Figure 1. Possible Types of Subunits Composing White-Albino Loci.

	Seed Phenotype	Seedling Phenotype					
- Functional unit	yellow	green					
- Inactivated unit	white	albino					
or or Partially inactivated unit	pale yellow	pale green (pastel)					
Types of Alleles							
	yellow	green					
	white	green					
	yellow	albino					
	white	albino					
or or	white	pale-green					
or or	yellow	pale-green					
or or	pale-yellow	albino					
or or	palc-yellow	green					
or or or	pale-yellow	pale-green					

4. Temperature sensitive alleles of the Y-1 locus.

Two mutants which are allelic to Y-1 were found at the California Institute of Technology by Dr. E. G. Anderson. One of these, pastel-8549, originated in the progeny of a seed exposed to a gamma ray dose of 40,000 r units. This allele has white endosperm but when germinated at high temperatures produces a pale green (pastel) seedling. The second allele, white-mutable, arose as a spontaneous mutant in one of Dr. Anderson's stocks and is similar to pastel-8549. However, it is unstable and regularly back mutates to normal in both endosperm and plant tissue. As a result, white-mutable seeds have a white endosperm with spots of normal yellow tissue. Grown under high temperature the seedlings are pale green with streaks of normal green tissue. Grown in the field both pastel-8549 and white-mutable plants are considerably paler than normal plants and have a distinct zebra phenotype, and white sheaths and leaf midribs. In addition white mutable plants have the streaks of dark green tissue. Only an occasional pastel-8549 plant can be grown to maturity, and these are extremely weak. In contrast, many white-mutable plants have been grown to maturity and some are strong enough to produce ears and set seed. The white-mutable plants are very similar to a mutant described by Dr. Dollinger (MGCNL 31 & 33), which he has shown to be allelic to y-1 and pb-1.

In seedling tests of the white-mutable and pastel-8549 mutants, it was frequently difficult to distinguish between normal and mutant seedlings. Since most of the difficulties in classification were encountered in winter and the best classifications were made in spring or early summer, it was felt that the lower temperature in the greenhouse during winter months might be responsible for the difficulty in classification.

To test the effect of temperature upon these mutants, seedlings were grown in a constant temperature box at 37° C and 19° C (\pm 2 $1/2^{\circ}$ C). Mutant and normal seedlings grown at 37° C could be readily distinguished while those grown at 19° C were difficult to separate consistently.

The pigments of normal and mutant seedlings grown at the two temperatures were extracted and total chlorophyll determined. Table I summarizes the results of these determinations for pastel-8549 seedlings.

Table I Summary of chlorophyll analyses of normal and pastel-8549 seedlings grown at 37° C and 19° C.

	37° C		19 ⁰ C			
-	Average Pigment Concentration mg/gm f.w.	Mutant Normal	Average Pigment Concentration mg/gm f.w.	Mutant Normal	Calc. Mut 19° C (mut 37°) (Nor. 37° : Nor. 19°) mg/gm f.w.	obs. Mut 19° C Calc. Mut. 19° C
Normal	.74	10 3%	. 090	73.3%	.009	7.3
Pastel-8549	.076	<u> </u>	.066			

At 37° C the mutants have about 10% the chlorophyll content of the normals, while at 19° C the chlorophyll content of the mutants is about 73% that of the normals. If one takes the behavior of normal seedlings as standard, a marked temperature effect is observed upon the concentration of chlorophyll. In normal seedlings the chlorophyll level at 37° C is 8.2 times the level at 19° C. If temperature had

the same effect on the mutant, a chlorophyll concentration of .009 mg/gm f.w. would be expected (Table I, column 6) at 19^{0} C. The value of .066 mg/gm f.w. observed for the mutant at this temperature is seven times higher than expected.

A similar temperature effect upon the content of the yellow pigments of mutant seedlings also has been demonstrated although the exact quantitative relationships have not been worked out as yet.

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5. The use of w-3 to study the role of carotenoids in the protection of chlorophyll from photodestruction.

The studies of Cohen-Bazire and Stainer, (Nature 181:250-252. 1959), and Fuller and Anderson (Nature 181:252-254. 1958), have shown that colored carotenoids are required for the protection of bacteriochlorophyll from photodestruction in the photosynthetic bacteria. It has been proposed that this is a unique role of carotenoids in all phototrophys. The white-albino mutant, w-3, provides a tool for determining if carotenoids might serve such a protective function in higher plants. This mutant is a chalky-white albino when grown in the light. However, when grown in the dark it produces protochlorophyll which is converted to chlorophyll on short exposure to light, but upon continued illumination the chlorophyll is bleached.

To determine the carotenoid content of white-3 and normal seedlings, plants grown in the greenhouse seedling bench were ground in a mixture of acetone and hexane. The hexane fractions containing carotene and colorless precursors of carotene were isolated and their spectra were made with a Beckman DU spectrophotometer. The visible and ultraviolet spectra of white-3 seedlings did not show any absorption typical of carotenoid as was found for the extract of normal seedlings. Instead, the w-3 extracts had large amounts of a substance which absorbed light in the ultraviolet region with peaks at 275, 285, and 297 mm. These peaks are similar to those reported for the carotenoid precursor, phytoene. Extracts of normal seedlings showed no absorption at these wave lengths.

Koski and Smith (Arch. Biochem. Biophys. 34:189-195. 1951) reported that dark grown white-3 seedlings contained as much and usually more protochlorophyll than did normal seedlings and that the protochlorophyll of both normal and mutants was readily converted to chlorophyll upon exposure of the seedlings to light. To determine if oxygen was necessary for the photodestruction of chlorophyll observed in white-3 seedlings, one group of normal and mutant dark-grown plants was exposed to light in an atmosphere of air and another group of normal and mutant dark-grown seedlings was exposed to light under anaerobic conditions. When normal seedlings were exposed to light in the anaerobic environment, the chlorophyll level diminished slightly for the first twenty minutes, after which stabilization was observed, followed by an eventual increase in chlorophyll content. The chlorophyll level of dark-grown normal seedlings, exposed to light in an anaerobic environment, remained essentially constant for the 100 minutes of the experiment. The chlorophyll of dark-grown white-3 seedlings was completely destroyed after a twenty minutes exposure to light under aerobic conditions. However, upon exposure to light under anaerobic conditions the chlorophyll level of dark-grown white-3 seedlings remained stable.

The chlorophyll content of white-3 seedlings grown in dim-light (less than 0.5 foot candle) increased over a two-week period. Visual observations of chloroplasts from sectioned leaves of such mutant material reveal that they are as numerous and of the same size as those of normal seedlings. Free chloroplasts obtained from grinding mutant leaves in sand and 0.35 Na Cl and centrifuging were a little more opulescent but otherwise they appear to be normal.