.195	3.357	4.878	5.989	7.779	9.488	13.28
5	1.610	2.343	3.000	4.351	6.064	7.289	9.236	11.07	15.09
6	2.204	3.070	3.828	5.348	7.231	8.558	10.65	12.59	16.81
7	2.833	3.822	4.671	6.346	8.383	9.083	12.02	14.07	18.48
8	3.490	4.594	5.527	7.344	9.524	11.03	13.36	15.51	20.09
9	4.168	5.380	6.393	8.343	10.66	12.24	14.68	16.92	21.67

Put Your Results to the Test!

Put your claims to a test! Was the ratio of the phenotypes in the F_2 generation what you predicted it would be? Was it even close? A χ^2 will compare your observations with your hypothesis.

The χ^2 test calculates (1) the deviation between your observed numbers and your expected numbers and (2) the probability that the deviation is due to **chance** or that the deviation is significant. If the deviation is merely due to chance, then the results do not disprove your hypothesis. If the deviation is **significant**, then your results do not support your hypothesis and you **reject** your hypothesis. In either case, a scientist would then run more tests to see if the results were repeatable.

STEP 1 Determine the ratio of phenotypes you expected in the F_2 generation, based on your hypothesis.

Phenotype	Expected Ratio	Expected Number of Plants (e)
1. _____	_____	_____
2. _____	_____	_____
3. _____	_____	_____
4. _____	_____	_____

STEP 2 Record the ratio of phenotypes you observed in the F_2 generation.

Phenotype	Observed Number of Plants (o)
1. _____	_____
2. _____	_____
3. _____	_____
4. _____	_____

STEP 3 Calculate the χ^2 value. Fill out the following table, using numbers from the entire class. For o and e values, use the actual numbers of plants, not percentages or ratios.

List Each Phenotype:	1	2	3	4
Observed Value (o)				
Expected Value (e)				
Deviation (d) = $o - e$				
Deviation Squared (d^2)				
d^2 / e				

STEP 3 Calculate the χ^2 value, continued.

Add all of the d^2/e values together to get the χ^2 value:

STEP 4 Calculate the degrees of freedom.

To calculate the degrees of freedom, subtract one from the number of phenotypes possible.

Number of phenotypes possible Degrees of freedom

— 1 =

STEP 5 Determine whether to accept or reject your hypothesis.

Find the probability that the deviation of the observed values from the expected values was a chance occurrence. Look up your degrees of freedom in the table below. Find where your χ^2 value falls in that row.

Degrees of Freedom	Probability of Chance Occurrence								
	90%	80%	70%	50%	30%	20%	10%	5%	1%
1	0.016	0.064	0.148	0.455	1.074	1.642	2.706	3.841	6.635
2	0.211	0.446	0.713	1.386	2.408	3.219	4.605	5.991	9.210
3	0.584	1.005	1.424	2.366	3.665	4.642	6.251	7.815	11.341
4	1.064	1.649	2.195	3.357	4.878	5.989	7.779	9.488	13.277

Probability value:

If the probability is 5% or greater, then you can accept your hypothesis.
 If the probability is less than 5%, then reject your hypothesis.

Do you accept or reject your hypothesis?







Phenotypes and Genotypes





Rapid-cycling *Brassica rapa* has been designated the cultivar name “RCBr.”





Purple stem Wisconsin Fast Plants™ contain anthocyanin, a purple pigment found in many plants, including Wisconsin Fast Plants™. Anthocyanin is best observed when the plants are 4-7 days old. Look on the stems and hypocotyls, under the cotyledons, and at the leaf tips. A single gene, the anthocyaninless gene (*anl*), in Wisconsin Fast Plants™ regulates whether or not anthocyanin will be expressed. In the homozygous, recessive form (*anl/anl*), anthocyanin expression is completely suppressed, and the plants appear a bright green color (which is the “non-purple stem” phenotype). If the genotype is *anl/ANL* or *ANL/ANL*, then anthocyanin is expressed at varying levels and the plants are the “purple stem” phenotype. The genotype of the wild type is *ANL/ANL*.

The yellow-green gene (*ygr*) in Wisconsin Fast Plants™ determines whether the leaves will be yellow-green or green in color. In the homozygous, recessive form (*ygr/ygr*), the leaves appear a pale, yellow-green color (which is the “yellow-green leaf” phenotype). If the genotype is *ygr/YGR* or *YGR/YGR*, then the leaves appear green (which is the “green leaf” phenotype). The genotype of the wild type is *YGR/YGR*.













Three Monohybrid Options

F ₁ male gametes	Purple 1/2 wild-type	Non-Purple 1/2 mutant
F ₁ female gametes		
Purple 1/2 wild type		
Non-Purple 1/2 mutant		

F ₁ male gametes	Green 1/2 wild-type	Yellow-green 1/2 mutant
F ₁ female gametes		
Green 1/2 wild type		
Yellow-green 1/2 mutant		

F ₁ male gametes	Standard 1/2 wild-type	Rosette 1/2 mutant
F ₁ female gametes		
Standard 1/2 wild type		
Rosette 1/2 mutant		

Dihybrid F₂ Phenotypes

F ₁ male gametes	Purple, Green 1/4 2 wild type traits	Non-Purple, Green 1/4 1 mutant, Non-Purple 1 wild type, Green	Purple, Yellow-green 1/4 1 wild-type Purple 1 mutant, Yellow-green	Non-Purple, Yellow-green 1/4 2 mutant traits
F ₁ female gametes				
Purple, Green 1/4 2 wild type traits				
Non-Purple, Green 1/4 1 mutant, Non-Purple 1 wild-type Green				
Purple, Yellow-green 1/4 1 wild-type Purple 1 mutant, Yellow-green				
Non-Purple, Yellow-green 1/4 2 mutant traits	