

Two-gene segregation in barley

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Contents

The material includes seeds for one experiment, i.e. about 200 seeds.

Purpose

The goal of the experiment is to show how chlorophyll mutations appear, how each mutant gene is passed on to the next generation (3:1 segregation) and how two mutant genes are passed on simultaneously (9:3:4 segregation).

TEACHER'S GUIDE

About the materials in the set

The seeds provided have been harvested after natural self-pollination on barley plants which were heterozygous type AaBb for two recessive, independently inherited genes (unlinked genes).

The one mutant gene is referred to as "a", the other as "b". The seedlings with genotype aaBB will be white chlorophyll mutants (*Albina*), and seedlings with genotype Aabb will be yellow chlorophyll mutants (*Xanta*). Since chlorophyll mutants die in the seedling stage, the mutant genes must be passed on in heterozygous plants with genotype AaBb. These plants will be green and therefore survive to bear seeds and mature.

Background for the experiment

The double heterozygous plants with genotype AaBb were originally produced by crossing one plant heterozygously for one gene (AaBB) with another plant heterozygously for the other gene (AABb). One fourth of the F1-plants which were formed by this crossing of single heterozygotes will be double heterozygotes (AaBb). These double heterozygotes were identified by allowing each F1-plant self-pollinate naturally and sow 25 of the F2 seeds which result (each plant can yield about 300-500 seeds). By means of the division in F2 the genotypes in F1 can be determined. This part of the production process can be illustrated schematically as follows:

Crossing of two single-heterozygotes: AaBB x AABb

F1-genotypes:	AaBB	: AaBB	: AABb	: AABb
	↓	↓	↓	↓
F1-division:	all green	3 green 1 white	3 green 1 yellow	9 green 3 yellow 4 white

The F1-plants with genotype AaBb were thus identified. The remainder of the 300-500 seeds from just these plants were then used to grow double heterozygotes. This was done by sowing the seeds. Among the seeds sown the green plants (9 out of 16) will survive to maturity and be harvested. Of these four will be double heterozygotes, as identified by the descendant test (see above). The remaining seeds from this batch are used for school experiments where they can be used for a new production run.

STUDENT GUIDE

Day 1: Sowing and cultivation
Day 6-10: Observation

Sowing and cultivation

The approximately 200 seeds should be sown in a planting box or in small pots in a layer of soil or gravel at least 1-2 cm deep. The seeds should be covered with about 1 cm of gravel, and the soil/gravel should be moistened with tap water. The material should be placed in a bright, warm (17-23 degrees C) location such as a window sill to germinate. Avoid direct sunlight. The soil/gravel should be kept moist during germination. After germination at 17, 20 or 23 degrees C the material should be ready for observation after 10, 8 or 6 days respectively. The first leaves should then be adequately developed (6-9 cm high). If the plants have germinated without adequate illumination they can be difficult to evaluate. Therefore, make sure that there is enough light.

Observations

The number of seedlings with the three different phenotypes: green, yellow and white should be counted. The counting process is easiest to do if the plants are pulled up (the leaves are pulled off) and sorted into three piles (green, yellow, white). The numbers can then be entered into the following table:

COUNTING OVERVIEW:

	Green	Yellow	White	Total
Number found (green:yellow:white)		:	:	=
Number found (non-white:white)	}		:	=
Number found (green:yellow)		:	-	=

THEORETICAL BACKGROUND

The following 9 genotypes, 3 phenotypes and ratios of occurrence are expected:

Genotype	Phenotype	green	yellow	white
AABB	green	1	-	-
AABb	green	2	-	-
Aabb	yellow	-	1	-
AaBB	green	2	-	-
AaBb	green	4	-	-
Aabb	yellow	-	2	-
aaBB	white	-	-	1
aaBb	white	-	-	2
aabb	white	-	-	1

Theoretical division: green:yellow:white $\underline{9} : 3 : 4 = 16$

Theoretical division: non-white:white $12 : 4 = 16$

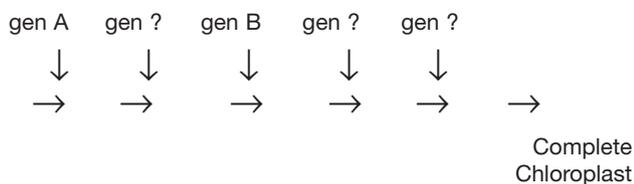
Theoretical division: green:yellow $9 : 3 = 16$

Two different mutant types in barley

The two different chlorophyll mutation types can be seen directly. The chlorophyll defects are due to the presence of one or another of the two recessive mutant genes. Only when these genes are homozygotically present (aa or bb) is the normal development of chloroplasts hindered. The chlorophyll mutants are able to germinate, but when the stored nutrients in the seed have been consumed they die, because they lack the chlorophyll necessary for photosynthesis.

Why does the genotype aabb have a white phenotype?

The double recessive genotype aabb sets the conditions for both white and yellow simultaneously. The chloroplasts develop, however, through a number of steps, each controlled by one or more genes:



In the genotype aa (*Albina*) this gene determines the phenotype regardless of whether the gene bb (*Xanta*) is present or not. The genotype aa stops the development of the chloroplasts at an earlier stage than bb does. The genotype aa "suppresses" the phenotype condition set by the bb gene (gene a is epistatic).

Separation ratio 3:1 for each of the two recessive genes

The a-gene: The number of green and yellow plants (genotypes AA and Aa) will occur compared to the number of white plants (genotype aa) in the ratio 3:1.

The b-gene: Because the phenotypes of the white plant "hide" the phenotypes determined by the genotypes BB, Bb and bb, the white plants should not be included in the count. This will however not alter the result, since the non-white plants will occur in the expected ratio 3:1 (for green and yellow respectively).

Division ratio 9:3:4 for two recessive, non-coupled genes

A prerequisite in this experiment for the division ratio is that the genes are inherited, i.e. that they are non-coupled.

The division ratio for both genes taken together will - due to the epistasi of the a-gene in relation to the b-gene - be a modified 9:3:3:1 division. The number of plants with the three phenotypes green, yellow and white will occur in a ratio which is expected to be 9:3:4.

The divisions are subject to the laws of chance, so that deviations from the theoretically expected ratios will occur. If 192 seeds germinate (96% of 200 seeds), then the expected number would be 108 green (9/16), 36 yellow (3/16) and 48 white (4/16). The number of white plants could deviate by 12 and the yellow plants by 10 due to random variations.

A X2 test can be used to check whether or not deviations in the numbers found in the experiment are statistically significant.

The seeds were produced by the Research Center at Risø, DK-4000 Roskilde, Denmark.