Chlorophyl mutants in barley

RISØ

21.12.10 Ae 7791.05

Contents

Materials are included for one experiment.

Ca. 40 Albina seeds, ca. 40 Viridis seeds, ca. 120 Xanta seeds.

Purpose

The purpose of the experiment is to show how chlorophyl mutants can look like and how mutant properties are inherited. The experiment also illustrates the interaction of inheritance and environment.

TEACHERS' GUIDE

About the materials

The material in the package consists of three varieties of barley (genetically identical barley plants) each containing a recessive mutant gene. The three genes are at different locations on the chromosomes and they are inherited independently of one another.

Albina (ca. 40 seeds) become white seedlings.

Viridis (ca. 40 seeds) become light green seedlings.

Xanta (ca. 120 seeds) become yellow seedlings.

Background for the experiment

The mutant genes are induced experimentally by irradiation of the previous generation. The three seed samples in the package have not been irradiated.

The following table shows the production process for the seeds in the *Albina* seed sample with a mutated A-gene. A, B and C stand for the dominant normal genes, and a stands for the recessive *Albina* mutant gene. Similar tables can be constructed for the *Viridis* seed sample (AABBCc) and the *Xanta* (AABbCC) seed sample where b and c represent the recessive *Xanta*- and *Viridis* mutant genes.

The irradiated seeds are denoted M1 in the table. Their descendants (after self-pollination) are denoted M2 and successive generations are denoted M3 and M4, etc.

Seeds of the barley stain Carlsberg II	genotype AABBCC		
1. The mutation A → a is induced	genotype AaBBCC		
2. The M1 generation is planted and harvested			
3. Descendant test: 20 M2 seeds form each M1 plant are sown. In the M1 plants where mutant genes are present the following are found in M2	AaBBCC		
genotypes: phenotypes: frequency:	AABBCC green 1	AaBBCC green 2	aaBBCC white 1
4. Green M2 plants are planted:	harvested	harvested	die
5. Descendant test: 20 M3 seeds are sown from each harvested M2 plant The M3 phenotypes and genotypes become:	all plants 2 green AABBCC	1 green AABBCC 2 green AaBBCC 1 white aaBBCC	
6. Remnants of M3 seeds:	thrown away	used for experiments and new germination	

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STUDENT INSTRUCTIONS

1st day: Sowing and growing (steps 1-4 below).

9th day: Pots with Xanta-b are moved (step 5 below).

10th day: Perform observations (see step 6 below).

1. The seeds in the bags marked Xanta are divided up into three samples of equal size and marked a, b and c. Each sample of about 40 seeds is sown independently, e.g. in flower pots or in a layer of soil or gravel at a depth of about 1-2 cm. The seeds are covered with a layer of gravel about 1 cm deep, and the earth/gravel is moistened using a watering can.
2. The three flower pots with Albina, Viridis and Xanta-a are placed in a bright window sill (17-23 degrees C) to germinate. Avoid direct sunlight on the pots to prevent the soil and plants from drying out.
3. The two flower pots with Xanta-b and Xanta-c are set to germinate in the dark, e.g. in a dark closet, or cover them with another flower pot with a plug in the bottom hole. Press the edge of the flower pot about 1 cm down into the gravel/soil so that it is light tight. The seedlings germinating in darkness can be examined from time to time, but do not expose them to light for more than a few minutes.
4. During germination the gravel/soil should be kept moist. The growth rate is temperature dependant. At temperatures of 17, 20 and 23 degrees C the results can be examined after 10, 8 or 6 days respectively.
5. The day before the observations are to be performed the pot with Xanta-b should be removed from the darkness and placed in a bright environment.
6. Observations and counting. The pot with the Xanta-c is removed from darkness. The number of normal, green plands and of chlorophyl mutations are counted up and the data is entered in a table like the one shown below. The percentages of chlorophyl mutations is computed.

TABLE OF RESULTS

Test no.	Number			Percent
	Normal	Mutations	Total	
Albina *Viridis* *Xanta-a*				
Total				

The divisions obey the law of averages. Normally deviations will be observed. If a total of 40 plants are examined, it is to be expected that 30 plants will be green, and 10 will be mutants. Deviations of up to 6 from the expected values can occur.

THEORETICAL BACKGROUND

Various chlorophyl mutations

In the three seed samples which germinate in light one can directly observe and compare the three different chlorophyl mutants. The Albina mutant is white, Viridis is light green, and Xanta is yellow.
Each of these chlorophyl defects is due to one of the three different mutant genes. When one of the mutant genes is present homozygotically, the normal development of the chloroplasts is blocked. The mutant plants are able to germinate, but when the stored nutrients in the seed are used up they die because the chlorophyl is missing. The three mutant types are typical, damaging mutations which are of no value to the plants or to the planter.

Division of the individual gene

The three mutant types are each conditional on a single recessive gene. We can derive the division from the following table (for self-pollination of AaBBCC). Because B and C are homozygote, the following hereditary pattern is only valid for the gene pair Aa. (Similar tables can be shown for the Xanta heterozygote AABbCC and the Viridis heterozygote AABBCc.

♀ \ ♂	A	a
A	AA	Aa
a	Aa	aa

Descendants: 75% normal, green plants (AA and Aa) and 25% white mutants (aa). I.e. a 3:1 division of properties.

Interaction between inheritance and environment

In the *Xanta-c* sample which germinated in darkness all of the plants are yellow. In the “normal” plants the development of the chlorophyl has been blocked by the lack of light and in the mutant plants due to the missing mutant gene. On the other hand the yellow xantofyl has been formed in both genotypes. During the germination process in darkness it is thus not possible to distinguish the mutant genotype from the normal genotype because the phenotype is identical for each.
In the *Xanta-b* sample germinated in darkness which had been placed in the light about 24 hours before examination the normal plants attained an almost normal green color, while the mutations remained yellow. The phenotype differs for the two genotypes after the exposure to light.
In the *Xanta-a* sample germinated in a bright environment the two phenotypes corresponding to the two genotypes have been apparent all along.
The frequency of naturally occurring chlorophyl mutations in barley is about 1 mutation for every 6300 plants. In a newly germinated barley field (ca. May 1st or October 1st) once will be able to locate at least one chlorophyl mutant for every 50 square meters. (They are easiest to find under overcast skies.)

The seeds have been produced by The Research Center at Risø in Roskilde, Denmark.

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