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Genetic Relationships among Three Chlorophyll-Deficient Mutants in Peanut, *Arachis hypogaea* L.

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Summary. The genetic relationships of three chlorophyll-deficient mutant peanuts, lutescens (lu), aureus (au), and virescent (v) were studied under field and greenhouse conditions. The F_1 plants from crosses between these mutants produced phenotypically normal green. In F_2, aureus × virescent segregated 675 normal green : 225 virescent : 45 aureus : 15 virescent aureus : 64 seedling lethal, and lutescens × virescent segregated 45 normal green : 15 virescent : 3 lutescens : 1 seedling lethal. (Lutescens peanuts were seedling lethal in the field.) As previously reported, the F_2 of aureus × lutescens gave 225 normal green : 15 aureus : 15 lutescens : 1 seedling lethal. The three chlorophyll-deficient factors (au, lu, and v) show independent inheritance. The recessive combinations from the parental types between aureus and virescent and between aureus and lutescens would produce plants with a combination of their respective parental characteristics, but the recessive combination between lutescens and virescent was nearly albino. The *v-au* and *lu-au* seedlings have a longer life span than the *v-lu* seedling has. The genotypes for the three mutants are tentatively identified as lutescens $VV\ Au_1Au_1\ Au_2Au_2\ lu_1lu_1\ lu_2lu_2\ L_1L_1L_2L_2$, aureus $VV\ au_1au_1\ au_2au_2\ Lu_1Lu_1\ Lu_2Lu_2\ L_1L_1\ l_2l_2$, and virescent $vv\ Au_1Au_1\ Au_2Au_2\ lu_1lu_1\ Lu_2Lu_2\ l_1l_1\ L_2L_2$.

Key words: Peanut - Chlorophyll - Mutants

Introduction

In infraspecific hybridizations between *Arachis hypogaea* ssp. *hypogaea* and *A.hypogaea* ssp. *fastigiata* var. *vulgaris*, lethals that range from zygotic stage to yellow seedling are usually observed in the F_2 generation under field conditions. A review on chlorophyll-deficient inheritance in this crop has been made by Hammons (1973).

The objective of this study was to examine the inheritance model and to determine the genetic relationship of three chlorophyll deficient mutants in peanuts.

Materials and Methods

Three chlorophyll-deficient mutants, virescent, aureus, and lutescens, and the normal green krinkle leaf peanut, were studied. Virescent, which originated from a radiation-induced mutation (W.C. Gregory, personal communication), shows the yellowish-light-green in the seedling stage and the young leaflets with somewhat albino rachises and midrib, as reported by Patil and Bora (1963) and Patil (1969). Young leaflets become greener with age, starting from the tip and around the midribs. The youngest three to four leaves continue to be chlorophyll-deficient, but the lower leaves appear to be normal green. Benedict and Ketring (1972) reported that chlorophyll synthesis in the virescent leaves shows a 72-hour lag period before the onset of a phase of rapid chlorophyll accumulation as compared to the normal green leaves. The change to green is hastened as temperature increases and sunlight intensifies. Aureus, which was found in a plot of PI 268637 (Stone 1968), has young leaves of normal green color that turn yellow as the leaf ages. Lutescens 0026 was isolated from F_2 progeny of the cross PI 259662 × krinkle leaf (Tai and Todd 1972). Krinkle lutescens was found in a nursery plot of PI 234422 (Tripp 1968). Both lutescens mutants show a pale green leaf-color throughout all stages of leaf development. Lutescens 0026 has smooth leaflets, whereas krinkle lutescens has crinkled leaflets. Krinkle leaf (Hammons 1964) has crinkled leaflets with normal green color.

Crosses among mutants and with normal green were made in the green-house. F_1 plants were grown either in the greenhouse or in the field as necessary to develop F_2 seeds. Four F_2 populations, two each from aureus × virescent (C 298-2 and C 298-3) and krinkle × virescent (C 296-2 and C 296-3) were field-grown; the other F_2's were grown in the greenhouse for phenotypic classification. To ensure uniform germination, the F_2 seeds were treated with ethylene and kept for 24 hours in the seed germinator.

F_2 phenotypic frequencies were tested with chi-square for goodness-of-fit to proposed genetic ratios.

Results and Discussion

In the very early seedling stage, both lutescens and virescent plants are a "white" phenotype similar to albino, but pigment development characterizes their differences not only from albino, but also from each other as soon as light intensifies and temperature increases. Aureus plants begin as normal green seedlings. The complete development of the chlorophyll type is best seen in the mature plant. However, the golden yellow character of this mutant type appears in the cotyledons at the seedling stage. The aureus cotyledons are golden yellow at cracking time, but they turn green after exposure to light. The chlorophyll pigment persists approximately two weeks and then decomposes, leaving the cotyledons yellow again. Aureus can be distinguished unambigiously from green as seedlings.

Inheritance and Genetic Factors for the Three Chlorophyll Mutants

1. *Lutescens*. Both lutescens 0026 and krinkle lutescens have been found genetically by Tai et al. (1970) to be double recessive to normal green. The F_2 plants from normal green × lutescens segregated for green and lutescens in a 15:1 ratio. Both mutants have an identical genotype for the chlorophyll-development factors.

Two symbols, $Lu_1 lu_1 Lu_2 lu_2$, have been assigned for the green-lutescens relationship.

2. *Aureus*. The aureus × normal green (krinkle) cross gave 15 green: 1 aureus in F_2 (Table 1) as previously obtained by Matlock et al. (personal communication). Two factorial pairs, $Au_1 au_1 Au_2 au_2$, were previously assigned (Tai et al. 1970).

3. *Virescent*. This character is expressed as a 3:1 recessive (Table 2). Another phenotypic class, albino seedling, which is lethal, was produced in the F_2 population from crosses between krinkle and virescent. The phenotypic segregation ratio for green: virescent: lethal is 45:15:4, but differential viability of the lethal phenotype was presumably responsible for the variableness of chi-square for different progenies from the same cross-combination. The aberrant segregation may be attributed to the fact that the virescent used

Table 1. F_2 phenotypes and χ^2 analyses for leaf color in the peanut cross krinkle × aureus

Population	Frequency		χ^2	P
	Green	Aureus	(15:1)	
C 302-1	435	27	0.130	.718
C 302-2	515	30	0.517	.473
Pooled	950	57	0.597	.440
Homogeneity	-	-	0.049	.824

for this research is a radiation-induced mutant. Such genetic aberration has previously been described by Gustafsson (1938) and Loesch and Hammons (1968). Moreover, Hull (1937) found deficiencies of yellow seedlins and suggested possible causes as unequal numbers of effective gametes or differential viability of zygotes. The gene symbol v, devised by Patil (1969, 1973) for the green-virescent relation was adapted in this study.

Because neither parent could produce the albino (lethal) seedling per se, each parent presumably contributed a lethal factor. Based upon the present results and their botanical types, the lethal seedling genotypes $L_1L_1l_2l_2$ and $l_1l_1L_2L_2$ might be assigned respectively to krinkle and virescent. The complete genotypes might be $vv\ l_1l_1L_2L_2$ for virescent and $VV\ L_1L_1l_2l_2$ for krinkle.

Interrelation Between the Aureus and Virescent Types

It has been shown that virescent is a simple recessive and aureus is a doubly recessive to normal green and F_1 plants are green. Under cool temperature and low light intensity, the first two new young leaves on the F_1 plant show the "virescent" characteristics, but this less distinct character vanished as soon as the leaves became older. In the F_2 generation, five distinct types of plants were recognized (Table 3). Two were parental types, one was like the F_1 hybrid, and two were entirely new types. One was a re-combination of the two recessives, virescent-aureus; the other was lethal. Although virescent-aureus plants resemble virescent in the early seedling stage, because of their poor vigor, "aureus" trait becomes

Table 2. F_2 phenotypes and χ^2 analyses in the peanut cross krinkle × virescent

Population	Frequency			χ^2		χ^2	
	Green	Virescent	Lethal	(45:15:4)	P	(3:1)*	P
C 296-1	477	164	16	16.451	.0003	0.117	.943
C 296-2†	240	92	20	1.5096	.470	0.301	.521
C 296-3†	342	103	14	8.8574	.012	0.816	.665
Pooled	1059	359	50	20.3548	.00001	0.076	.962
Homogeneity	-	-	-	6.4632	.049	2.158	.707

* Lethal plants excluded
† Field-grown

Table 3. F_2 phenotypes and χ^2 analyses for leaf color in the peanut cross virescent × aureus (Expected ratio = 675:225:45:15:64)

Population	Frequency					χ^2	P
	Green	Virescent	Aureus	Virescent -Aureus	Lethal		
C 298-1	296	87	15	6	16	6.276	.179
C 298-2†	366	96	21	6	29	5.363	.252
C 298-3†	394	149	18	12	18	16.341	.003
Pooled	1056	332	54	24	63	5.047	.283
Homogeneity	-	-	-	-	-	22.933	.004

† Field grown

evident much earlier than would be usual. Due to other genetic factors, these plants varied in viability, ranging from the seedling lethal to mature-plant under favorable greenhouse culture. Under field conditions, all virescent-aureus plants died as seedlings.

As noted previously for crosses between krinkle and virescent, because neither parent could produce the albino seedling per se, both virescent and aureus carry lethal gene(s). We assumed that aureus and krinkle both carry the same lethal genes because both are Spanish (*A. hypogaea* ssp. *fastigiata* var. 'vulgaris') type.

Based on the results of our study, the F_1 genotype $Vv\ Au_1au_1Au_2au_2\ L_1l_1L_2l_2$ was proposed. F_2 progenies of green, virescent, aureus, virescent-aureus, and seedling lethal are expected in the ratio of 675:225:45:15:64. While the data shown in Table 3 do not fit this ratio closely, the deviations may be largely explained by the deficit of lethals. Lethality may occur anytime from zygotic to seedling stage, but only the seedling lethals are detected and counted. According to the hypothesis, the double recessive factors $l_1l_1l_2l_2$ produces lethality, irrespective of other chlorophyll factors present. Theoretically, the cross would give 27 genotypes of lethality, depending on the combination of the other three chlorophyll factors (V, Au_1 and Au_2). Hull (1937) also suggested that unequal numbers of effective gametes or differential viability of zygotes could cause the deficiency of yellow seedlings. Furthermore, the possibility of variant behavior with virescent has been discussed previously in the krinkle × virescent cross. It might act the same way in causing abnormal segregation in the F_2 of virescent × aureus.

Interrelation Between the Lutescens and Virescent Types

In crosses between lutescens and lirescent, the F_1 plants were green, but the virescent (not lutescens) characteristic could be seen in the first one and two young leaves on these plants under favorable conditions. The F_2 segregated four apparent types -- green, virescent, lutescens, and the new combination seedling lethal. The latter died in the early seedling stage, but could be identified. At first, the seedlings were nearly albino, but low concentration of

Table 4. F_2 phenotypes and χ^2 analyses for leaf color in two peanut crosses, lutescens 0026 × virescent (C 321) and krinkle lutescens × virescent (C 322) (Expected ratio = 45:15:3:1)

Population	Frequency				χ^2	P
	Green	Virescent	Lutescens	Lethal		
C 321-1	57	16	5	1	0.878	.831
C 321-2	105	37	7	6	5.454	.142
Pooled	162	53	12	7	3.213	.360
Homogeneity	-	-	-	-	3.119	.374
C 322-1	46	14	6	2	3.613	.316
C 322-2	51	22	5	4	7.288	.063
Pooled	97	36	11	6	8.644	.045
Homogeneity	-	-	-	-	2.257	.521
Pooled total	259	89	23	13	10.015	.018
Homogeneity	-	-	-	-	7.218	.614

Table 5. F_2 phenotypes and χ^2 analyses for leaf color in the peanut cross aureus × krinkle lutescens (Expected ratio = 225:15:15:1)

Population	Frequency				χ^2	P
	Green	Aureus	Lutescens	Lutescens		
C 323-1	64	3	3	0	0.965	.810
C 323-2	82	6	5	1	1.142	.767
Pooled	126	9	8	1	0.403	.940
Homogeneity	-	-	-	-	1.704	.636

chlorophyll developed in a pattern as does the virescent (*vv*) plant. Due to the extensive albinism, the new biparental combination ($vv\ lu_1lu_1lu_2lu_2$) died before expressing the lutescens ($lu_1lu_1lu_2lu_2$) characteristic in the older leaves even when light and temperature conditions were very favorable. The action of genes "*v*" and "*lu*" on chlorophyll development, as shown in this study, was independent, but that of gene "*v*", which requires the lag period in chlorophyll accumulation (Benedict and Ketring 1972), extends chlorophyll deficiency. Therefore, the plants died in the early seedling stage, as soon as food stored in the cotyledons was exhausted.

F_2 progenies from both lutescens 0026 × virescent (C 321) and krinkle lutescens × virescent (C 322) segregated in the ratio of 45 green : 15 virescent : 3 lutescens : 1 lethal with a fairly close fit as shown in Table 4. Lutescens 0026 × virescent, however, produced a smaller chi-square value in F_2 populations than did the cross of krinkle lutescens × virescent.

Interrelation Between the Lutescens and Aureus Types

Extensive genetic investigations on the interrelation between the lutescens and aureus have been conducted by Tai et al. (1970). Our additional results are shown in Table 5. As has been shown, lutescens and aureus are controlled by double recessive, duplicate unlinked loci, symbolized $lu_1lu_1lu_2lu_2$ and $au_1au_1au_2au_2$, respectively. F_1 plants from crosses between these mutants are all fully green, both in the seedling and in the mature growth stages, indicating complementary gene action between *Au* and *Lu*. Four phenotypes occur in F_2, green, aureus, lutescens, and the new combination lutescens-aureus, and segregation fits the expected 225:15:15:1 phenotypic ratio. The present results confirm the previous conclusion (Tai et al. 1970). The new combination ($lu_1lu_1lu_2lu_2$ $au_1au_1au_2au_2$) of the two parental types produced a plant with lutescens characteristics in early seedling stage, but the chlorophyll was gradually decomposed

Table 6. Genetic constitution of a normal green and three chlorophyll deficient mutant types in peanuts

Chlorophyll types	Chlorophyll factors
Green (Krinkle)	$VV\ Au_1Au_1Au_2Au_2\ Lu_1Lu_1Lu_2Lu_2\ L_1L_1l_2l_2$
Aureus	$VV\ au_1au_1au_2au_2\ Lu_1Lu_1Lu_2Lu_2\ L_1L_1l_2l_2$
Lutescens (0026 and Krinkle)	$VV\ Au_1Au_1Au_2Au_2\ lu_1lu_1lu_2lu_2\ L_1L_1L_2L_2$
Virescent	$vv\ Au_1Au_1Au_2Au_2\ Lu_1Lu_1Lu_2Lu_2\ l_1l_1L_2L_2$

as leaves aged, presumedly due to the "*au*" genes. Even under favorable environments, "*lu-au*" plants turn golden yellow in color and die in the late seedling stage.

The seven recessive factors from three mutants discussed in this paper interact to give the chlorophyll abnormality. The normal green peanut plant must then possess the dominant alleles of these factors. However, undesirable alleles can accumulate in self-pollinating amphiploid species if there are traits controlled by duplicate factors (Ashri 1968; Hull 1937). The genetic constitution of these mutants is presented in Table 6.

The present data indicate that both aureus and lutescens give consistently better fits of the theoretical segregation ratios than does virescent. Thus, the virescent mutant may carry residual background genotypic changes from chromosome structural damage, whereas the aureus and lutescens mutants would not have such alterations.

The three chlorophyll-deficient factors (*au*, *lu*, and *v*) show independent inheritance. The recessive combinations from the parental types between $au_1au_1au_2au_2$ and vv and between $au_1au_1au_2au_2$ and $lu_1lu_1lu_2lu_2$ would produce plants with combination of their respective parental characteristic, but combination between $lu_1lu_1lu_2lu_2$ and vv was nearly albino. The aureus is a mature-plant type mutant and can overcome the lag period in chlorophyll accumulation of both the lutescens (Tai and Todd 1972) and virescent (Benedict and Ketring 1972) types; therefore, the lutescens-aureus (*lu-au*) and virescent-aureus (*v-au*) phenotypes are expressed. Lutescens may not have the distinct lag period of chlorophyll accumulation which virescent has, but its chlorophyll accumulation cannot increase (Tai and Todd 1972). When these two parental types are combined, they produce albino through the action of genes "*lu*" and "*v*", causing imbalance in both chlorophyll formation and accumulation. The *v-au* and *lu-au* seedlings have a longer life span than the *v-lu* plant has.

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