INHERITANCE OF A MELANINLIKE PIGMENT IN THE GLUMES AND CARYOPSES OF BARLEY [1]

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INTRODUCTION

Although black barleys are rarely grown commercially in the United States, they have been used in hybridization for many years. Many valuable characters, including smooth awns, have been transferred from varieties with black hulls and caryopses. A large number of varieties and strains of black barley have been introduced or selected from crosses involving black and white color. The factor pair responsible for the production of black pigment in barley has been studied by a number of workers, but no reference has been made to the various degrees of black coloration that exist. This paper presents the results of a study of the inheritance of the black or melaninlike pigment that occurs in various intensities in the glumes and caryopses of barley (*Hordeum* spp.) seed in some 8,000 F_3 progenies from more than 50 crosses.

REVIEW OF LITERATURE

The character black vs. white flowering glumes has been reported to be controlled by a single factor pair in several publications reviewing genetic studies in barley. Black has been considered completely dominant over white, giving F_2 ratios of 3 black to 1 white. Tschermak (*13*)[2], Biffen (*1*), Griffee (*4*), Hayes et al. (*6*), Ubisch (*14*), Hor (*7*), Robertson (*10*), Sigfussen (*12*), Buckley (*2*), and Kuckuck (*9*) have all reported monogenetic ratios of black vs. white caryopses or glumes. Harlan (*5*) reported the black color to be the result of a melaninlike pigment found in the lemma, palet, and pericarp only, and the color in the aleurone to be due to anthocyanin pigments. A special study of color inheritance in barley and possible linkage relationships has been reviewed and discussed by Buckley (*2*). He concluded that black pericarp is always associated with black lemma, that either the same gene is responsible for the coloring in both the lemma and the pericarp or that very close linkage between two separate genes must exist. No linkage was found between the genes controlling black vs. white glume and pericarp color and any of the anthocyanin factors. The genes for black and white color were reported by Buckley to be inherited independently when tested with long vs. short rachilla

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[2] Italic numbers in parentheses refer to Literature Cited, p. 28.

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hairs, hooded vs. awned, and hulled vs. hulless. Gaiser (*3*) had previously reported that black lemma was linked with the texture of awn, rachilla hairs, color of caryopsis, one albino factor, and one factor for the resistance to *Helminthosporium*. Hor (*7*) reported black lemma linked with texture of awn and length of rachilla hairs. Buckley (*2*) found three linkage groups and in addition suggested that the factor pairs black vs. white and hulled vs. naked caryopsis represented two additional linkage groups. More recent work by Robertson, Wiebe, and Immer (*11*) indicates that the factor pair black vs. white is in linkage group No. 2 along with $A_t\, a_t$, an albino factor found in Trebi I. They list a factor responsible for the production of a third outer glume in the same linkage group with black vs. white lemma. Ivanova (*8*) describes this third outer glume as a new character in barley. Seed of this particular barley has not been available to workers in the United States, so that the genes controlling black vs. white and $A_t\, a_t$ are the only genes in linkage group No. 2 available to them.

Sigfussen (*12*) has pointed out that the black pigment does not express itself until just before ripening, making it difficult to classify immature plants.

No reference was found in the literature to studies of crosses between colored parents that differed in the intensity of black pigment in the lemma and caryopsis.

MATERIAL AND METHODS

A number of varieties and strains containing anthocyanin and the melaninlike pigment were used in this study.[3] A few white-glumed varieties thought by some workers to produce occasional dark types also were included in an observational nursery. These varieties have been grown for periods of 7 to 9 years. Head selections also have been made from them, some of which appeared to show greater color variation than others.

After preliminary studies all of the varieties were arbitrarily classified into five groups based on the intensity of the melaninlike pigment. Two of the varieties in each group breeding most uniformly for color intensity were chosen for the genetic studies. These groups were designated as (1) dense black, (2) black, (3) medium black, (4) gray, and (5) white.

Although the climatic conditions were generally favorable for color classification, it was difficult in some crosses. Attempts were made to extract the black pigment so that the extracts might be used as a basis for classifying color, but with no success.

Most of the more than 100 black-glumed varieties and strains were found to be relatively stable and uniform for color intensity. A few varieties such as Blackhull had previously been reported to contain some individuals with lighter colored glumes and caryopses than the variety as a whole. In a study of a large number of head rows of Blackhull (C. I.[4] 878), a light-colored type was isolated and found to breed true. This light-colored strain of Blackhull (C. I. 878) was used as one of the parent strains in the gray group. A number of the color variants found in the original Blackhull variety were definitely

[3] Obtained from H. V. Harlan, principal agronomist, in charge of barley investigations, Division of Cereal Crops and Diseases, Bureau of Plant Industry.
[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

identified as mixtures or hybrid segregates. A few other varieties, including C. I. 875 and 3204, contained two or more color classes which, when isolated, have continued to breed true for dark and light glumes, respectively. There is no evidence from a 9-year study that any of the varieties grown under Utah conditions have thrown mutants of either lighter or darker color intensity than was found in the original material after pure color types were established.

The varieties used in this study to represent the five color-intensity groups are shown in table 1, and some of them are illustrated in figure 1. Group 1, arbitrarily classed as dense black, is characterized by its

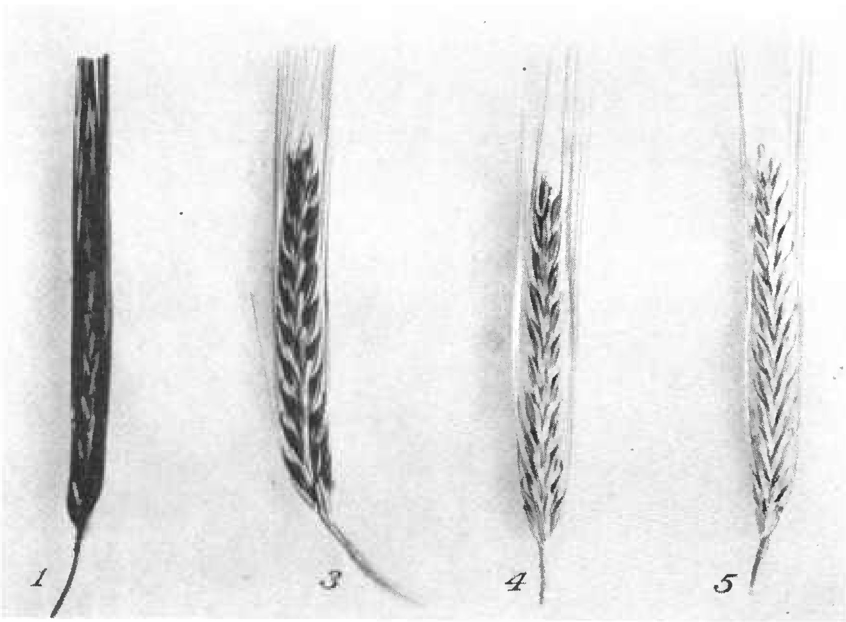

FIGURE 1.—Spikes of barley illustrating four of the five color-intensity groups: Group 1, dense black (*BB*); group 3, medium black ($B^{mb}B^{mb}$); group 4, gray (B^gB^g); and group 5, white (*bb*).

extreme black pigmentation in the flowering glumes, pericarp, and awns. Blackhull (C. I. 878) and Black Algerian (C. I. 708) are representative of this group. Group 2, classed as black, appeared to be slightly lighter in color intensity than group 1, the awns often showing a yellowish color. Bucher (C. I. 1461), Donjon (C. I. 1264), an unnamed variety (C. I. 3910–1), and Jet (C. I. 2222) are listed as representative varieties in group 2. Group 3, described as medium black, shows slightly less pigmentation than group 2. The lateral florets are generally light-colored in the two-rowed and intermediate segregates found in this group. C. I. 4376 and 2970 are representative varieties. Group 4 is best described as gray in color and generally shows sufficient contrast to the other groups to be easily identified. A light-colored type isolated from Blackhull (C. I. 878) and Dentil (C. I. 1260) was chosen to represent this group. In group 5 are included varieties with white glumes and pericarp, of which C. I. 4354 and Blackhull 1178 (C. I. 5679) are representative.

All combinations among 2 of the above varieties from each of the 5 color classes were made. The total of 45 crosses involved combinations of all fertility classes such as two-rowed, six-rowed, intermedium, and deficiens, as well as other character differences. Other crosses also were made for additional information on the inheritance of black color.

TABLE 1.—*Color-intensity groups and descriptions of the varieties selected to represent them*

Group and variety	C. I. No.	Varietal characters			
		Glume and pericarp color	Awn color	Fertility type of lateral florets	Caryopsis
1, Dense black:					
Blackhull	878	Dense black	Black	Deficiens	Hulled.
Black Algerian	708	do	do	Vulgare	Do.
2, Black:					
Jet	2222	Black (lateral florets gray).	Dark to light	Intermedium	Hulless.
Bucher	1461	Black	do	Vulgare	Hulled.
Unnamed	3910-1	Brown to black	do	do	Hulless.
Donjon	1264	Black	do	do	Hulled.
3, Medium black:					
Unnamed	4376	Dilute black	Gray	Deficiens	Do.
Do	2970	Dilute black (laterals yellow).	do	Distichon	Do.
4, Gray:					
Blackhull (light-colored).	878	Gray	Colorless	Deficiens	Do.
Dentil	1260	do	do	Distichon	Do.
5, White:					
Unnamed	4354	White	do	Vulgare	Do.
Blackhull selection 1178.	5679	do	do	Intermedium	Do.

Experience indicated that observations on the primary spikes were more reliable than those on spikes chosen at random and that all material should be thoroughly ripened before harvest in order to classify color density accurately. This latter fact also was pointed out by Sigfussen (*12*).

All crosses were studied through the F_3 generation as a check on F_2 classifications. Numerous doubtful types were continued in head rows for 2 or 3 years longer or until they could be identified more positively. Normal color densities failed to develop in seasons of excessive moisture or cloudy weather. Such seasons sometimes necessitated the study of large populations in the F_4 and F_5 generations in order to determine the breeding behavior of the F_2 plants.

The material was space-planted in rows 8 to 17 feet long. A large part of the material was studied in the field.

EXPERIMENTAL RESULTS

COLORED × WHITE

Pigmented varieties representing the four color-intensity groups were crossed with varieties having white or colorless glumes and pericarps. In each case F_2 ratios of 3 black to 1 white resulted, as is shown in table 2. This is in agreement with the ratios generally obtained by previous workers. No intermediate color classes could be found, regardless of the intensity of the black parent used.

TABLE 2.—*F_2 segregations in crosses between colored and white varieties*

Cross	Genetic symbols	F_2 segregation based on F_3 breeding behavior		Deviation / Standard error
Dense black × white	$BB \times bb$	Dense black	White	
Blackhull × Blackhull selection 1178		230	75	0.17
Blackhull × C. I. 4354		340	113	.03
Black Algerian × C. I. 4354		211	80	.97
Black Algerian × Blackhull selection 1178.		156	64	1.40
Black × white	$BB \times bb$	Black	White	
C. I. 3910–1 × C. I. 4354		527	185	.61
Bucher × C. I. 4354		205	71	.28
Jet × C. I. 4354		353	124	.50
Donjon × C. I. 4354		110	46	1.29
C. I. 3910–1 × Blackhull selection 1178		257	93	.68
Jet × Blackhull selection 1178		153	72	2.43
Donjon × Blackhull selection 1178		51	21	.82
Medium black × white	$B^{mb}B^{mb} \times bb$	Medium black	White	
C. I. 2970 × C. I. 4354		262	99	1.25
C. I. 2970 × Blackhull selection 1178		194	83	1.91
Gray × white	$B^gB^g \times bb$	Gray	White	
Blackhull gray segregate × C. I. 4354		216	79	.71
Dentil × C. I. 4354		253	96	1.08
Dentil × Blackhull selection 1178		326	126	1.41
White × white	$bb \times bb$	No segregation		
Blackhull selection 1178 × C. I. 4354				
Total		3,844	1,427	

It should be noted that the white segregates are in excess in all but two of the crosses. In all crosses studied the deviation in favor of light-colored segregates is still more pronounced, giving 6,166 dark to 2,234 light, with a deviation of 134 ± 40. No satisfactory explanation has been found for this behavior.

COLORED × COLORED

Tabulated results of the segregations from crosses of colored × colored are shown in table 3.

DENSE BLACK × DENSE BLACK

No indication of any segregation could be observed in the F_2 and later generations of crosses between dense black and dense black varieties.

DENSE BLACK × BLACK

Six crosses of dense black × black were studied, but classification was difficult. These two color groups differ so slightly in intensity of coloration, which expresses itself largely in the awns, lateral florets, and culms, that they may be included in one group.

DENSE BLACK × MEDIUM BLACK

With favorable seasonal conditions the segregating generations from the cross of dense black × medium black could be classified. A single-factor difference explains the results obtained. Varieties or segregates of the six-rowed type consistently have a slightly lighter coloration than those of the non-six-rowed types. When the color classes of the parents were similar, classification of the segregates was more difficult in the progeny from crosses involving six-rowed by non-six-rowed varieties than when both parents were of like fertility.

TABLE 3.—*F_2 segregations in crosses between pigmented varieties representing the different color-intensity groups*

Cross	Genetic symbols	F_2 segregation based on F_3 breeding behavior		Deviation / Standard error
Dense black × dense black	$BB \times BB$			
Blackhull × Black Algerian		No segregation		
Dense black × black	$BB \times BB$			
Blackhull × Bucher		No segregation		
Blackhull × Jet		do		
Black Algerian × Jet		do		
Black Algerian × C. I. 3467		do		
Blackhull × C. I. 3910–1		do		
Black Algerian × C. I. 3910–1		do		
Dense black × medium black	$BB \times B^{mb}B^{mb}$	Dense black	Medium black	
Blackhull × C. I. 2970		176	72	1.47
Black Algerian × C. I. 2970		Difficult to classify		
Dense black × gray	$BB \times B^{g}B^{g}$	Dense black	Gray	
Blackhull × C. I. 1260		215	80	.84
Black Algerian × Blackhull gray segregate.		176	70	1.25
Black Algerian × Dentil		74	23	.29
Black × black	$BB \times BB$			
Donjon × C. I. 3910–1		No segregation		
Jet × C. I. 3910–1		do		
C. I. 4363 × Jet		do		
Jet × Bucher		do		
Black × medium black	$BB \times B^{mb}B^{mb}$			
Jet × C. I. 2970		Difficult to classify		
C. I. 3910–1 × C. I. 2970		do		
Black × gray	$BB \times B^{g}B^{g}$	Black	Gray	
Bucher × Blackhull gray segregate		187	67	.51
C. I. 3910–1 × Blackhull gray segregate.		228	87	1.07
Donjon × Blackhull gray segregate		125	43	.18
C. I. 3467 × Dentil		166	42	1.60
Bucher × Dentil		188	57	.63
C. I. 3910–1 × Dentil		215	68	.38
Medium black × medium black	$B^{mb}B^{mb} \times B^{mb}B^{mb}$			
C. I. 2970 × C. I. 4376		No segregation		
Medium black × gray	$B^{mb}B^{mb} \times B^{g}B^{g}$	Medium black	Gray	
C. I. 2970 × Blackhull gray segregate		305	117	1.29
C. I. 4376 × Dentil		94	28	.52
C. I. 2970 × Dentil		173	53	.54
Gray × gray	$B^{g}B^{g} \times B^{g}B^{g}$			
Blackhull gray segregate × Dentil		No segregation		

DENSE BLACK × GRAY

Crosses involving dense black × gray were extremely clear-cut in their segregation. A monofactorial condition was suggested by all such crosses.

BLACK × BLACK

No indication of segregation was observed in any of the four crosses studied involving black × black. In C. I. 3910–1, a hulless variety, the pericarp was more densely colored at full maturity than the glumes.

BLACK × MEDIUM BLACK

Differences in the parents chosen for black and medium black could be seen when conditions for color development were favorable. Crosses involving these two groups, although indicating segregation, did not permit clear-cut separation in the F_2 or later generations.

BLACK × GRAY

Each of the six crosses studied indicated a single-factor difference between the color intensities black and gray. In seasons when bleaching was abnormal it was necessary to continue considerable material into the F_4 or F_5 generation in order to separate the classes.

MEDIUM BLACK × MEDIUM BLACK

No segregation was evident when two varieties of this color-intensity group were crossed.

MEDIUM BLACK × GRAY

Crosses between varieties of the medium black and the gray classes yielded monofactorial segregations in the F_2 generation.

GRAY × GRAY

A cross between C. I. 1260 × Blackhull (C. I. 878) (gray) showed no indication of segregation in later generations.

DISCUSSION

Although five color-intensity groups were described and studied, the problem would have been simplified if the group listed as black had been eliminated or combined with dense black. Great difficulty was encountered in classifying the plants in crosses involving black × dense black or black × medium black. It appears that the black group may differ slightly from either of these other dark-colored groups and that possibly still other color groups might be found by a positive method of determining color intensity. The medium-black group appears to be distinguishable from the other groups but is not too well established as yet.

Using only data from crosses in which the segregates could be classified by inspection, the results definitely establish three color groups, the dense black (*BB*), gray (B^gB^g), and white (*bb*). The factors responsible for the production of the melaninlike pigment appear to represent an allelomorphic series, since only monofactorial ratios were obtained from any combination showing color-intensity differences. In all crosses the darker color class showed complete dominance.

These studies also suggest that certain other varieties might give more clear-cut results in crosses.

The Dentil variety displayed a winter-grown habit in two seasons, which complicated the studies, but early seeding eliminated further difficulty in this respect.

Buckley (*2*) concluded that black pericarp is always associated with black lemma and that either the same gene is responsible for the coloring or close linkage exists. No segregates exhibiting recombinations of glume and pericarp colors were obtained in these experiments.

SUMMARY

No mutation or new off-color type was observed during a study of more than 100 varieties and strains of barley, although in some varieties individual plants segregated for two color classes.

Barley varieties were grouped into five color classes, based upon the intensity of the black melaninlike pigment in the glume and pericarp. These groups were (1) dense black, (2) black, (3) medium black, (4) gray, and (5) white. More than 50 crosses involving all group combinations were made and studied in the segregating generations. Eliminating group 2 (black), all crosses among the remaining four groups yielded monofactorial segregations in the F_2 generation. More refined classification might establish one or more additional color intensities.

The medium-black group appeared to be distinct from the others, but its relationship could not be established.

The data suggest an allelomorphic series of factors causing various color-intensity expressions. The factors definitely established are black (*BB*), gray (B^gB^g), and white (*bb*). The denser color was always completely dominant over the lighter one.

Despite attempts to isolate other color groups from the progeny of certain crosses, the parental types only were recovered in the homozygous condition in later generations.

Pigment formation in both the pericarp and the flowering glume appears to be controlled by a single series of allelomorphic genes.

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