

# ALFALFA GENETIC MUTANTS

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## Introduction

Alfalfa mutants studied prior to 1967 are reported in USDA Bulletin No.1370 by D. K. Barnes. This report illustrates genetic mutants that have been used in research since 1967.

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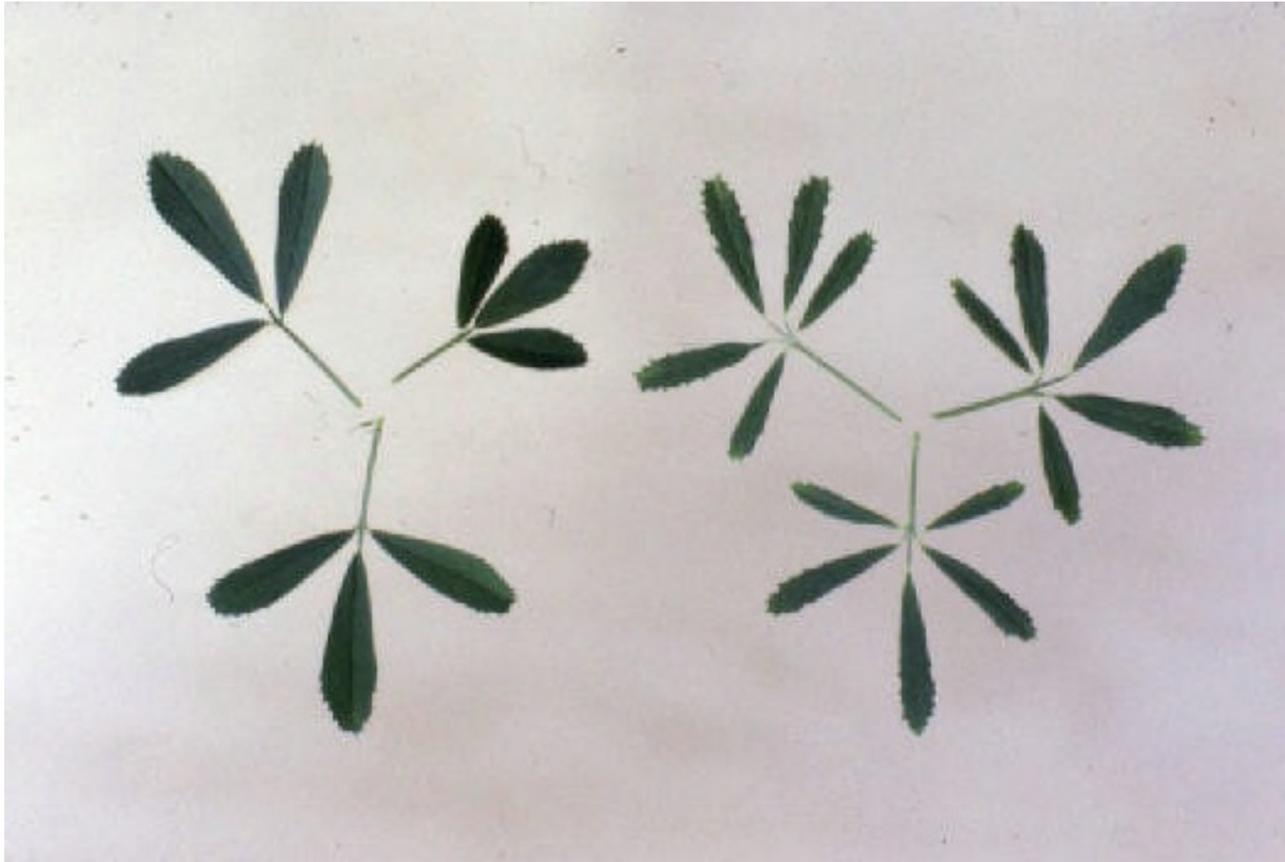
21-23: Plastid Mutation – Albino Sectors

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Normal *Medicago sativa* leaves on the left and the multifoliate leaves on the right.

References to multifoliate leaves:

Crop Science 5:233-235 (1965), and 13:463-465 (1973).

Botanical Gazette 27:221-225 (1966).



***Medicago sativa***



***Cannabis sativa***

This *Medicago* multifoliolate leaf bears a striking resemblance to a multifoliolate leaf in another genus. It is interesting that they both have the same species name. Multifoliolate leaves occur predictably after wide crosses of subspecies or even within a species where the lines have been isolated for a long time. We consider them products of recombination involving diverse chromosome blocks. This probably accounts for why the trait is difficult to stabilize.



This picture illustrates the range in leaf types observed in *Medicago sativa*. The simple leaf-type on the left is associated with the cauliflower head-trait. It is sterile and must be manipulated in the heterozygous condition. Normal trifoliate in the center and an extreme multifoliate on the right. The simple leaf, cauliflower head-trait was discussed in volume 3 of this website with regard to a sterile trait for gene containment.



This picture was taken from an old slide and shows much of the genetic variation that we have worked with over the years. The white flowered examples on the left are homozygous recessive at the basic color factor locus C2. They lack anthocyanin in all parts of the plant including the hypocotyl which makes the trait a useful seedling marker. The purple in the center is the normal type. Cream-flowered on the right is homozygous recessive at the P locus.

References to flower color from this project:

Crop Science 13:393-394 (1973), and 32:73-77 (1992).



A close-up of three hypocotyls on the left illustrate the lack of pigment in the hypocotyl when the C-locus is in the recessive condition (cccc).

Normal seedlings are on the right. Seedlings can be classified soon after emergence, making this a very useful seedling marker.



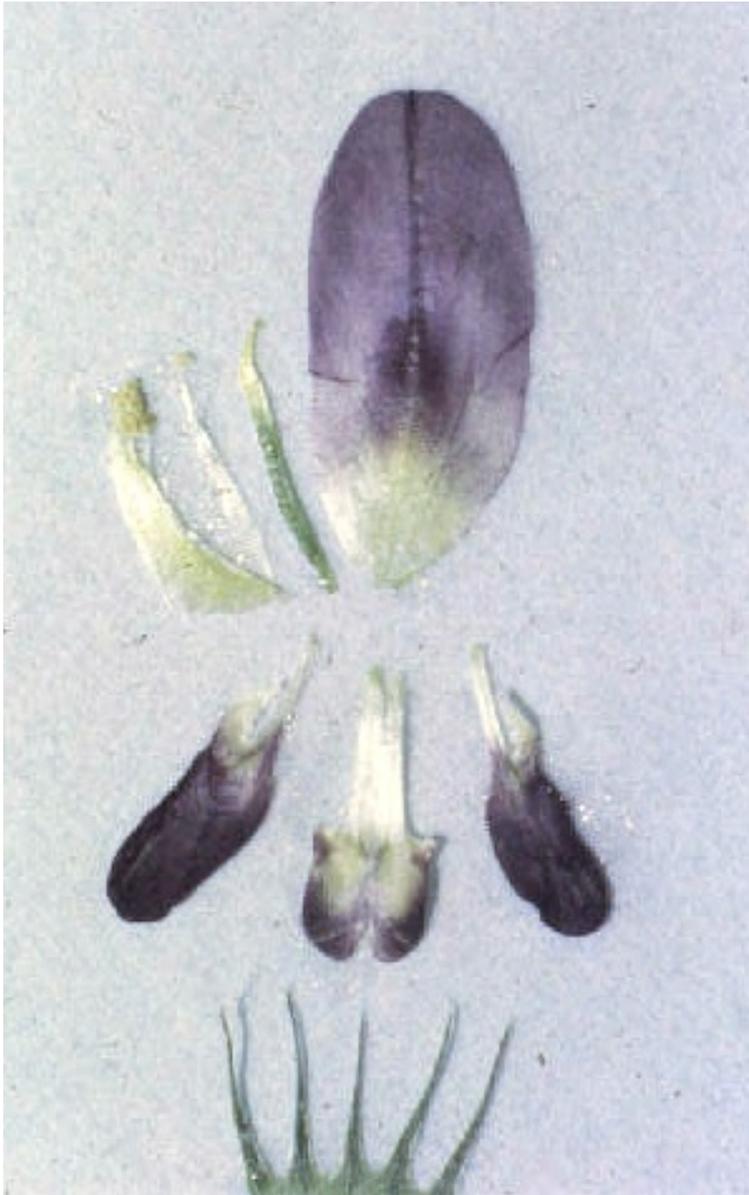
The purple flowers are from clone 6-4ms, referenced in:

[www.medicago-reports.org](http://www.medicago-reports.org)

Volume 3, with information on microsporogenesis, and Volume 5 with information about crosses with *M. arborea*.

White flowers (c2c2c2c2) were backcrossed into clone 6-4ms for use as a recessive tester. Clone 6-4ms has been maintained by shoot cuttings for 35+ years.

See: American Journal of Botany 83:383-404 (1996) for information on plastid transmission in the megaspore. Clone 6-4ms is a strong female plastid transmitter. This paper is a review of cytoplasmic inheritance. Clone 6-4ms was used in at least five other journal articles.



The parts of the flower.

The standard, wing and keel petals radiate from the center along with the carpel containing the ovary and about ten ovules. The stamen has ten anthers, nine of which are fused, and one is free.

A sheath formed by the fused anthers surrounds the carpel and is part of the tripping mechanism.

The sepals are at the bottom.



The mutable flower condition at the C2 locus. The c2-m1,2,3, mutable conditions are all similar in that they all have the same range in variation and transmit similarly. They are given three different designations because they represent three independent discoveries. Alfalfa genetic stocks segregating for c2-m1, c2-m2 and c2-m3 were released to the public and placed in the Plant Introduction System as PI 605704, PI 619433 and PI 619434. See *Crop Science* 42:1386 (2002).

Additional references for the mutable loci:

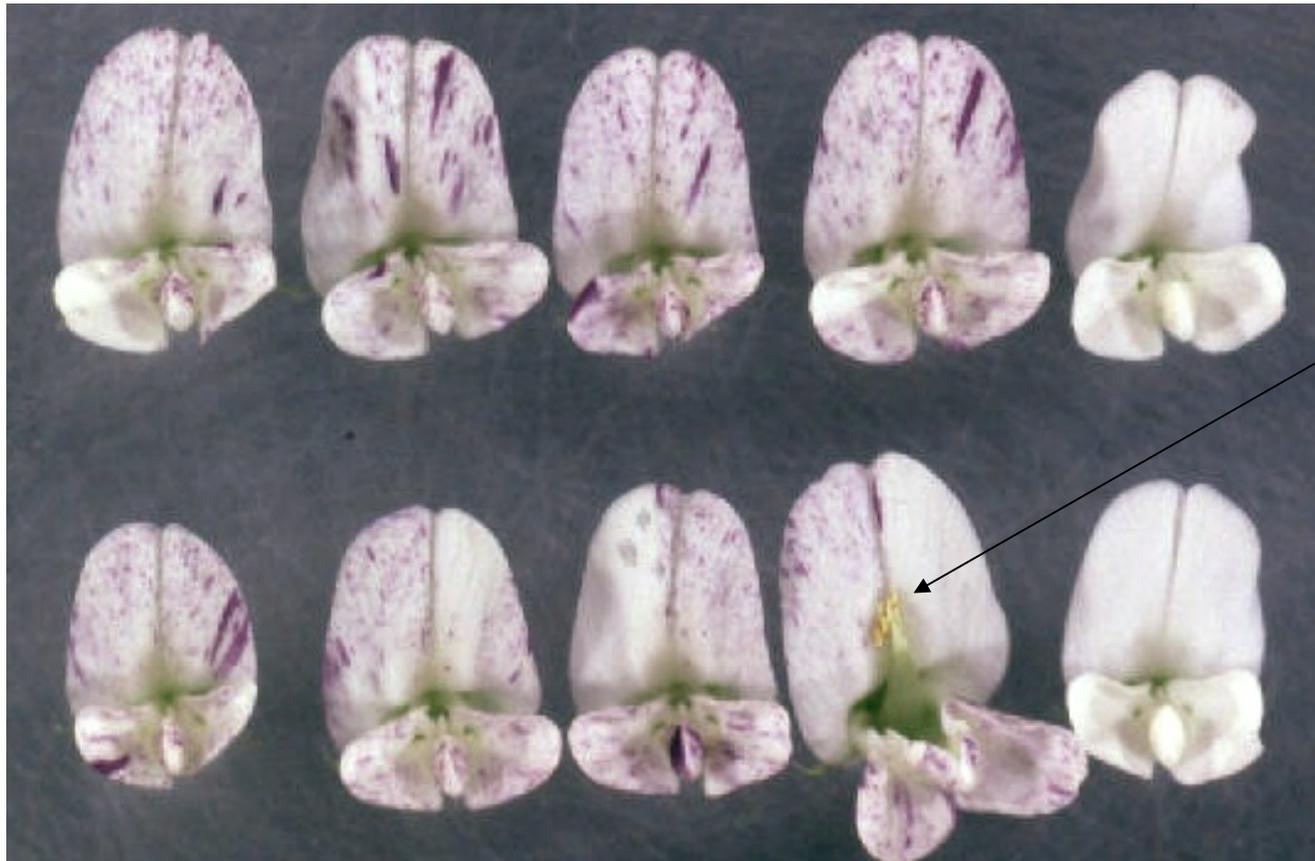
*Crop Science* 26:823-827 (1986).

*The Journal of Heredity* 86:407-410 (1989).

Report in Volume 1 of [www.medicago-reports.org](http://www.medicago-reports.org)



Another example of the mutable condition expressed in the mutant flowers on the left. A stable white flowered control is on the right. A review of the mutable loci in alfalfa can be found in: *Developmental Genetics* 10:552-560 (1989).



Note the  
tripped flower

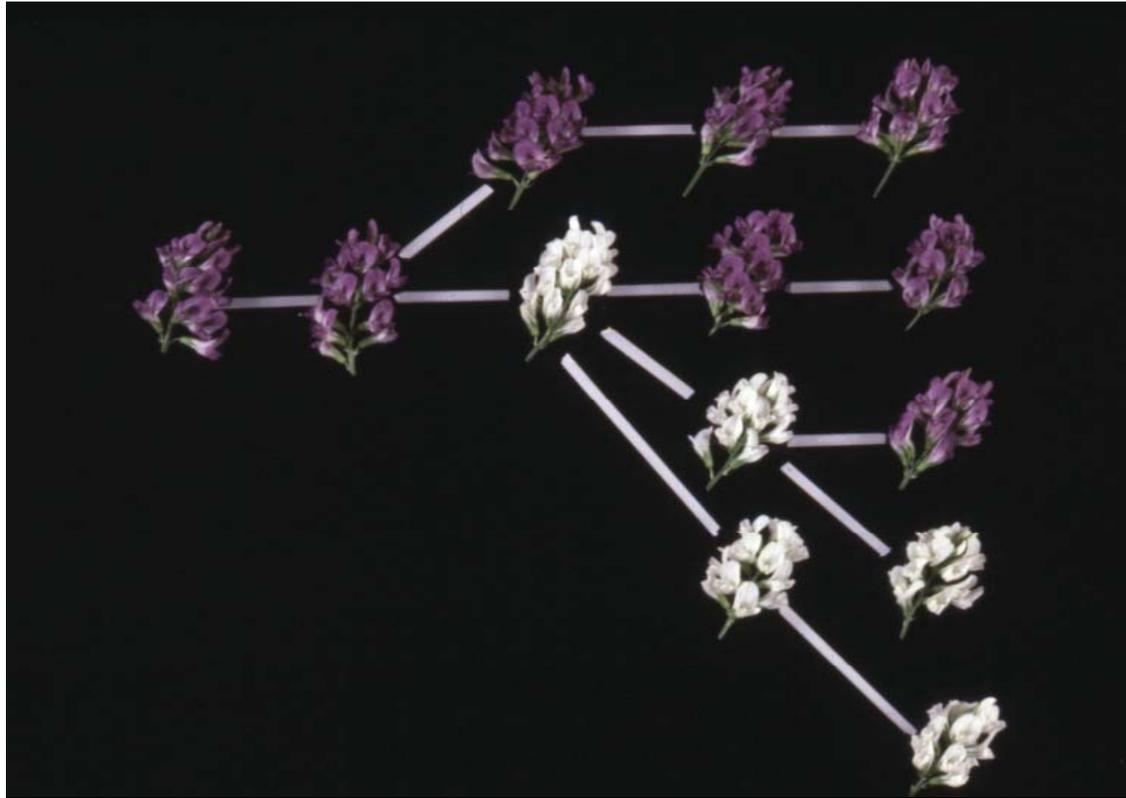
These flowers are all from one plant. They illustrate the variation that can occur on single plants due to timing of the mutation in development.



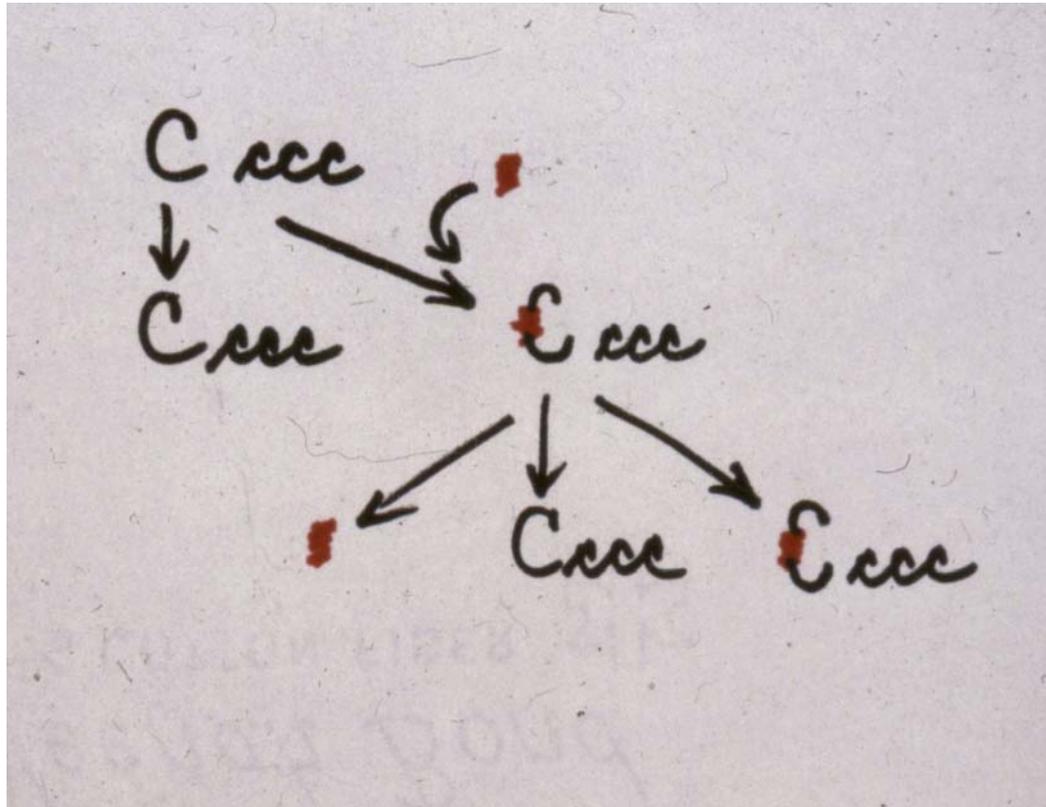
Another mutable genotype illustrating variation due to timing of the mutation in development.



This picture illustrates the separate genetic control of pigmentation in the wing petals. The top row of flowers demonstrates fusion of petals to form each wing and separate genetic control in each fusion product. Note the keel petals in the top row versus the bottom row; genetic control of pigmentation in the keel also is independent.



This illustrates the discovery of c2-m4 in tissue culture. A purple flowered tissue donor (left) was cultured and regenerated. Most of the regenerated plants were purple but one white variant was recovered. When the white variant was put through another cycle of tissue culture, some of the regenerated plants reverted to purple. A genetic model for what appeared to take place is on the next page. Interestingly, clone 6-4ms was the female parent of the plant in which c2-m4 arose.



In this model, an element jumped into the C locus in culture and blocked its function. The regenerated plant was white flowered. Upon reculture of the white plant, white and purple revertants were regenerated, probably because the element left the locus. This exercise was repeated many times.

References to c2-m4:

Crop Science 24:655-658 (1964).

Plant Cell Reports 5:104-107 (1986), and 5:108-110 (1986).

Genome 34:35-40 (1991).



This is a special case involving white streaks in sectors that occur in purple flowers. This represents mutation from dominant purple to recessive white. This type of mutable was discovered twice and the lines designated mutable 5 and mutable 6. This is a very symmetrical example; most flowers have random sectors.

See: *Developmental Genetics* 10:552-560 (1989).



Anthocyanin sectors in leaves. These occur on plants that have the mutable flower condition. Thus, the mutable condition can be studied in any tissue that produces anthocyanin, including the seed coat.



Additional examples of the mutable anthocyanin condition in leaves.



Anthocyanin sectors in a leaf of *Medicago sativa*.



Anthocyanin sectors in leaves of *Medicago truncatula*.  
Is this the same system as *M. sativa*?



An albino sectors on  
an otherwise normal  
plant.



This plant with albino sectors illustrated how the albino sectors can be studied and can even produce seed because they are crossed-fed by the normal green shoots.



Examples of albino sectoring in leaves, and a normal leaf.

References to plastid transmission in *Medicago sativa*:  
Journal of Heredity 77:35-38 (1986), 80:214-217 (1989),  
and 83:342-345 1992).  
European Journal of Cell Biology 59:211-218 (1992).



This is a plastid mutation conditioning chlorophyll deficiency. The same rules of plastid transmission and sectoring apply to chlorophyll deficient and albino mutants. See references on previous pages.



The dominant red root trait in alfalfa. The picture illustrates the variation in the degree of red pigment along with two normal white roots. Red root is a useful seedling marker that can be classified at about six weeks. The genetics of red root were reported in USDA Technical Bulletin 1370 (1967), and Volume 1 of [www.medicago-reports.org](http://www.medicago-reports.org)



Normal alfalfa seeds are on the left with a vertical row of brown seeds on the extreme left that are dead. The viability of a seed lot of alfalfa can be estimated by the content of the yellow and brown seeds. The white seeds are from plants homozygous recessive for one of the basic color factors either C1 or C2. Basic color factors C1 and C2 were named by S.B. Gupta in a paper on the biochemistry of anthocyanins published in *Genetics* 65:267-278 (1970). C1 and C2 are non-allelic as reported in *Crop Science* 13:393-394 (1973). The mixture of brown and black seeds is from a population segregating for brown and black seeds. The black seeds on the right were discovered in three different populations of crosses between diploid alfalfa and wild *M. falcata*. The black seed trait was then transferred to the tetraploid level via 2x-4x crosses and reported in *Crop Science* 37:1376-1378 (1997). Discussion of the black seed trait discovered in 'Grimm' alfalfa may be found in USDA Technical Bulletin #1370 (1967) by D.K. Barnes.

**List of mutants to be reported at future date.**

Genetic Male Sterility – Single nuclear genes; and Hybrid breakdown.

Cytoplasmic Male Sterility (CMS) – One main type (6-4ms is typical).

Restorers/Maintainers of CMS – Dormant alfalfa contains mostly restorers, while non-dormant alfalfa contains mostly maintainers.

Female Sterility – Single recessive gene affecting ovule development.  
See: Agronomy Monograph No. 29, Alfalfa and Alfalfa Improvement. Chapter 24:737-776 (1988).

Restitution Pollen (2n pollen) – See: Ibidum

Jumbo Pollen (4n pollen) – See: Ibidum

Restitution Eggs (2n eggs) – See: Ibidum

Albinos and Dwarfs – See: Ibidum. Chapter 29:903-929.