

# An Allotetraploid Model for the Inheritance of Wine Testa Color in Peanut<sup>1</sup>

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## ABSTRACT

Wine-colored peanut seeds originate as mutants and appear later as segregates in farmer's fields. Off-color seeds are unwanted by shellers and manufacturers. The inheritance of wine testa is complex and among the least understood of the basic testa colors. The objective of this study was to determine the inheritance of wine testa color. True breeding genotypes for tan and wine testa color were crossed and progenies analyzed for testa color. A new genetic model is presented to explain the inheritance of the wine-colored seed trait. A tetragenic model, based on a 207:49 (tan to wine) F<sub>2</sub> segregation pattern, best described the data and assumes two sets of paired genes representing two genomes. At least one pair of homozygous recessive alleles in each set is necessary for expression of the wine-colored trait. Using parents in peanut breeding programs that are homozygous dominant at all four loci will help reduce the rate at which wine-colored seeds appear in farmer's fields.

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Key Words: *Arachis hypogaea*, seed color.

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The peanut industry has specific interest in a naturally occurring spontaneous mutants for wine-colored testa. Wine testa, as used here, is not to be confused with some of the similar appearing purple colors. Purple testae are phenotypically and genetically distinct from wine. Wine-colored seeds occur in farmer stock peanuts at shelling plants as contaminants in the normal tan-colored types of commercial trade. The natural mutation rate for wine is not known and wine mutants have no special marketing value. Conversely, the color is highly undesirable because it goes undetected until the pods are shelled. Electric eye sorters, common in modern shelling plants in the U.S., are adjusted to remove diseased seeds based on dark color and various extraneous material during the shelling operation. At present, however, they cannot distinguish diseased seeds from the off-colored types such as wine. Therefore, economic losses to the sheller occur because considerable quantities of otherwise sound, wine-colored

seeds are diverted to stocks used for crushing into oil. Peanut seeds used for oil stock receive a much lower price on the world market than those used in edible confectionary food products such as salted nuts, candy, and peanut butter.

Reviews of peanut genetics by Hammons (1973) and Wynne and Coffelt (1982) discussed testa color expression and concluded that seven loci were known to control the solid phenotypes of white, tan, red, and purple. Branch (1985) studied the inheritance of purple and purple-striped testa colors. He observed incomplete dominance for solid purple and purple stripes in peanuts where the segregation of F<sub>2</sub> populations appeared to support a digenic ratio of 3:6:7, which is a modification of the 9:7 ratio. The solid purple color and the purple-stripe characters were inherited independently by one and two genes, respectively. Branch (1997) studied the inheritance and possible allelic interactions of wine testa color in peanuts. Branch observed that the cv. Makulu Red (which is a recessive red-colored peanut) is dominant to wine. The F<sub>2</sub> segregation showed a good fit for the 3:1 ratio with Wine-Frr or PI 264549 × Krinkle-Leaf. F<sub>2</sub> segregation with Wine-Frr × Makulu Red showed a good fit to a 13:3 red to wine ratio. This implies that one or possibly two sets of homozygous recessive alleles control wine test color. Later, Branch (2001) studied the relationship between purple and wine testa color and found that the F<sub>2</sub> progeny segregated into wine and/or purple to pink and/or tan in a 13:3 digenic model.

The objective of this study was to determine the inheritance of wine testa color in cultivated peanuts. Results will lead to a better understanding of the reason for its appearance in farmer's fields so that strategies can be developed to diminish its frequency and increase profitability.

## Material and Methods

Crosses were made among five tan and two wine testa genotypes (Table 1) by employing the techniques of Banks (1976). The wine genotypes, P-291 and P-2577, used in the study were spontaneous mutants from a Krinkle leaf line (Hammons, 1964) and from cv. Chico (Bailey and Hammons, 1975), respectively. The mutants are phenotypically identical to their original sources except for the testa colors. The F<sub>1</sub> hybrids and subsequent F<sub>2</sub> segregating populations were grown in field plots at Perkins, OK in various years where normal cultural practices were employed. Pod samples from single plants were harvested, bagged, and transported to the laboratory for visual classification of the testa color after drying and shelling. Segregation data among progenies were

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**Table 1. Parents used in peanut testa color crosses.**

OSU acc. no. <sup>a</sup>	Testa color	<i>Arachis</i> botanical variety	Source
P-151	Tan	<i>A. hypogaea</i> ssp. <i>fastigiata</i> var. <i>vulgaris</i>	Krinkle-leaf
P-291	Wine	<i>A. hypogaea</i> ssp. <i>fastigiata</i> var. <i>vulgaris</i>	Mutant from P-151
P-936	Tan <sup>b</sup>	<i>A. hypogaea</i> ssp. <i>fastigiata</i> var. <i>vulgaris</i>	PI 262129
P-1286	Tan	<i>A. hypogaea</i> ssp. <i>fastigiata</i> var. <i>fastigiata</i>	Narrow leaflet
P-1452	Tan	<i>A. hypogaea</i> ssp. <i>hypogaea</i> var. <i>hypogaea</i>	F416 (Florida)
P-2577	Wine	<i>A. hypogaea</i> ssp. <i>fastigiata</i> var. <i>vulgaris</i>	Mutant from PI 565455
P-3767	Tan <sup>c</sup>	<i>A. hypogaea</i> ssp. <i>fastigiata</i> var. <i>vulgaris</i>	Mutant from PI 565443

<sup>a</sup>Oklahoma State Univ. Accession Number.

<sup>b</sup>Has small purple stripes.

<sup>c</sup>Has "rusty" spots.

analyzed by the CHISQA computer program of Hanna *et al.* (1978). Thirty genetic ratios (i.e., 244:1, 253:3, 247:9, 246:10, 243:13, 229:27, 225:31, 207:49, 139:117, 63:1, 61:3, 60:3, 59:5, 57:7, 55:9, 54:10, 49:15, 45:19, 39:25, 37:27, 36:28, 15:1, 13:3, 11:5, 10:6, 9:7, 7:1, 5:3, 3:1, and 1:1) were tested in an attempt to identify the best model to explain the inheritance of wine testa color.

## Results and Discussion

All F<sub>1</sub> hybrid testae produced from tan × wine testa parents showed tan color indicating that wine is recessive to tan. These results are in agreement with those reported by Harvey (1967). The F<sub>2</sub> segregation data from four cross combinations of tan × wine testa parents are presented in Table 2. The data is supportive of a 207:49 (tan to wine) genetic ratio; but conflicts with the 3:1 ratio hypothesis of Harvey (1967). Likewise, F<sub>2</sub> segregation data from two additional cross combinations showed a 15:1 (tan to wine) genetic ratio which conflict with that proposed by Harvey (1967). When the pooled data were

analyzed, only the 207:49 and 13:3 ratios yielded acceptable Chi-squares (Table 2). The 13:3 ratio seems unsatisfactory because some otherwise acceptable one- and two-gene ratios do not fit easily into a 13:3 ratio concept. Acceptance of our 207:49 model is strengthened by recognizing that the 3:1 ratio of Harvey (1967) and our 15:1 (Table 2) are compatible with the proposed four-gene model. For example, AABbCdd (tan) × AAbbccdd (wine) and AABBCDD (tan) × aaBBCCdd (wine) crosses would be tan; and when selfed the crosses would produce 3:1 and 15:1 ratios, respectively. In addition, there are several other parental genotypes that would produce the same results.

The above ratios are easily explained by studying Table 3. The model assumes that wine testa color is controlled by four genes (arbitrarily designated A, B, C, and D). However, the genes exist as pairs (i.e., A with B and C with D) and may represent the two genomes in the cultivated peanut. Furthermore, each gene is comprised of two alleles, dominant and recessive (e.g., A and a, B and b, etc.). We believe that wine testa color is dependent

**Table 2. F<sub>2</sub> segregation and chi-square values for tan and wine testa colors in peanut crosses with expected ratios of 13:3, 15:1, and 207:49.**

Group	Cross	Tan	Wine	Chi-square					
				13:3	P	15:1	P	207:49	P
1	P-291 × P-3767 <sup>a</sup>	320	74	0.00	0.99			0.03	0.86
	P-291 × P-1286	263	61	0.00	0.97			0.02	0.89
	P-2577 × P-151	397	81	1.02	0.31			1.49	0.22
	P-291 × P-1452	219	62	2.03	0.15			1.55	0.21
2	P-2577 × P-936	176	10			0.24	0.62		
	P-936 × P-291	282	20			0.07	0.79		
1	Total			3.05				3.09	
	Pooled			0.00	0.94			0.10	0.76
	Homogeneity			3.04	0.39			3.00	0.39
2	Total					0.31			
	Pooled					0.01	0.93		
	Homogeneity					0.31	0.58		

<sup>a</sup>Includes reciprocal crosses.

**Table 3. Various tan-by-wine crosses that could be made and the segregation ratios expected among their F<sub>2</sub> progeny.**

Wine parent genotype	Tan parent genotype						
	AABBCCDD	AABBCCdd	AABBccDD	AABBccdd	AAbbCCDD	aaBBCCDD	aabbCCDD
AAbbCCdd	(1-1) 15:1 <sup>a</sup>	(1-0) 3:1	(1-2) 57:7	(1-1) 3:1	(0-1) 3:1	(2-1) 57:7	(1-1) 3:1
AAbbccDD	(1-1) 15:1	(1-2) 57:7	(1-0) 3:1	(1-1) 3:1	(0-1) 3:1	(2-1) 57:7	(1-1) 3:1
AAbbccdd	(1-2) 57:7	(1-1) 3:1	(1-1) 3:1	(1-0) 3:1	(0-2) 9:7	(2-2) 207:49	(1-2) 9:7
aaBBCCdd	(1-1) 15:1	(1-0) 3:1	(1-2) 57:7	(1-1) 3:1	(2-1) 57:7	(0-1) 3:1	(1-1) 3:1
aaBBccDD	(1-1) 15:1	(1-2) 57:7	(1-0) 3:1	(1-1) 3:1	(2-1) 57:7	(0-1) 3:1	(1-1) 3:1
aaBBccdd	(1-2) 57:7	(1-1) 9:7	(1-1) 3:1	(1-0) 3:1	(2-2) 207:49	(0-2) 9:7	(1-2) 9:7
aabbCCdd	(2-1) 57:7	(2-0) 9:7	(2-2) 207:49	(2-1) 3:1	(1-1) 15:1	(1-1) 3:1	(0-1) 3:1
aabbccDD	(2-1) 57:7	(2-2) 207:49	(2-0) 9:7	(2-1) 3:1	(1-1) 15:1	(1-1) 3:1	(0-1) 3:1
aabbccdd	(2-2) 207:49	(2-1) 57:7	(2-1) 57:7	(2-0) 9:7	(1-2) 9:7	(1-2) 9:7	(0-2) 9:7

<sup>a</sup>(x-y) indicates the number of loci at which segregation will influence expression of wine testa in the AB and CD sets; w:z indicates the segregation ratio of tan to wine testa expected in the F<sub>2</sub> generation.

on the presence of at least one homozygous recessive gene for each of the gene pairs in the set. More specifically, the combinations of aa and/or bb and cc and/or dd results in wine color. Other combinations result in tan testa. Table 3 shows five different genotypes that produce wine testa. It should be noted that all of these wine genotypes breed true for the wine trait when selfed (i.e., will not segregate for tan testa). This feature is an important consideration for understanding the proposed genetic model because in previous observations we have never recovered tan testa segregates directly from descendants of wine seed (data not shown). This feature also eliminates other genetic models in which some wine phenotypes could segregate into tan phenotypes in the following generation. Exceptions might be expected to occur in extremely rare cases of simultaneous double mutations of recessive to dominant alleles.

The validity for accepting a paired, four-gene model to explain tan vs. wine-colored testa inheritance is strengthened by realizing that peanut is an allotetraploid. Acceptance of the 207:49 ratio also is rational because it is the product of two 9:7 (two-gene) ratios. Branch (1985) reported an unusual 3:6:7 testa color segregation ratio and noted that it was a 9:7 ratio modification. Such observations lend considerable support to the potentiality for 9:7 ratios in peanuts.

Except for the report of Banks and Kirby (1981), we have failed to find additional references supporting 207:49 ratios in peanut genetic studies. It seems likely that the previously recognized (Higgins, 1940) tan testa alleles, F<sub>1</sub>F<sub>2</sub>F<sub>2</sub>, are good candidate symbols for one of the gene pairs (e.g., AABB). The two other genes in our model (CCDD) may be duplicate sets of the previously described wine (W) gene. Consequently, we propose that these genes be symbolized, W<sub>1</sub>W<sub>1</sub>W<sub>2</sub>W<sub>2</sub>. We assume that at least one of the color developmental genes (D<sub>1</sub>D<sub>1</sub> or D<sub>2</sub>D<sub>2</sub>) is necessary for the wine testa trait.

The proposed model helps to explain the occurrence

of wine segregates from tan seed stocks where occasional spontaneous mutations occur. There are only 31 tan testa genotypes that would fail to segregate upon selfing. All of the remaining tan genotypes [176, or 85%, from Punnett Square Table (not shown)] would be expected to produce wine phenotypes in subsequent generations.

Although it appears that the inheritance of wine testa has been identified, we can offer no practical solution to completely eliminate them in farmers' fields except to emphasize to peanut breeders the importance of using parents that are homozygous dominant for tan testa at all four loci when developing future cultivars. Such a procedure will slow down the rate of occurrence of wine testa phenotypes in tan-seeded cultivars and will help maintain phenotypic purity. As an aid in combating future problems, we suggest developing genetic stocks which are properly documented and cataloged for use in cultivar development and for use in test crosses involving testa color inheritance studies. Difficulty will be encountered in establishing and maintaining genetic purity in peanut parents because of the potential for natural outcrossing, primarily by bees (Coffelt, 1989), when peanuts are grown in field plots. However, the maintenance of stocks of known genetic purity is imperative for the development of accurate models in future investigations.

Fortunately, our understanding of testa color inheritance in peanuts will be greatly enhanced as new gene-identification technologies (He and Prakash, 1997; Hopkins *et al.*, 1999) are applied to the species. However, investigators presently must rely on indirect approaches that utilize phenotypic ratios to predict the consequences of genetic recombinations.

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