

Inheritance of the White Seed Character in Alfalfa¹

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SYNOPSIS

White seed in alfalfa is conditioned by a duplicate set of homozygous recessive genes for colorless and one dominant color inhibitor gene. This gene complex also inhibits anthocyanin production throughout the plant. The distribution of the offspring of a white-seeded hybrid having pure alfalfa parentage was interpreted as resulting from selective pairing of chromosomes, and that of a white-seeded hybrid, having as one parent an artificially induced autotetraploid from diploid *Medicago falcata*, as being the result of random chromosome pairing. These results are taken as evidence that alfalfa is of autotetraploid origin, and has evolved into a functional diploid.

THE purpose of this study was to gather evidence concerning the homology between the two sub-genomes in cultivated alfalfa, a natural tetraploid. The inheritance of the white seed character was determined as a means of inferring the manner of chromosome pairing in two different types of F_1 hybrids. Both types of hybrids contained two sub-genomes from a white-seeded alfalfa plant in a cultivated variety. In one type of hybrid the other two genomes came from tan-seeded alfalfa plants of the same general origin. In the second type of hybrid, however, two identical genomes were supplied from tan-seeded artificially induced autotetraploid *Medicago falcata* plants.

The autotetraploid makeup of alfalfa is well supported by earlier cytogenetic evidence (4, 5, 7, 9 and 10). Armstrong (1) postulated that alfalfa is an allopolyploid derivative of closely related diploid species. The somatic chromosome number in cultivated alfalfa is 32, the basic chromosome number in the genus *Medicago* being 8. With two exceptions, all investigators, however, have interpreted genetic data from alfalfa on a diploid basis, that is, assum-

ing that preferential pairing of the chromosomes within sub-genomes regularly occurs. Stanford (11 and 12) presented evidence for inheritance in cultivated alfalfa suggestive of random pairing between chromosomes in sub-genomes. Dudley and Wilsie (2), more recently, have explained their genetic data by assuming both preferential and random pairing.

The data presented here are of interest also for the light they throw on the inheritance of seed coat color and anthocyanin formation as such, in alfalfa.

LITERATURE REVIEW

MacVicar (8) reported that white seed is recessive to the ordinary tan seed and that the difference is conditioned by either one or two genes. Lepper and Odland (6), using Hansen's white-seeded, white-flowered strain, reported an F_2 ratio of 15 colored-flowered to 1 white-flowered, following a white-flowered by blue-flowered cross. Inasmuch as white-flowered plants of the Hansen strain are white-seeded, it can be assumed from these results that recessive genes at two independent loci condition the white seed character.

The white flower character studied by Stanford (11), as judged by his description, is probably phenotypically the same as the white seed character studied in this investigation, and probably is conditioned by recessive genes on duplicated chromosomes.

MATERIALS AND METHODS

An alfalfa plant, with white seeds and white flowers, obtained from the Colorado Agricultural Experiment Station (white-flower No. Colo. 117-49) (13) was mated with three autotetraploid *M. falcata* plants which had been produced by colchicine treatment of diploid ($2n = 16$) individuals (S.P.I. 84336). The *M. falcata* plants bore yellow flowers and tan seeds. The white-seeded plant was also mated with four *M. sativa* plants having blue flowers and tan seed. The tan-seeded alfalfa plants selected varied in depth of flower color and plant pigmentations. One tan-seeded plant from the Buffalo variety had intensely pigmented stems and very dark purple flowers. The other three, from the Cossack variety, had moderate stem pigmentation and flower colors which ranged from cream, with a tint of blue, to dark blue.

Twenty-four F_1 hybrids, 12 each from the *M. sativa* and 4n *M. falcata* matings in which hybrids from each tan-seeded parent were represented, were backcrossed to the white-seeded parent. After suction emasculation of the flowers, pollen collected from the Colorado white-seeded plant on a toothpick tipped with fine emery cloth was applied to the stigmas of 10 to 15 flowers in each of

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10 to 20 racemes per F_1 plant. The remaining flowers on the F_1 plants were self-pollinated by tripping the flowers with a clean toothpick.

All matings were made in the greenhouse in the absence of pollinating insects. A family, as referred to in table 1, represents the progeny from a particular backcross mating or selfing.

Seed from the backcross matings and self-pollinations was planted in the field in June, 1949. When the resulting plants began flowering in August, they were classified for flower-color—blue, yellow, or white. As the pods matured, the plants were classified for seed-color—white or tan.

White seed can be distinguished from tan seed in most cases with the naked eye. Weakly pigmented tan seeds were found to be tan colored in the crease near the junction of the cotyledons and hypocotyl when observed with a low-power binocular magnifier. There is no pigment in this region in white seeds.

After many plants had been classified for seed color, it became evident that white-seeded plants invariably lack anthocyanin pigment. Plants which were difficult to bring into flower and contained anthocyanin pigment in the vegetative tissues, henceforth were classified as tan-seeded. The other plants were forced into flower in the greenhouse and classified directly for seed color. The flowers of white-seeded plants having *M. falcata* ancestry were sometimes light yellow but contained no blue or brown pigment.

RESULTS AND DISCUSSION

All white-seeded \times tan-seeded F_1 hybrid plants were tan-seeded, indicating that white seed is recessive to tan seed.

The following scheme of tetrasomic inheritance may be assumed for the white seed character. White seed results only when all four color (*C*) genes are present in recessive form, that is *cccc*. The presence of a dominant inhibitor gene, *I*, is required also for expression of the white seed phenotype. When one or more of dominant *C* genes is present or when the *I* locus is homozygous recessive, anthocyanin and consequently tan seed is produced by the plant.

To explain the distributions obtained, the white-seeded parent, which was also used as the recurrent parent in the backcross, was assumed to be nulliplex for *C* and monoplex for *I*, *ccccIiii*, thus resulting in two types of F_1 progeny. The ratios expected in the backcross progenies are shown below.

Genotypes			Tan to white seed ratios of backcross progeny		
tan-seeded	white-seeded	F_1	<i>M. sativa</i> hybrids	<i>4n M. falcata</i> hybrids	
			<i>CCCCiiii</i>	<i>ccccIiii</i>	<i>CcCcIiii</i>
			<i>CcCciii</i>	7:1	11:1

The distributions in the backcross generations were calculated on the basis of preferential pairing of the chromosomes bearing the *c* locus in the *M. sativa* hybrids and on the basis of random pairing of the chromosomes in the case of $4n M. falcata$ hybrids.

The observed distributions of the backcross and F_2 generations are summarized in table 1. The deviations of the observed distribution of tan to white seed from the calculated for each group, as defined by origin and ratio, are within the accepted limits as tested by the Chi-square method. Also, with one exception, to be discussed later in the text, the deviations from expectation within individual families were within the limit of random sampling.

Tan-seeded individuals appeared in the few progenies which were obtained following self-pollination of the white-seeded parent plant. This result would be expected on the basis of the hypothesis outlined above. A satisfactory determination of the frequency of tan-seeded offspring was not possible, however, because of the extreme self-incompatibility of this plant.

An interesting exception to the regular behavior of white seed was not included in table 1. The distribution of plants in one backcross family (*M. sativa* \times white-seeded hybrid) had to be rejected as a 7:1 ratio, $P = 0.05$. The observed distribution, 113 tan to 6 white seed, is much nearer a 15:1 ratio, $P = 0.5$ to 0.7, than to a 7:1 ratio, $P = 0.01$ to 0.02. A simple and reasonable, although not proven, explanation exists for this exception when the characteristics of the parent plants are considered. The hybrid which produced progeny in this ratio was, like its tan-seeded parent, a dark-purple flowered, highly pigmented plant. If a single gene caused this intense pigmentation and was complementary to *C*, resulting in tan seed regardless of the state of *C*, a 15:1 ratio would result in the backcross generation of the hybrid containing it. The above ratio could also have resulted from random pairing of the chromosomes bearing the *c* locus. Other F_1 plants from the same mating, however, gave ratios characteristic of selective pairing.

Another exception, to which there is no simple explanation based on the data at hand, is the existence of white-seeded plants in the F_2 progeny of a $4n M. falcata$ hybrid assumed not to contain the inhibitor gene, *I*, and which therefore was not expected to yield white-seeded progeny. There are possible explanations involving double reduction and two alleles of the color gene; the data necessary to test this explanation are not available.

The genetic behavior of the *M. sativa* hybrids suggests that the four genomes are differentiated into two subgroups. On the other hand, the genetic behavior of hybrids

Table 1.—Distributions of progeny in the F_2 and backcross generations of Colorado white-seeded \times tan-seeded alfalfa hybrids in which the Colorado white-seeded plant was the recurrent parent.

Tan-seeded parent	Generation	No. of families	Assumed Ratio	Observed No. of plants		Calculated No. of plants		P range
			Tan:White	Tan	White	Tan	White	
<i>M. sativa</i> -----	Backcross	6	13:3	684	165	689.81	159.19	0.50-0.70
		5	7:1	533	76	532.88	76.12	0.98-0.99
$4n M. falcata$ -----	Backcross	5	7:1	625	97	631.75	90.25	0.30-0.50
		7	11:1	1113	96	1108.25	100.75	0.50-0.70
$4n M. falcata$ -----	F_2	2	141:3	367	9	368.17	7.83	0.50-0.70
		1	$\alpha : 0$	85	2	87	0	

containing $4n$ *M. falcata* parentage indicates that there is no differentiation of the genomes in alfalfa, particularly when they are placed in combination with two genomes, identical with each other, derived from $4n$ *M. falcata*.

Earlier investigators have found that genetic ratios in alfalfa conformed to those characteristic of diploid organisms. Improvement of the long established alfalfa varieties has been primarily by intravarietal selection and, therefore, any chromosome differentiation existing in these varieties has not been changed. In recent years, however, alfalfa varieties have been synthesized from strains more diverse in origin. The characteristics of the near-homologous chromosomes which cause differentiation of sub-genomes may be different in the various strains entering into the newer synthetic varieties. The sub-genomes from these unrelated strains would lose their identity in a synthetic variety, resulting in random pairing of the four homologous chromosomes. This could account for the recent reports of inheritance based on random chromosomal pairing.

It is reasonable to assume that alfalfa is of autotetraploid origin and has evolved into a functional diploid, as suggested by Dudley and Wilsie (2) and others. Gillis and Randolph (3) have presented evidence of decreasing quadrivalent formation at diakinesis in advanced generations of autotetraploid maize. Perhaps reduction in the frequency of quadrivalent formation is the first step in the evolution from autotetraploid to diploid genetic behavior. Differentiation of homologous genomes into independent sub-genomes as the result of the accumulation of genetic mutations and small structural changes causing a reduction in homology could be the final step in the evolution from the autotetraploid to the diploid type of chromosome behavior.

Some hybridization and testing of the Hansen strain of white-seeded alfalfa was done, and the results suggest that its genotype is slightly different from that of the Colorado white-seeded plant used in this study. Additional investigation of the inheritance of the white seed character should reveal some interesting relationships and perhaps explain the exceptions found in this investigation.

A few observations, not pertinent to this study but nevertheless helpful to investigators of the inheritance of flower color in alfalfa, were made during the course of this study. (A) Genes for blue flower color are distinct from the basic genes conditioning anthocyanin pigmentation but dependent on the presence of at least one dominant basic gene, *C*, for expression. Blue flowers were present on F_1 hybrid plants from the mating $4n$ *M. falcata* \times white-seeded; therefore, it is assumed that the basic genes, *CC*, from the $4n$ *M. falcata* allowed the expression of the blue-flower genes which were completely masked in the white-seeded parent. (B) The system of genes which control anthocyanin production is independent of the sys-

tem of genes which govern yellow flower pigment. White-seeded plants with *M. falcata* parentage generally had yellow flowers, although they were somewhat lighter in color than those of the parent stock and lacked the characteristic maroon markings in the flower.

SUMMARY

White seed color in alfalfa is conditioned by genes which also inhibit anthocyanin synthesis throughout the plant. A white-seeded plant (*M. sativa*) obtained from the Colorado Experiment Station was mated to tan-seeded *M. sativa* plants and to autotetraploid *M. falcata* plants which had been produced by colchicine treatment of a diploid. From the analysis of the distributions in the backcross generation in which the white-seeded parent was the recurrent parent, it was determined that white seed was conditioned by recessive genes at a duplicated locus and by a dominant color inhibitive gene. Tetrasomic inheritance was found. Ratios characteristic of preferential chromosome pairing were obtained from pure *M. sativa* hybrids and ratios characteristic of random chromosome pairing were obtained from the white-seeded *M. sativa* \times autotetraploid *M. falcata* hybrids.

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