Natural selection for blue and white aleurone color in barley, with yield and agronomic data
by Arthur L Dubbs

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University

Abstract:
This study was conducted to compare the performance of blue and white aleurone seed at different locations in the state. No differences were found in plant height, date of heading, tillering, percent protein or seed weight. Blue aleurone seed averaged higher in percent skinning when threshed, Test weight differences were small but in most cases favored the blue seed, which may have resulted from the higher skinning percent of the blue seed. Yield comparisons of blue and white isogenic lines showed no Significance due to, seed color; however, yearly differences did exist, but not significantly. Blue and white seed grown in 50-50 mixtures showed a natural selection against blue seed when grown at Moccasin for five years. Five bulked hybrids were grown at two locations. through various generations to determine natural selection of seed color. Two of the hybrids did not fit their expected ratios at Bozeman or Moccasin. Two had good fits at both locations. One performed as expected at Moccasin but not at Bozeman. When all hybrids were averaged on a yearly basis two significant losses in percent blue seed were obtained at At Moccasin a in 1955 and a Bozeman, one in 1953 and the other in 1957 significant loss in blue seed was recorded gain in 1957. No natural selection trends could be detected when the five bulked hybrids were grown at five locations in the state for two years.

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ABSTRACT

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INTRODUCTION

In a plant breeding program it is of interest to the plant breeder to know how certain plant characteristics perform under various climatic and soils conditions. By knowing this he is aided in making his plant selections. Plant characteristics often differ when grown under different climatic conditions. These differences may be detrimental or favorable. Plant height, for instance, under normal rainfall conditions may be satisfactory, but under drought conditions it can be too short for combining. Some varieties will yield higher than others during years of low rainfall but less when moisture is adequate.

It was the objective of this study to investigate one of these plant characteristics, blue as contrasted to white aleurone color, which is common in many barley varieties. This characteristic color of caryopsis is found in the aleurone layer of the endosperm.

In making barley selections where there is a choice of blue and white seeds, the question often arises as to which type should be selected. Perhaps one type yields higher under certain conditions or shows more drought resistance under other conditions. Many questions such as these could be asked, but only through applied research can they be answered.

LITERATURE REVIEW

The aleurone layer in barley is located in the outermost layer of cells of the endosperm. This consists of three (two to four) layers of cubical cells which contain no starch but aleurone grains composed principally of protein. (4)[1/]

Barley grains vary in color through white, violet, blue, blue-gray, violet-red, brown to black, with intermediate shades. These colors may occur in the hulls, pericarp, aleurone and occasionally the starchy endosperm. Harlan (3) reported that two pigments were responsible for seed color; a melanin-like compound, which is black; and anthocyanin, which is red in its acid and blue in its alkaline condition. A light deposit of the melanin-like compound in the hulls results in brown while a heavy deposit results in black. Anthocyanin in the hulls results in light violet-red. In hulless kernels the melanin-like compound in the pericarp results in black, while anthocyanin produces violet. The acid condition of the anthocyanin in the pericarp superimposed upon the alkaline condition in the aleurone layer gives the effect of purple. A blue aleurone beneath a colorless pericarp results in blue-gray. White hulls over a blue aleurone cause the grain to appear bluish-gray. Black hulls over a blue aleurone result in black.

1/ Numbers in parentheses refer to literature cited.

Inheritance of the different colors in barley have been reported by various authors. Daane (2) reported purple versus white pericarp (Pp) as due to a single factor. Myler and Stanford (6) obtained a ratio of 12 purple, 3 blue and 1 white in a cross with purple pericarp underlain with blue aleurone and a white pericarp. Buckley (1) reported white versus orange lemma (Oo) as being due to a single factor which is linked with long versus short haired rachilla (Ss) with a crossover value of 39.1 percent. Myler and Stanford (6) also found white versus orange lemma as being due to a single factor but inherited independently from factors in linkage groups I, III, IV, and V. A number of authors have reported blue versus white aleurone as due to a single factor. Buckley (1) placed (Bl bl) in linkage group IV as being linked with hooded versus awned (Kk) with a crossover value of 40.6 percent. Robertson (7) also placed this factor in the same group as being linked with hoods but with a crossover of 22.58 percent. Myler and Stanford (6) obtained a 3:1 segregation for blue aleurone in the F_2 of some crosses; but obtained a 9:7 ratio in the F_2 of a cross between two white varieties which indicated the presence of two complementary factors. One complementary factor was found linked with hoods in group IV with a crossover of 24.72 percent. The second factor was linked with hulled versus naked (Nn) and placed in group III with a 9.88 percent crossover value. Smith (8) in his summary placed (Bl_1 bl_1) in

group IV as being linked with hoods, light green seedling, zoned leaf, glossy seedling and susceptibility to mildew. He placed (Bl_2 bl_2) in group III as being linked to naked seed and red stem.

Lejeune and Parker (5) reported that aleurone color has no actual relationship to mellowness or starchiness in barley. However, they may be classed as non-mellow under the present interpretation of the Official Grain Standards of the United States. Most maltsters will pay equivalent premiums for the acceptable blue pearl varieties if marketed in unmixed carlots and of satisfactory malting quality. Mixtures of blue and white pearl varieties are discounted by buyers even though each may be an acceptable variety.

MATERIALS AND METHODS

Three different sources of blue and white aleurone isogenic material were tested in this study. One source consisted of seven lines developed by Dr. G. A. Weibe at Beltsville, Maryland. These lines were developed from the following crosses or composite crosses: Manchuria x C. I. 5719, 31-18, Manchuria x C. I. 4424, 32-18, Manchuria x C. I. 2384, 33-18, Manchuria x C. I. 4428, 34-18, C. I. 5461, C. C. sel. 35, 35-26, C. I. 5461, C. C. sel. 36, 36-26 and C. I. 5461, C. C. sel. 37, 37-26. The lines are identified by the numbers 31-18 through 37-26 with the first number representing the selection number and the second the year of selection. These lines were developed by selfing for 18 and 26 generations. Thus the percent of genes common to both pairs is greater than 99.99 percent with the linked genes having a minimum effect. Another source of isogenic lines was developed by Mr. R. F. Eslick at Montana State College by bulking the blue and white aleurone seed from a cross in the fourth generation. Although there are variations in genotypes in each line, the same plant to plant variation should be represented in each bulk, this being influenced by linked genes. The lines obtained by bulking are from the crosses Trebi-Dryland x Glacier, 52-5396, Glacier x Mars, 52-5397 and Glacier x Manchuria, 52-5398. The Titan isogenics were selected from drilled plots in which numerous blue aleurone

types were noted with the blue aleurone plants being indistinguishable from the normal Titan. To obtain the isogenic lines 200 plants of blue and 200 of white were selected and bulked.

Yields and other agronomic data collected from the isogenic lines were obtained from replicated nurseries grown at Moccasin and Bozeman. These nurseries consisted of single row plots, 18 feet long spaced 1 foot apart. The grain at both locations was threshed in a Vogel thresher. Seeding at both locations was accomplished with a belt seeder mounted behind a tractor.

Seed weights were determined by weighing six samples, each consisting of 100 randomly picked kernels.

In 1953, twelve 50-50 mixtures of blue and white aleurone kernels were compounded. Each mixture consisted of 200 seeds each of blue and white seed from the same isogenic. These were grown at Moccasin from 1953 through 1957 in single row plots, 18 feet long with no replication. Each year three 100 seed samples were randomly picked from the threshed grain and the number of blue aleurone kernels present were recorded as percent of the total. The seed planted each year was from seed harvested the previous year.

The four crosses, Glacier x Colsess, Glacier x Mars, Glacier x Manchuria and Trebi-Dryland x Glacier, were made by S. C. Litzenberger in 1943 at Bozeman. The F_1 plants were

grown in the greenhouse during the winter of 1944 and the F_2 plants in the field in 1944. The Glacier x Titan cross was made by Mr. Eslick at Bozeman in 1948. The generations of these hybrids were grown in bulk until 1957 at Bozeman. In 1948 the F_6 seed generation of Glacier x Mars, Glacier x Manchuria and Trebi-Dryland x Glacier were grown at Moccasin. In 1952 all five crosses were grown at Moccasin which included Glacier x Colsess F_5, Glacier x Titan F_4, Glacier x Manchuria F_7, Glacier x Mars F_7, and Trebi-Dryland x Glacier F_7. The generations grown at Moccasin were comparable to those grown at Bozeman. The expected ratios for each cross were determined by the aleurone color of each parent in the cross. Since the aleurone color is controlled by two complimentary genes with one gene located in linkage group III and the other in IV, a cross involving two white parents would have an expected ratio of 9 blue seed to 7 white, while a cross between a blue and a white seeded parent would result in a ratio of 3:1. The calculated percent blue kernels in each seed generation for 9:7 and 3:1 ratios are as follows:

Generation	9:7 Ratio	3:1 Ratio
F_3	39.06	62.50
F_4	31.64	56.25
F_5	28.22	53.13
F_6	26.59	51.56
F_7	25.79	50.78
F_8	25.39	50.39
F_9	25.20	50.20
F_{10}	25.10	50.10
F_{11}	25.05	50.05
F_{12}	25.02	50.02

Identification and plant characteristics of the varieties used in the crosses are as follows:

Variety	C.I. No.	Aleurone Color	Row No.	Awn Type	Awn Barbing
Glacier	6976	white	6	awned	semi-smooth
Colsess	2792	blue	6	hooded	rough
Mars	7015	white	6	awned	smooth
Titan	7055	white	6	awned	smooth
Manchuria	2947	blue	6	awned	rough
Trebi-Dryland	6981	blue	6	awned	rough

The generations grown at Moccasin were comparable to those grown at Bozeman. Plantings consisted of one row, 18 feet long with no replications. The percentage of blue aleurone seed in each generation was determined in the same manner as for the 50-50 mixtures.

In 1952 the five crosses were grown at five locations in the state under dryland and irrigated conditions. The following generation seed from Bozeman only was planted: Glacier x Colsess F_5, Glacier x Titan F_4, Glacier x Mars F_6, Glacier x Manchuria F_6, and Trebi-Dryland x Glacier F_6. Plantings were in single 18 foot rows with no replication. The seed harvested in 1952 at each location was again planted in 1953 at the location from which it was harvested. Samples of the seed harvested each year were sent to the author for determination of percent blue aleurone seed.

All material tested was grown under dryland at Moccasin and under irrigation at Bozeman.

RESULTS

Agronomic and Yield Comparisons of Isogenic Lines

The data shows no differences in plant height, date of heading or tillers per three feet of row at Moccasin (Table I), when the blue versus white aleurone pairs developed by selfing were compared. This could have been expected since these lines were developed after selfing for 18 to 26 generations. The percent skinning of seed at Bozeman as determined by three runs through a head thresher showed differences between pairs. Five of the seven lines showed a higher degree of skinned blue kernels. In six out of seven comparisons at Moccasin and in five out of seven comparisons at Bozeman, blue had higher test weight than white. (Table II) Thus eleven of the fourteen comparisons were in favor of the blue aleurone. This higher test weight may be explained by considering the higher percent skinning of the blue seed. The analysis of variance for yield showed no significant color or color x strain interaction.

Yields of the bulked blue and white isogenic pairs from the three crosses (Table III) show no significant difference. In 1953, at Bozeman the blue lines approached being significantly higher in yield than the white, but in 1954 the white lines on the average yielded higher than the blue, although not significantly so. Plant height, date of heading, tillers and percent protein (Table IV) show no great differences

Table I. Plant height, date of heading, tillers per three feet of row and percent skinning of blue and white isogenic lines obtained by selfing when grown at Moccasin in 1953 and Bozeman in 1954.

Isogenic Lines	Aleurone Color	Plant Height Inches	Date First Headed	Tillers per 3 ft. of row	Percent skinning
		Mocc.	Mocc.	Mocc.	Boz.
31-18	Blue	27	7-11	101	15
	White	28	7-11	97	15
32-18	Blue	29	7-8	80	24
	White	28	7-8	101	22
33-18	Blue	29	7-14	89	8
	White	29	7-14	58	9
34-18	Blue	31	7-10	83	18
	White	31	7-10	91	12
35-26	Blue	31	7-6	95	27
	White	29	7-6	62	26
36-26	Blue	29	7-11	63	11
	White	29	7-11	68	5
37-26	Blue	27	7-9	77	7
	White	27	7-9	54	3
Average	Blue	29	7-9	84	16
	White	29	7-9	76	13

Table II. Test weight and yield of blue and white aleurone isogenic lines obtained by selfing when grown at Moccasin in 1953 and at Bozeman in 1954.

Isogenic Line	Aleurone Color	Test Weight lb. per bu.		Yield Bu. per acre	
		Mocc.	Boz.	Mocc.	Boz.
31-18	Blue	42	50.8	28.9	61.7
	White	40	50.2	29.1	70.4
32-18	Blue	42	50.1	25.5	49.3
	White	40	49.0	24.6	57.7
33-18	Blue	45	50.1	21.3	50.5
	White	45	49.3	18.1	44.5
34-18	Blue	47	49.4	22.8	67.6
	White	44	49.5	32.6	64.1
35-26	Blue	41	47.1	35.9	67.8
	White	40	46.6	30.3	78.7
36-26	Blue	45	52.7	39.6	81.7
	White	44	52.6	36.2	86.8
37-26	Blue	43	50.2	31.8	85.1
	White	41	51.0	34.8	83.7
Average	Blue	44	50.1	29.4	66.2
	White	42	49.7	29.4	69.4

Analysis of Variance - Yield

Source	Degrees of Freedom		Mean Squares	
	Mocc.	Boz.	Mocc.	Boz.
Strains	6	7	9236.66	89251
Error (a)	6	21	2440.54	16646
Color	1	1	0.14	9097
Color x Strains	6	7	1678.56	4943
Error (b)	7	24	221562.01	9671

Table III. Yields of blue versus white aleurone isogenic lines obtained by bulking when grown at Moccasin in 1953, 1955 and 1956 and at Bozeman in 1953 and 1954.

Location	Year Grown	Aleurone Color	Yield-Bushels per Acre			Average Bushels per Acre
			Glacier x Mars	Glacier x Manchuria	Trebi-Dryland x Glacier	
Moccasin	1953	Blue	36.0	34.2	40.4	36.9
		White	36.5	33.7	39.3	36.5
Moccasin	1955	Blue	16.8	14.6	16.1	15.8
		White	16.8	14.5	17.6	16.3
Moccasin	1956	Blue	30.9	24.4	29.8	28.4
		White	30.3	24.6	28.1	27.7
Bozeman	1953	Blue	76.8	51.2	66.0	64.7
		White	73.1	46.6	48.8	56.2
Bozeman	1954	Blue	64.4	75.9	72.1	70.1
		White	78.8	78.0	73.3	76.7

Analysis of Variance - Yield

Source	Degrees of Freedom		Mean Squares	
	Mocc.	Boz.	Mocc.	Boz.
Years	2	1	204785*	171307
Color	1	1	56	1581
Crosses	2	2	9742	38297
Years x Color	2	1	163	49651
Years x Crosses	4	2	1590	79680
Color x Crosses	2	2	21	14232
Years x Color x Crosses	4	2	187	3707
Error	68	44	62715	397352

* Significant at the 5 percent level

Table IV. Plant height, date of heading, tillers per three feet of row and percent protein of three blue and white aleurone isogenic lines obtained by bulking when grown at Moccasin in 1953, 1954 and 1956, and at Bozeman in 1953.

Isogenic Lines	Aleurone Color	Plant Height Inches		Date First Headed		Tillers per 3 ft. of row	Percent Protein	
		1954	1956	1954	1956	1953	1953	
		Mocc.	Mocc.	Mocc.	Mocc.	Mocc.	Boz.	Mocc.
Glacier x Mars	Blue	24	21	7-5	6-28	65	11.6	11.8
	White	24	21	7-6	6-28	74	11.4	11.2
Glacier x Manchuria	Blue	24	21	7-7	6-28	74	12.0	11.7
	White	24	21	7-6	6-28	86	12.2	11.8
Trebi-Dryland x Glacier	Blue	23	21	7-7	6-26	103	12.3	12.8
	White	23	21	7-6	6-26	101	12.9	12.4
Average	Blue	24	21	7-7	6-28	81	12.0	12.1
	White	24	21	7-6	6-28	87	12.2	11.8

between pairs. Seven of the twelve test weights at Moccasin were higher for blue aleurone than white while three out of six at Bozeman were higher for blue aleurone. (Table V) The overall average test weight shows blue to weigh 0.5 pounds per bushel higher than the white. In Glacier x Mars the blue is always higher in test weight than the white while the other two crosses are variable.

Yields between the blue and white Titan were not significantly different. However, differences between years did exist. (Table VI) In 1952, at Moccasin the white yielded significantly higher than the blue. During the three years tested at both locations the white yielded higher than the blue in four out of six comparisons. On the average the white yielded higher than the blue at both locations. No differences between plant heights were recorded. (Table VII) The blue Titan showed some differences in date of heading, ranging from one to three days later than the white. Tillering was approximately the same. The protein content varied one percent in favor of the blue seed, which is perhaps associated with slightly later heading. Of the five comparisons only two favored the blue seed in test weight; however, the averages were only 0.1 pounds per bushel different.

Seed weights (Table VIII) showed no differences between blue and white seed. The small differences in test weight which favored the blue seed were not reflected in the seed weights.

Table V. Test weight of three blue and white aleurone isogenic lines obtained by bulking when grown at Moccasin in 1953, 1954, 1955 and 1956 and at Bozeman in 1953 and 1954.

Isogenic Lines	Aleurone Color	Test Weight - lb. per Bushel						
		1953		1954		1955	1956	Avg.
		Boz.	Mocc.	Boz.	Mocc.	Mocc.	Mocc.	
Glacier x Mars	Blue	48.8	46.0	47.7	40.2	42.0	43.6	44.7
	White	48.4	42.3	47.5	39.2	38.0	42.4	43.0
Glacier x Manchuria	Blue	47.9	41.7	47.6	37.8	40.0	42.3	42.9
	White	48.7	41.3	47.7	38.0	38.0	41.3	42.5
Trebi-Dryland x Glacier	Blue	48.5	40.3	47.4	39.0	42.0	42.8	43.3
	White	48.3	42.3	48.3	40.0	41.0	43.1	43.8
Average	Blue	48.4	42.7	47.6	39.0	41.3	42.9	43.6
	White	48.5	42.0	47.8	39.1	39.0	42.3	43.1

Table VI. Yields of blue versus white aleurone isogenic Titan when grown at Moccasin and Bozeman from 1952 to 1956.

Year Grown	Bushels per Acre			
	Moccasin		Bozeman	
	Blue	White	Blue	White
1952	20.2	26.7	48.2	57.1
1953	38.2	42.0	58.2	53.4
1954	29.3	29.3	68.8	71.5
1955*			75.3	74.8
1956*			83.0	88.8
Average	29.2	32.7	66.7	69.1

* Not included in Analysis of Variance

Analysis of Variance		
Source	Degrees of Freedom	Mean Squares
Location	1	1058000
Color	1	21060
Years	2	75313
Locations x Color	1	18
Locations x Years	2	50933
Color x Years	2	8684
Location x Color x Years	2	2996
Error	34	289297

Table VII. Plant height, date of heading, tillers per three feet of row, percent protein and test weight of blue and white aleurone isogenic Titan when grown at Moccasin and Bozeman in 1952, 1953 and 1954.

Character	Location	Year	Titan	
			Blue	White
Plant height inches	Mocc.	1952	22	22
		1954	29	29
First headed date	Mocc.	1952	6-25	6-22
		1953	7-5	7-5
		1954	7-5	7-4
No. tillers per 3 ft. of row	Mocc.	1953	94.0	96.2
Percent protein	Boz.	1953	14.3	13.3
Test weight lbs. per bu.	Mocc.	1953	44.8	43.0
		1954	47.0	48.2
	Boz.	1952	49.0	50.0
		1953	53.1	52.9
		1954	50.6	51.1
Average test weight			48.9	49.0

Table VIII. Seed weights in grams per 100 kernels of blue and white aleurone isogenic lines from seed grown at Moccasin and Bozeman.

Isogenic Line	Aleurone Color	Grams per 100 kernels					
		Moccasin			Bozeman		Average
		1953	1954	1955	1953	1954	
52-5396	Blue	2.90	2.70	2.60	4.63	4.47	3.46
	White	3.03	2.77	2.57	4.57	4.37	3.46
52-5397	Blue	3.17	2.98	2.65	4.80	4.52	3.62
	White	3.23	3.00	2.63	4.68	4.60	3.63
52-5398	Blue	2.93	2.37	2.18	4.28	3.97	3.15
	White	2.90	2.37	2.22	4.32	3.98	3.16
31-18	Blue	2.2				3.20	2.70
	White	1.9				3.03	2.47
32-18	Blue	2.1				3.33	2.72
	White	2.0				3.42	2.71
33-18	Blue	2.9				3.73	3.32
	White	2.9				3.70	3.30
34-18	Blue	2.4				3.05	2.73
	White	2.4				3.08	2.74
35-26	Blue	3.5				4.90	4.20
	White	3.5				5.10	4.30
36-26	Blue	3.6				4.52	4.06
	White	3.2				4.50	3.85
37-26	Blue	2.7				4.07	3.39
	White	2.9				4.02	3.46
Average	Blue	2.84	2.68	2.48	4.57	3.98	3.31
	White	2.80	2.71	2.47	4.52	3.98	3.30

Natural Selection for Aleurone Color in 50-50 Mixtures

Results obtained from the 50-50 mixtures indicate a natural selection against blue aleurone seed at Moccasin (Table IX). Chi-square tests were conducted on each mixture to determine whether the percentages were random variations from the expected. Since all percentages are expected to be 50 percent, a set of limits could be calculated which would apply to all figures in the table except the averages. The limits which can be calculated by the chi-square test are 50 $\pm$ 9.8 percent for a 5 percent level of significance and 50 $\pm$ 12.9 percent for the 1 percent level. In 1957 four of the mixtures were below the 50 percent expected blue seed with a probability of less than one percent that this was a random variation. The other mixtures showed the normal deviations with a few percentages being above or below the expected; however, these returned to normal the following year and may represent sampling variation. Three of the mixtures, 33-18, 34-18 and 35-26, remained near the expected 50 percent indicating equal competitive ability of the blue with the white seed. The blue in the Titan mixture showed the least competitive ability by dropping to 9.0 percent blue aleurone in 1956. All years, except 1953, showed an average loss in percent blue seed with the losses in 1955, 1956 and 1957 being significant. The greatest loss in percent blue seed was in 1955 with an average drop of 6.9 percent.

Table IX. The percent blue aleurone seed obtained from 50-50 mixtures compounded in 1953 from blue and white aleurone isogenic lines and grown in successive years at Moccasin from 1953 through 1957.

Isogenic Lines	Percent Blue Aleurone in Mixture				
	1953	1954	1955	1956	1957
31-18	54.0	70.0**	47.3	48.7	42.0
32-18	48.7	49.7	43.7	32.7**	25.7**
33-18	71.3**	59.0	60.0**	56.3	52.3
34-18	52.3	54.0	44.0	52.7	51.0
35-26	48.7	45.0	42.7	30.0**	49.7
36-26	49.7	48.0	40.7	42.7	40.7
37-26	46.0	40.0*	39.3*	54.7	34.7**
38-26	56.3	48.3	37.7*	23.7**	29.7**
52-5396	47.3	45.3	48.3	43.0	40.3
52-5397	51.7	48.7	40.3	33.3**	42.3
52-5398	51.3	48.3	40.3	47.0	41.0
Titan	45.3	40.3	29.0**	9.0**	11.3**
Average	51.9	49.7	42.8**	39.5**	38.4**

* Significant at the 5 percent level from the expected.

** Significant at the 1 percent level from the expected.

Table X represents the shifts in percent blue aleurone. Using the percentages obtained the previous year as the expected, a chi-square test was conducted on all figures in the table, except the averages, to determine if a significant gain or loss in percent blue seed occurred. The loss recorded in 1955 was the only year with a significant shift in blue seed as determined by student's pairing method of analysis.

Natural Selection for Aleurone Color in Bulked Hybrids

The results from the five bulked hybrids grown at Moccasin (Table XI) show considerable variation from the expected percentages. According to the chi-square test, Glacier x Titan had the best fit for the expected ratio of 9:7. Trebi-Dryland x Glacier had three generations which deviated from the expected, while the other four generations were normal shifts. Glacier x Manchuria had four generations in sequence that were below the expected percentage; however, in 1957 it was within the fiducial limits of the expected percentage. Glacier x Mars and Glacier x Colsess did not fit the expected ratios. Glacier x Mars ranged above the expected in percent blue seed while Glacier x Colsess was below.

Table XII presents the shifts in percent blue aleurone between generations. The figures shown are the percent above or below in percent blue seed from the expected after the deviations from a normal shift had been removed. This was calculated by determining the expected drop in percent blue

Table X. Yearly shifts in percent blue aleurone from 50-50 mixtures compounded in 1953 from blue and white aleurone isogenic lines and grown at Moccasin from 1953 through 1957.

Isogenic Lines	Yearly Shifts in Percent Blue Aleurone				
	1953	1954	1955	1956	1957
31-18	≠ 4.0	≠16.0**	-22.7**	≠ 1.4	- 6.7
32-18	- 1.3	≠ 1.0	- 6.0	-11.0*	- 7.0
33-18	≠21.3**	-12.3**	≠ 1.0	- 3.7	- 4.0
34-18	≠ 2.3	≠ 1.7	-10.0*	≠ 8.7	- 1.7
35-26	- 1.3	- 3.7	- 2.3	-12.7*	≠19.7**
36-26	- 0.3	- 1.7	- 7.3	≠ 2.0	- 2.0
37-26	- 4.0	- 4.0	- 0.7	≠15.4**	-20.0**
38-26	≠ 6.3	- 8.0	-10.6*	-14.0**	≠ 6.0
52-5396	- 2.7	- 2.0	≠ 3.0	- 5.3	- 2.7
52-5397	≠ 1.7	- 3.0	- 8.4	- 7.0	≠ 9.0
52-5398	≠ 1.3	- 3.0	- 8.0	≠ 6.7	- 6.0
Titan	- 4.7	- 5.0	-11.3*	-20.0**	≠ 2.3
Average	≠ 1.9	- 2.2	- 6.9**	- 3.3	- 1.1

* Significant at the 5 percent level from the expected.

** Significant at the 1 percent level from the expected.

Table XI. The percent blue aleurone kernels in five bulked hybrids when grown at Moccasin from 1948 through 1957.

Year Grown	Percent Blue Aleurone in Each Generation				
	Glacier x Manchuria 3:1 Ratio	Glacier x Mars 9:7 Ratio	Trebi-Dryland x Glacier 3:1 Ratio	Glacier x Colsess 3:1 Ratio	Glacier x Titan 9:7 Ratio
Percent Blue Planted	47.0	42.7	47.3	21.3	35.0
1948	47.3	51.3**	49.0		
1952	43.7	54.6**	43.7	33.3**	27.7
1953	39.3*	59.6**	40.0*	24.0**	32.7
1954	38.3*	64.7**	42.0	23.7**	26.0
1955	32.0**	57.3**	39.7*	17.3**	22.3
1956	33.7**	46.7**	34.7*	25.7**	21.0
1957	40.0	51.7**	41.3	32.0**	31.0

* Significant at the 5 percent level from the expected ratio.
** Significant at the 1 percent level from the expected ratio.

NOTE:
Crosses with Manchuria, Mars and Trebi-Dryland ranged in seed generations from F_6 through F_{12}.

Glacier x Colsess from F_6 through F_{11}.

Glacier x Titan from F_5 through F_{10}.

Table XII. Generation shifts in percent blue aleurone kernels from five bulked hybrids when grown at Moccasin from 1948 through 1957.

Year Grown	Deviations, as gain or loss of percent blue from the expected					
	Glacier x Manchuria	Glacier x Mars	Trebi-Dryland x Glacier	Glacier x Colsess	Glacier x Titan	Average
1952	- 2.9	≠ 4.8	- 4.6			- 0.45
1953	- 4.1	≠ 5.9	- 3.4	- 8.8	≠ 6.6	- 0.63
1954	- 0.9	≠ 5.6	≠ 2.2	- 0.1	- 5.7	≠ 0.18
1955	- 6.2	- 7.1	- 2.2	- 6.3	- 3.3	- 4.18**
1956	≠ 1.7	-10.5*	- 5.0	≠ 8.4*	- 1.1	- 1.08
1957	≠ 6.3	≠ 5.1	≠ 6.6	≠ 6.3	≠10.1*	≠ 5.73**
Average	- 1.02	≠ 0.63	- 1.07	- 0.08	≠ 1.10	- 0.09

* Significant shift at the 5 percent level.

** Significant shift at the 1 percent level.

for that generation and applying this percentage to the observed with the difference recorded in this table as generation shift. Although two of the crosses did not fit the expected ratio, shifts between generations were quite normal with only three shifts deviating from the expected. For a perfect shift all averages should be zero. The gain or loss in percent blue for all hybrids between years shows a significant loss in 1955, a year favoring natural selection for white seed, and a significant gain in 1957, a year favoring natural selection for blue seed.

These same hybrids were grown at Bozeman (Table XIII) during the same years and generations as at Moccasin. Glacier x Titan and Glacier x Manchuria performed as expected while the other three hybrids did not fit their respective ratios. Trebi-Dryland x Glacier was the only cross which did not perform at Bozeman as at Moccasin.

The shifts between generations (Table XIV) were calculated in the same manner as for Moccasin. At Bozeman the significant shifts were more numerous than at Moccasin; however, the accumulations of these shifts for each hybrid was nonsignificant. When comparing all the hybrids on a yearly basis two significant losses in percent blue aleurone were obtained, one in 1953 and the other in 1957. These two losses in percent blue at Bozeman did not occur at Moccasin for the same years.

Table XIII. The percent blue aleurone kernels in five bulked hybrids when grown at Bozeman from 1945 through 1957.

Year Grown	Percent Blue Aleurone in Each Generation				
	Glacier x Manchuria 3:1 Ratio	Glacier x Mars 9:7 Ratio	Trebi-Dryland x Glacier 3:1 Ratio	Glacier x Colsess 3:1 Ratio	Glacier x Titan 9:7 Ratio
1945	58.3	68.0**	32.7**		
1946	52.7	52.0**	45.7*	43.0**	
1947	47.0	42.7**	47.3	41.3**	
1948	44.7	46.3**	34.7**	21.3**	35.0
1952	55.7	46.7**	39.7	46.3	31.7
1953	43.7	41.0**	29.3**	35.3**	19.7
1954	56.7	42.0**	29.7**	25.0**	15.7*
1955	61.3*	52.3**	33.3**	24.3**	13.3**
1956	58.0	43.0**	33.3**	25.3**	20.7
1957	51.3	38.0**	29.7**	21.3**	25.3

* Significant at the 5 percent level from the expected ratio.
** Significant at the 1 percent level from the expected ratio.

NOTE:
Crosses with Manchuria, Mars and Trebi-Dryland ranged in seed Generation from F_3 through F_{12}.

Glacier x Colsess from F_3 through F_{11}.

Glacier x Titan from F_4 through F_{10}.

Table XIV. Generation shifts in percent blue aleurone kernels from five bulked hybrids when grown at Bozeman from 1945 through 1957.

Year Grown	Deviations, as gain or loss of percent blue from the expected					Average
	Glacier x Manchuria	Glacier x Mars	Trebi-Dryland x Glacier	Glacier x Colsess	Glacier x Titan	
1946	≠ 0.2	- 3.1	≠16.3**			≠ 4.47
1947	- 2.8	- 3.6	≠ 4.1	≠ 2.6		≠ 0.08
1948	- 0.9	≠ 6.1	-11.2*	-17.7**		- 5.93
1952	≠11.7*	≠ 1.8	≠ 5.5	≠25.6**	≠ 0.2	≠ 8.96
1953	-11.6*	- 5.0	-10.1*	-10.3*	-10.2*	- 9.44**
1954	≠13.2**	≠ 1.3	≠ 0.5	-10.0*	- 3.4	≠ 0.32
1955	≠ 4.7	≠10.5*	≠ 3.7	- 0.6	- 2.2	≠ 3.22
1956	- 3.2	- 9.2	≠ 0.03	≠ 1.1	≠ 7.5*	≠ 0.76
1957	- 6.7	- 5.0	- 3.6	- 4.0	≠ 4.6	- 2.94**
Average	≠ 0.51	- 0.69	≠ 0.58	- 1.66	- 0.58	- 0.37

* Significant shift at the 5 percent level.

** Significant shift at the 1 percent level.

To obtain additional data concerning natural selection for blue and white aleurone seed, plantings for two years were made at three other locations in the state. (Table XV) Bozeman seed was used as the seed source. At Huntley and Creston the hybrids were grown under both dryland and irrigated conditions. Since only two years data were obtained, no general trends for natural selection of blue and white aleurone seed can be detected. The chi-square test was used in this table to obtain significant deviations from the expected ratio for the two generations. Glacier x Mars, Trebi-Dryland x Glacier and Glacier x Colsess did not fit their respective ratios at all locations, while Glacier x Manchuria shows a better fit under irrigation than under dryland. Glacier x Titan, as at Moccasin and Bozeman (Tables XI and XIII), showed a good fit.

Table XVI presents the generation shifts in percent blue at the various locations. These shifts were determined in the same manner as for Tables XII and XIV. When comparing the accumulated gains or losses of each hybrid under dryland or irrigation, only one accumulation was significant. That was Glacier x Mars under dryland, which had a significant gain. When the accumulated gain or loss by years is compared, Bozeman showed a significant loss in percent blue in 1953 while Sidney irrigated and Huntley dryland had gains in 1952 and 1953 respectively.

Table XV. The percent blue aleurone kernels in five bulked hybrids when grown at five locations under irrigated and dryland conditions in 1952 and 1953 from seed grown at Bozeman.

		Percent Blue Aleurone				
		Glacier x Manchuria F_6	Glacier x Mars F_6	Trebi-Dryland x Glacier F_6	Glacier x Colsess F_5	Glacier x Titan F_4
Percent Blue Planted		44.7	46.3	34.7	21.3	35.0
Year	Location					
	Irrigated					
1952	Bozeman	55.7	46.7**	39.7*	46.3	31.7
1953		43.7	41.0**	29.3**	35.3**	19.7*
1952	Huntley	41.0*	50.0**	25.7**	34.7**	38.0**
1952	Creston	41.3	59.7**	27.7**	28.3**	23.0
1953		38.0**	52.7**	37.3**	33.7**	28.0
1952	Sidney	45.7	46.0**	39.3*	24.7**	37.7**
1953	" (early)***	51.0	45.7**	29.7**	25.3**	30.0
1953	" (late) ***	42.7	47.0**	34.0**	25.0**	35.0
	Dryland					
1952	Moccasin	38.0**	52.3**	40.0*	33.3**	27.7
1953		39.3**	59.6**	40.0*	24.0**	32.7
1952	Huntley	27.7**	46.0**	30.0**	21.0**	24.0
1953		44.7	58.3**	28.0**	34.7**	37.0*
1952	Creston	38.0**	45.7**	30.3**	36.7**	26.7
1953		33.3**	47.7**	31.7**	25.3**	22.6

* Significant at the 5 percent level from the expected.
** Significant at the 1 percent level from the expected.
*** Early and late plantings.

Table XVI. Generation shifts in percent blue aleurone kernels in five bulked hybrids when grown at five locations under irrigated and dryland conditions during 1952 and 1953.

Year	Location	Deviations, as gain or loss in percent blue from expected: Glacier x Manchuria	Glacier x Mars	Trebi-Dryland x Glacier	Glacier x Colsess	Glacier x Titan	Avg.
	Irrigated						
1952	Bozeman	≠11.7*	≠ 1.8	≠ 5.5	≠25.66**	≠ 0.2	≠ 8.96
1953		-11.6*	- 5.0	-10.1*	-10.3*	-10.2*	- 9.44**
1952	Huntley	- 3.0	≠ 5.1	- 8.5	≠14.0**	≠ 6.8	≠ 2.89
1952	Creston	- 2.7	≠14.8**	- 6.5	≠ 7.6	- 8.2	≠ 1.00
1953		- 2.7	- 5.8	≠ 9.9*	≠ 6.0	≠ 5.7	≠ 2.62
1952	Sidney	≠ 1.7	≠ 1.1	≠ 5.1	≠ 4.0	≠ 6.5	≠ 3.82*
1953	(E)***	≠ 5.8	≠ 1.1	- 9.2	≠ 1.1	- 6.6	- 1.56
1953	(L)***	- 2.5	≠ 2.4	- 4.9	≠ 0.8	- 1.6	- 1.16
	Average	- 0.41	≠ 1.94	- 2.34	≠ 6.10	- 0.89	≠ 0.88
	Dryland						
1952	Moccasin	- 6.0	≠ 7.4	≠ 5.8	≠12.6**	- 3.5	≠ 3.26
1953		≠ 1.7	≠ 8.3	≠ 0.4	- 8.6	≠ 5.8	≠ 1.52
1952	Huntley	-16.7**	≠ 1.1	- 4.2	≠ 0.3	- 7.2	- 5.34
1953		≠17.3**	≠13.2**	- 1.7	≠14.1**	≠13.7**	≠11.32*
1952	Creston	- 6.0	≠ 0.8	- 3.7	≠16.0**	- 4.5	≠ 0.52
1953		- 4.3	≠ 2.9	≠ 1.7	-10.7*	- 3.3	- 2.74
	Average	- 2.33	≠ 5.62*	- 0.28	≠ 3.95	≠ 0.22	≠ 1.44

* Significant shift at the 5 percent level.
** Significant shift at the 1 percent level.
*** Early and late plantings.

DISCUSSION

When the isogenic lines of blue and white aleurone seed were compared only small differences were found in plant height, date of heading, tillering, percent protein and kernel weight during the period tested at Moccasin and Bozeman. Test weight differences were small but in most cases this difference was consistently in favor of the blue seed. Since the preliminary information indicates the blue seed shows a tendency to have a higher degree of skinning, this may account for the higher test weight. No differences in yield were obtained over a period of years. However, in certain years the blue aleurone seeds yielded higher than the white, while the reverse would happen in others. The bulked isogenics showed a consistently high yield for blue aleurone at Bozeman in 1953 and consistently low yield in 1954. Since these lines were bulked in the fourth generation, linked genes could be making this difference.

The 50-50 mixtures grown at Moccasin show a natural selection against blue aleurone seed. This significant loss in blue seed occurred after the third year. Since these mixtures were made from isogenic lines which had been selfed for 18 and 26 years, linkages should have no effects. Therefore, this natural selection is probably caused by pleotropism or very closely linked genes. In 1955 at Moccasin, which was the year with the greatest loss in blue seed, all top growth

was destroyed by hail on June 26 when the plants were in the jointing stage; however, the plants recovered and produced seed. If the loss in blue seed was due to non-development of color because of lateness in maturity, the percentages should have been higher the following year. Since this was not the case, other factors must have been involved.

Comparing the results of the five bulked hybrids grown at Moccasin and Bozeman, Glacier x Mars and Glacier x Colsess did not fit their respective ratios at either location. Trebi-Dryland x Glacier had a good fit at Moccasin but a poor fit at Bozeman. Glacier x Titan had a good fit at both locations. It is suspected that linked genes account for these differences when testing for natural selection in a bulked population. Colsess, for example, is a blue seeded variety with hoods and one color factor linked. This would cause a decrease in percent blue seed if there was a natural selection which did not favor the hooded types. Another factor which may contribute to the loss in blue seed is the amount of shattering. Since the blue seed shows a higher degree of skinning, there is a possibility that it will have higher amounts of shattering in the field.

When the bulked hybrids were grown at five locations, no trends for selection could definitely be detected. However, differences between locations did exist, which was the purpose of this test. From the data collected, Glacier x

Manchuria showed a greater loss of blue seed under dryland conditions than under irrigation. Glacier x Mars gained in percent blue seed under both dryland and irrigation with the gain on dryland being significant. All locations expect Bozeman showed a loss in blue color for Trebi-Dryland x Glacier. Glacier x Colsess gained in percent blue seed under dryland and irrigation. Glacier x Titan showed a loss of blue seed under irrigation and a gain on dryland.

If selections were to be made for competitive types based on seed color in the five bulked hybrids, it appears that only blue aleurone plants would be selected from the Glacier x Mars cross, since the percent blue seed was higher than the expected at both locations. The white aleurone plants of Glacier x Colsess would be selected for both locations since the blue seed showed the least competitive ability. At Moccasin either blue or white seeded plants could be selected from the Trebi-Dryland x Glacier cross, but only the white seeded plants at Bozeman. In the Glacier x Titan cross either seed color could be selected for both locations since this cross performed as expected.

In 1955 at Moccasin, white seeded showed significant gains in both the bulked hybrids and the 50-50 mixtures while the white bulked isogenics yielded approximately one bushel per acre more. Such relationships were not consistent however, perhaps due to the difficulty of measuring yield.

Of the five crosses tested, it was noticed that Glacier x Titan had the most variability in seed color, especially when grown under irrigation. Many seeds were found with only a small patch of color near the germ end, which could cause variations in seed counts.

SUMMARY

No differences in plant height, date of heading, tillering, percent protein or seed weight were obtained when isogenic lines of blue and white aleurone seed color were compared.

Test weight differences were small but in most cases favored the blue seed.

Blue seed on the average had a higher percent of skinned kernels after threshing than the white, which may have caused the higher test weights of the blue seed.

Yields of isogenic lines showed no significant differences due to seed color. Yearly differences did exist which favored the blue seed in some years and white the others.

The 50-50 mixtures of blue and white aleurone seed showed a natural selection against blue color when grown at Moccasin. The year in which the greatest loss in blue seed occurred a hail storm early in the growing season destroyed all top growth. This caused the resulting regrowth to produce significantly less blue aleurone seed than the previous year. During the five years these mixtures were grown, the average loss in blue seed was 11.6 percent.

Five bulked hybrids were grown at Moccasin and Bozeman through various generations to determine the natural selection for aleurone seed color. Two of the hybrids, Glacier x Colsess and Glacier x Mars, did not fit their respective ratios

at either location. Glacier x Colsess ranged below the expected in percent blue seed while Glacier X Mars ranged above. Trebi-Dryland x Glacier had a better fit to its expected ratio at Moccasin than at Bozeman. Glacier x Titan and Glacier x Manchuria performed as expected at both locations.

Five bulked hybrids were grown for two years at five locations using a common seed source to determine their natural selection under dryland and irrigated conditions. Two years data was not sufficient to determine natural selection trends. The data collected during this period, however, indicate differences could exist between locations when grown under dryland and irrigation.

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