A STUDY OF PLANT COLOR IN CRESTED WHEATGRASS, AGROPYRON CRISTATUM (L.) GAERTN.

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INTRODUCTION

Chlorophyll mutants have been noted in many agricultural crops and have proved useful in inheritance studies. There have been few inheritance studies in the wheatgrasses, partly because of a lack of distinctive characters. The presence of grey-green and bright-green spike colors in diploid crested wheatgrass Agropyron cristatum (L.) Gaertn., suggested that simple genetic differences might be responsible.

The frequencies of grey-green and bright-green plants in Fairway-type strains were noted and the inheritance determined from test crosses. An assessment was made to determine if spike color types were related to agronomic worth. This included an appraisal of heterozygous plants for heterosis effects. Preliminary observations were made of differences in chlorophyll pigments of the various color types.

LITERATURE REVIEW

Differences in green pigmentation of plants have been studied quite extensively in a wide variety of crops. The earliest work dates back to 1901 with the classical study of Oenothera by De Vries. In corn alone, 400 papers were published on chlorophyll mutants by 1933 (5).

In grasses, there are few studies of chlorophyll mutants reported in the literature. Nielsen and Smith (15) studied albino seedlings in selfed and outcrossed progenies of timothy. When plants were selfed there were no definite segregation ratios in the progeny, but a continuous series from 4.6 green : 1 albino to 572 green : 1 albino. Progenies of outcrosses ranged in ratios of green vs. albino from 1.8 : 1 to 244 : 1. On the basis of these two groups of segregation ratios they assumed that the type of inheritance was tetrasomic to hexasomic. They were also able to determine that seven to eight percent selfing occurred in timothy.

A dominant yellow mutant in bromegrass was used by Ghosh and Knowles (6) for inheritance studies in that species. The character was found to be tetrasomically inherited and was subsequently used in pollination distance studies.

Cuany and Kalton (4) used chlorophyll deficiency markers to determine genetic behavior in orchardgrass, Dactylis glomerata L. They found that the various chlorophyll mutants were tetrasomically inherited. Using these chlorophyll mutants as testers, it was found that up to 98 percent outcrossing occurred in that species.

In Paspalum, Bennett (3) found single gene inheritance of leaf

color. Paspalum glabratum arose as a dominant mutant of the dark green Paspalum floridanum. In crosses between the two species, the whitish-green color was dominant in the F_1 population. There was no hybrid vigour in the F_1 and the hybrids were fully fertile. The F_2 population gave a good fit to a 3 : 1 ratio for whitish-green vs. green plants. It was postulated on the basis of these results that the two species had similar genomes with only a single gene difference.

Lawrence, (14) at Swift Current studied plants with grey and green foliage color in intermediate wheatgrass, Agropyron intermedium (Host.) Beauv. These were scored for 15 agronomic characteristics such as height, leafiness, extent of creep, forage and seed yield. He found no significant correlations of foliage color with any of the 15 agronomic characters. He concluded that foliage color had no agronomic value, but could be used as a variety identification marker. To date there have been no reports of inheritance studies on foliage color of intermediate wheatgrass.

Highkin (8) studied eight chlorina mutants in barley. He used ether to extract chlorophyll from the leaves of these plants. Quantitative determinations of chlorophyll components were made on a spectrophotometer. One of the chlorina mutants did not show the normal 3 : 1 ratio for chlorophyll a vs. chlorophyll b. Chlorina mutants one, six and eight showed less total chlorophyll than normal green plants. Chlorina number three was phenotypically similar to chlorina one but had a higher chlorophyll content than the normal green plant used as a check. The effect of the mutant gene on chlorophyll formation was either on total chlorophyll or on the ratio of chlorophyll a vs. chlorophyll b.

MATERIALS AND METHODS

Description of grey spike color

Inheritance studies were carried out on diploid Fairway type plants with intense grey spike colors (Figures 1, 2, 3). These were first noted in 1958 in a strain test of crested wheatgrass. The pigment appeared to be in greatest concentration in the outer glumes of the spikelets, with lesser amounts in the rachis and upper portions of the lemmas. The lower portions of the lemmas were relatively free of pigment (Figure 1).

The grey spike color showed maximum expression between heading and flowering stages. The intensity of color decreased slightly at the time of flowering and very markedly afterwards. It was not determined whether temperature affected grey or green spike color, but it was noticed that grey and green spikes could be classified equally well under either greenhouse or field conditions. On plants whose spikes were a very distinct grey, the leaves also had a greyish cast, but generally plants could not be classified on the basis of leaf color alone.

There was considerable variation in the intensity with which the progeny of certain plants expressed their pigment. Generally, in the intercrosses of grey plants all the progeny appeared to be distinctly grey. However, the progenies of intercrosses of certain green plants or of grey x green crosses produced some plants that were an intermediate grey-green for spike color. For this reason, segregating generations were placed in three classes. The symbol G was assigned to plants with a normal green spike. GY was used to indicate a spike color intermediate between grey and green. The intermediate color

Fig. 1. Comparison of spike color types in crested wheatgrass 2X normal

A - C157Y Typical grey spike

B - C1G Typical green spike

C - C198Y Typical grey spike

Fig. 2. Typical grey and green spikes in crested wheatgrass 6X normal

A - C157Y Typical grey spike

B - C1G Typical green spike

Figure 1

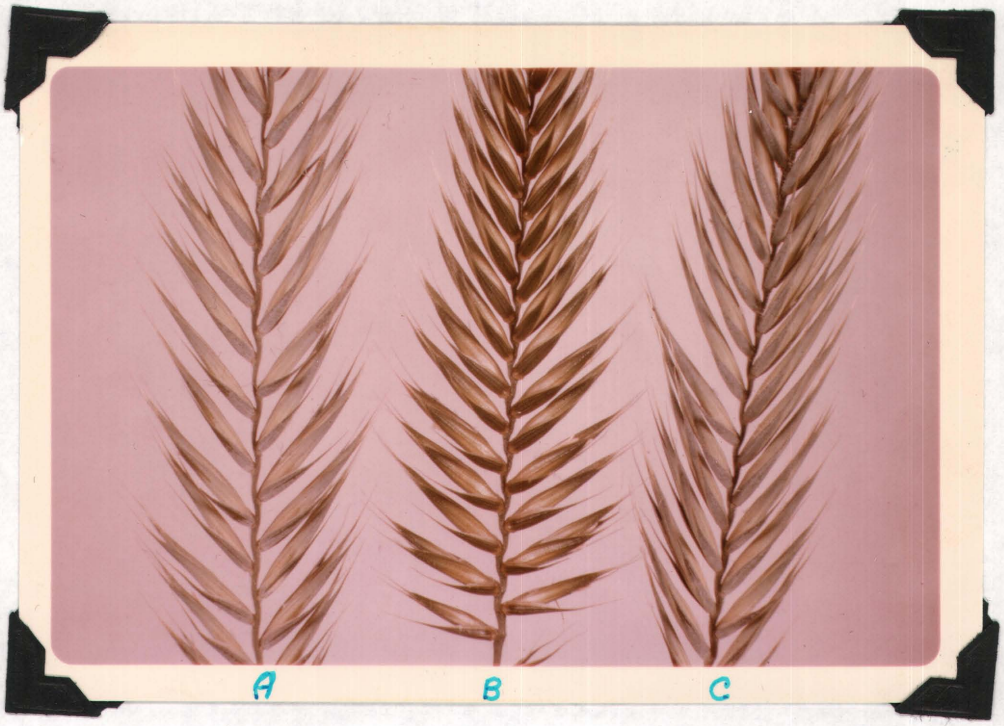

Figure 2

Fig. 3. Comparison grey and green crested wheatgrass progenies in the field.

A - Row with pure grey spike color

B - Row with pure green spike color

Figure 3

expression was much more prevalent in the progeny of certain plants than of others. The symbol Y was used to indicate the grey spike phenotype. Some introduced strains of crested wheatgrass were uniform for grey spike color. Occasionally the leaves of grey-spiked plants were of a glaucous-green color.

Grey spike frequencies

The frequency of plants with grey spikes in the Fairway variety was determined from space planted nurseries. The frequency of grey plants in Fairway was compared to frequencies of grey plants in newly-developed synthetics and composites. The synthetics and composites were formed from superior hay and seed yielding clones, with no regard given to spike color. The constituent clones were classified for spike color when inheritance studies were begun.

The synthetic 1 (syn. 1) was formed by harvesting in bulk the seed from a selected group of plants that were randomized, space planted and allowed to interpollinate under isolation. Synthetic 2 (syn. 2) was grown from the seed harvested from seeded rows of syn. 1 plants. The composites were formed by mixing equal quantities of polycross seed harvested from selected clones in polycross nurseries.

Grey spike frequencies of two composites and two synthetics were obtained from a replicated strain test. The plots of each strain in the test consisted of 11 plants and the plots were replicated six times.

Genetic studies

Plants with distinctive grey spikes were first noted in 1958,

in a strain test of crested wheatgrass. Five of these plants were interpollinated and all the progeny were found to have grey spike colors. Methods of pollen transfer were those described by Knowles and Horner (10). These authors also showed that crested wheatgrass was almost completely self-sterile. Therefore no emasculation was done in this study when controlled crosses were made.

More detailed studies were commenced in which green plants were intercrossed with greys to produce F_1 populations. F_1 plants were intercrossed to produce F_2 progenies. Twenty-five green parent plants in a polycross nursery were test-crossed to C198Y and C157Y recessive grey selections to determine whether these green plants were homozygous or heterozygous. Plants of grey-spike introductions were test-crossed to C198Y and C157 grey tester plants. This was done to determine whether the gene for grey spike color in the introductions was the same as the one causing grey spikes in the material currently studied.

When crosses were made, spikes of the female parent were enclosed in parchment bags prior to flowering. Controlled pollinations were done at the time of flowering. When mature, the bagged spikes were brought into the laboratory to be hand-threshed and cleaned. Seeds were germinated in moistened paper towels in April, then planted into greenhouse flats. Each flat contained 260 seedlings. In June, the seedlings were transplanted to the field with a mechanical tobacco planter. Plants were spaced two feet within rows and three feet between rows. Crested wheatgrass planted in June heads out the same year so that plants were classified for spike color in the year of planting.

Cytological studies

Spikes of grey and green parents and their F_2 progenies were collected for cytological studies. Collecting was done approximately one week after the spikes had completely emerged from the sheaths. Spikes were fixed in Carnoy's solution (6 ethanol : 3 chloroform : 1 glacial acetic acid) and later preserved in 70 percent ethanol. Temporary smear preparations of the anthers were made in acetocarmine and meiotic behavior in the P.M.C.s was observed.

Physiological studies

Quantitative determinations of chlorophyll a, chlorophyll b and carotene were made on spikes of grey and green plants and their F_2 segregates. The plants were taken from the field to the greenhouse in late October, by which time natural floral induction had taken place. These plants were brought into flower by placing them under appropriate conditions (11). They were planted into soil or vermiculite in plastic pots. No fertilizer was used. The potted plants were placed in a growth chamber under an 18-hour day. Cool-white fluorescent lamps supplemented with incandescent lamps provided constant intensity light of 2500 foot candles at plant height. The day and night temperatures were 69°F and 59°F respectively. Prior to pigment extraction the pots were uniformly watered. The method of pigment analysis was performed as outlined by Holm (9). A Unicam spectrophotometer with a 1 cm. absorption cell was used and optical density readings were taken at 440.5 mu, 644.0 and 662.0 mu. The concentrations of each pigment were obtained from the formulae:

$$Ca = 9.78\ D\ 662.0 - .99\ D\ 644.0$$

$$Cb = 21.4\ D\ 644.0 - 4.65\ D\ 662.0$$

$$Cc = 4.69\ D\ 440.5 - C\ (a + b)\ .268$$

where Ca, Cb and Cc represent milligrams of chlorophyll a, chlorophyll b and carotene respectively per litre of solution. Corrections were made for the weight of green material used and concentrations of pigments finally stated as micrograms (u.g.) of pigment per gram of fresh weight (F.W.).

Leaves from plants with grey and green spikes were also analyzed for contents of the three pigments.

Agronomic characteristics

(a) Parents

Agronomic data were obtained from clonal and polycross progeny tests conducted by the Research Station, Canada Department of Agriculture, Saskatoon and completed for the most part before spike color studies were begun by the author. As plant materials were classified for spike color they were evaluated according to these agronomic data.

Agronomic characteristics of grey and green parents were obtained from material grown in a polycross nursery of selected clones. These represented superior Fairway type plants selected from various sources on the basis of their apparent good forage and seed yielding characteristics. Following progeny evaluations the best ones were selected to produce the 16-clone synthetic S-55-65 and the 16-clone composite S-5566. Two smaller synthetics, S-5832 and S-5833 also were formed from the same 16 clones, with eight going into each synthetic strain.

There was a total of 213 clones in the polycross nursery. These

were arranged in plots of four plants spaced two feet by three feet in three replicates. Thirty-three of these clones were test-crossed to grey plants C157Y and C198Y to determine whether they were homozygous or heterozygous green or grey.

The plots were harvested for forage and seed yield. Forage yield was expressed as pounds of dry matter (D.M.) per plot, as an average of three replicates. Seed yield was expressed as grams per plot, and heights in inches, each as an average of three replicates. An unpaired "t" test was used to compare the homozygous green, heterozygous green, and homozygous grey group with respect to each agronomic characteristic.

(b) Progeny

Polycross progeny data likewise had been obtained by the Research Station, Canada Dept. of Agric., Saskatoon and Melfort Experimental Station before spike color studies were begun by the author. Therefore as the parents were classified for spike color the appropriate polycross progeny results were selected from existing data and applied to the parents in question.

Polycross seed harvested from 49 superior clones in the polycross nursery was used to seed polycross progeny tests at Saskatoon and Melfort in 1956. The test consisted of 49 entries arranged in a seven-by-seven simple lattice repeated design with six replicates. The plots were composed of three rows, twenty feet long and one foot apart. From 1957 to 1959 inclusive, data were obtained on hay yield, rate of recovery, lodging and heights. Seed yields were obtained in 1960. In 1961 the test at Melfort was grazed to determine whether animals had a preference for herbage with grey or green spikes. Six pure bred Rambouillet ewes were used for grazing. The test area was fenced and

grazing was started when the grass had begun to head-out. Grazing was discontinued at 36 days when preferences had been well established. Following grazing the stubble was harvested and yields of ungrazed residue expressed as pounds of dry matter (D.M.) per acre. A low yield of ungrazed residue for a strain was taken to indicate that a greater amount had been grazed off by the animals, that is, the strain had good palatability.

An analysis of variance was used to compare the three spike-color groups with respect to each agronomic characteristic. The three groups were composed of (1) polycross progenies from 10 homozygous green plants, (2) polycross progenies from 15 heterozygous green plants and (3) polycross progenies from 8 homozygous grey plants.

RESULTS

Frequency of grey spikes in Fairway-type strains

The frequencies of grey spikes in diploid crested wheatgrass strains are shown in Table 1. The frequency for the Fairway variety represents an average of five tests covering a three year period. The grey spike frequency in the Fairway variety was eight percent. The synthetic strains showed a frequency of grey spikes proportional to the frequency of grey-spiked parental plants. For example, S-5832 Syn. 1 with three-eighths basic grey clones showed approximately three times as many grey plants as S-5833 Syn. 1 in which only one-eighth of the basic clones was grey. Of the 16 basic clones used to form S-5565 and S-5566, four were grey and 12 were green. There is only a small difference in the frequency of grey spikes between the synthetic S-5565 and the composite S-5566 in both first and second generations. Composite S-5566 was formed from the reserve polycross seed from the same clones as made up the synthetic S-5565. Synthetic 2 showed a four percent increase in grey spikes over synthetic 1. The increase in frequency of grey spikes for composite 2 was five percent over composite 1. It appears that S-5565 and S-5566 attained equilibrium for grey spike frequency at about the same rate.

Table 2. Segregation of intercross progenies following crosses between parents with grey and green spikes in the greenhouse.

Cross	Number of plants in F_1		
	Green (G)	Intermediate (GY)	Grey (Y)
Intercrosses of grey x grey plants			
C198Y x C157Y	0	0	50
C38Y x C152Y	0	0	20
C157Y x C198Y	0	0	30
Intercrosses of green x green plants			
C204G x C156G	43	0	0
C156G x C46G	19	0	0
C156G x C204G	23	0	0
C212GY x C46G	1	0	0
C46G x C204G	8	0	0
Intercrosses of grey x green plants			
C204G x C157Y	32	0	0
C212GY x C157Y	34	0	0
C196G x C198Y	61	0	0
C35G x C198Y	67	0	0
C46G x C157Y	38	0	0
C1G x C198Y	31	0	0
C204G x C198Y	16	0	0
C38Y x C204G	20	0	0
C38Y x C193G	10	0	7

Table 3 shows the results of additional intercrosses made in the greenhouse among plants with green spike color. Clones C46G, C204G and C212GY had previously been crossed to grey plants (Table 2) and found to be homozygous green. Clones C204G and 808-36-39G produced progenies with only green spikes. The remainder showed low frequencies of GY plants, that is plants whose spike color was intermediate between grey and green. This may be explained by

variable expressivity of the gene for green spike color. Results in Table 4 show that grey-spiked plants when intercrossed did not produce any progenies with GY spike color. Variable expressivity therefore appeared to be confined to the allele carried by plants with green spike color.

In Table 4 grey-spiked plants C38Y, C157Y and C198Y showed a low frequency of progeny with green spikes when intercrossed in the greenhouse. In Table 2, when pair-crossed, these same clones gave no green progeny. The occurrence of green-spiked progeny in these intercrosses may be a result of contamination by green pollen at the time of intercrossing or to technical errors such as seed mixing or planting errors. However, the clones in Table 3 may be called homozygous green and those in Table 4, homozygous grey.

Table 3. Intercross progenies of seven green spiked parents intercrossed in the greenhouse.

Parent	Intercross segregation		
	G	GY	Y
C18G	31	2	0
C46G	25	4	0
C204G	35	0	0
C212GY	27	6	0
608-125-37G	25	6	0
608-125-40G	22	4	0
808-36-39G	35	0	0

Table 4. Intercross progenies of three grey spiked parents intercrossed in the greenhouse.

Parent	Intercross segregation		
	G	GY	Y
C198Y	1	0	37
C157Y	5	0	29
C38Y	2	0	29

(b) F_2 populations

Green plant 608-125-37 (Table 3) also was reciprocally crossed to grey plant 608-124-37 to produce an all green F_1. F_1 plants were pair crossed in the field to produce F_2 populations. Crossing was done by mutually bagging spikes from plants in adjacent rows or spikes from adjacent plants within rows. The F_2 plants were classified for spike color (Table 5). The cross between F_1 plants 1207-96-18 x 1207-96-19 produced two GY spikes in their progeny. For Chi square determinations these were included in the green spike group. This was done, since as shown in Table 4, plants with grey spikes produced no GY progeny whereas plants with GY spikes were produced by green plants (Table 3).

Of the 8 F_2 families shown in Table 5, 7 showed a satisfactory fit to the expected ratio of 3 green : 1 grey ($P = .05$). In Table 6 the heterogeneity Chi square is non-significant which indicates that all the F_2 populations studied are under similar genic control with respect to grey spike color. The deviation Chi square is likewise non-significant, since there are no deficiencies of plants in either class.

The F_1 plants used for intercrossing were sibs, therefore the frequencies of grey and green spikes of the 8 families were totalled.

Table 7 presents the F_2 segregation ratios for the cross of C38Y x C204G reported previously in Table 2. All 7 families show a good fit to the expected ratio of 3G : 1Y . The totals show a good fit to a 3 : 1 ratio (P = .90).

Table 7. Segregation for spike color in F_2 progenies of cross C38Y x C204G.

F_1 sib matings	F_2 segregation			P value
	G	GY	Y	3G : 1Y expected
1207-100-23 x 1207-100-24	39	1	12	.90 - .80
-25 x -26	42	0	15	.90 - .80
-27 x -26	40	5	17	.50 - .30
-29 x -28	41	0	16	.70 - .50
-30 x -29	49	0	15	.80 - .70
-31 x -32	30	0	8	.70 - .50
-36 x -37	27	0	11	.95 - .90
Total	268	6	94	.90 - .80

In Table 8 the heterogeneity and deviation Chi squares are non-significant indicating that the 7 F_2 populations are under the control of the same gene and that the population is homogeneous.

Table 8. Summary of X^2 test for heterogeneity of 7 F_2 populations segregating green vs. grey spike.

Item	X^2	N	P
Deviation	.0570	1	.90 - .80
Heterogeneity	1.7621	6	.95 - .90
Total	1.8191	7	

Table 9 presents results for the third F_2 population from grey x green crosses. These F_1 plants were sibs from a cross of previously untested plants 808-36-38Y and 808-36-39G. Eleven F_1 plants were isolated in the greenhouse in the winter of 1963-1964 and allowed to intercross at random. As shown in Table 9 none of the F_2 progeny of any of these 11 plants produced any GY spikes. Nine of the 11 families shown in Table 9 gave a good fit to the expected ratio of 3G : 1Y. The heterogeneity Chi square given in Table 10 is non-significant, indicating that the F_2 families are under similar genic control for grey spike color. The deviation Chi square is significant and examination of the data revealed a pronounced deficiency in the grey spike class. The progenies of plants 1207-114-25 and 1207-114-31 showed a poor fit to expected ratios because of an excess of green plants. The total frequencies would have shown a better fit to expected ratios if the progeny of the above two plants had not deviated so widely from the expected.

Table 11. Progenies of Plants which proved homozygous green when test crossed to grey plants C198Y and C157Y.

Green parent	Male parent	Number of plants		
		G	GY	Y
C1G	C198Y	31	2	0
C18G	C157Y	31	0	0
C35G	C198Y	66	0	0
C55G	C157Y	28	0	0
C55G	C198Y	31	0	0
C151G	C157Y	28	0	0
C151G	C198Y	31	0	0
C189G	C157Y	22	0	0
C189G	C198Y	20	0	0
C190G	C157Y	24	0	0
C190G	C198Y	30	1	0
C196G	C198Y	61	0	0
C204G	C157Y	32	0	0
C204G	C198Y	25	0	0
C212GY	C157Y	34	0	0
C212GY	C198Y	31	0	0
Totals	C157Y	199	0	0
	C198Y	326	3	0

Table 12. Test-cross progenies of plants which proved heterozygous green when test-crossed to grey plants C198Y and C157Y.

Green parent	Male parent	Test-cross segregation G	GY	Y	P 1G : 1Y expected
C2G	C157Y	13	2	7	.1 - .05
C25G	C157Y	15	0	17	.80 - .70
C25G	C198Y	17	1	11	.50 - .30
C27G	C157Y	11	0	11	1.0
C27G	C198Y	14	3	14	.70 - .50
C59GY	C157Y	12	1	4	.05 - .02
C59GY	C198Y	12	10	8	.02 - .01
C60G	C157Y	5	0	8	.70 - .50
C60G	C198Y	17	0	15	.80 - .70
C61G	C157Y	14	1	8	.20 - .10
C61G	C198Y	17	0	18	.90 - .80
C63G	C157Y	27	6	6	<.01
C155G	C157Y	25	0	7	<.01
C155G	C198Y	16	4	9	.05 - .02
C159G	C157Y	21	2	3	<.01
C159G	C198Y	17	2	10	.20 - .10
C161G	C157Y	13	6	4	<.01
C161G	C198Y	11	0	18	.2 - .1
C166G	C157Y	17	0	17	1
C166G	C198Y	19	3	10	.05 - .02
C182G	C157Y	17	0	17	1
C182G	C198Y	20	1	11	.10 - .05

Cont'd .....

Table 12 continued. Test-cross progenies of plants which proved heterozygous green when test-crossed to grey plants C198Y and C157Y.

Green parent	Male parent	Test-cross segregation G	GY	Y	P 1G : 1Y expected
C193G	C198Y	32	0	32	1
C200G	C157Y	22	4	7	<.01
C200G	C198Y	13	0	16	.70 - .50
Totals	x C157Y	212	22	116	<.01
	x C198Y	205	24	172	<.01
Grand Total		417	46	288	<.01

Table 13. Summary of the X^2 test for heterogeneity of 25 test-cross populations segregating green vs. grey spike.

Item	X^2	N	P
Deviation	40.78	1	<.005
Heterogeneity	54.96	24	<.005
Total	95.74	25	

small. Larger populations might show a better fit to the expected test-cross ratios. There also is the possibility that some clones are slightly self-fertile thus giving deviating ratios. These clones and their polycross progenies will likewise be utilized to evaluate the agronomic properties of the heterozygous green genotype.

(d) Test-crosses of grey introductions

The C198Y plant was used as the tester grey for introduced strains of crested wheatgrass which had a grey spike color. Results of the test-crosses are shown in Table 14. Introductions S-5610, S6241, S-6286 and S-6291 showed no green-spiked plants in their test-cross progenies, indicating that the genes responsible for grey spikes in both C198Y and these introductions are likely allelic.

In other introductions such as S-6285, S-6287 and S-6293, the test-cross progenies showed grey-green and green spikes. Hence grey spike color in these introductions is likely conditioned by a non-allelic gene. The occurrence of green spikes in the test-cross progeny also may be explained by the epistatic action of another grey gene, found in S-6293. The introduction S-6293 gave green, grey-green and grey progeny. The large number of grey progeny produced, relative to the green suggests that this introduction may be partially self-fertile. The present results indicate that the grey spike color is quite widespread in populations of diploid crested wheatgrass. These results are in agreement with Konstantinov (13) who claimed that grey spikes were commonly found in crested wheatgrass in Europe.

Table 14. Test-cross progenies of grey tester (C198Y) x grey introductions.

Grey introduction	Source	Grey tester	Test-cross segregation		
			G	GY	Y
S-5610	Berlin, Germany	C198Y	0	0	83
S-6241	Dresden, Museume de Histoire Naturelle	C198Y	0	2	54
S-6285	Keszjhely, Hungary	C198Y	6	7	0
S-6286	Bot. Garden Acad. Sci., Azerbajan, U.S.S.R.	C198Y	0	13	0
S-6287	Univ. of Toronto	C198Y	11	11	0
S-6291	Bot. Garden, Sofia, Bulgaria	C198Y	0	0	22
S-6293	Bot. Garden, Sofia, Bulgaria	C198Y	2	6	10

Cytological studies

Chromosome behavior was observed at meiosis of P.M.C.s. Anthers from grey and green parent plants were examined as well as anthers from their grey and green F_2 progeny plants. In each case, six plants of each type were examined. At metaphase, seven bivalents were always observed. These occurred in ring and rod configurations. There were no differences detected between grey and green plants in numbers of rings and rods. The chromosomes were observed to disjoin normally at anaphase. Baenziger (2) reported the occurrence of supernumerary chromosomes in some diploid strains of crested wheatgrass, but none was detected in this material.

Table 15. Comparison of chlorophyll a, chlorophyll b and carotene content in the spikes of green and grey parents and their progeny.

Material	Stage of development of spike	ug. pigment per gram fresh wt.			Percent of a + b + c		
		a	b	c*	a	b	c*
Green parent							
C63	Preflowering +	157	393	212	20.6	53.1	26.3
C63	Flowering	149	343	186	22.0	50.6	27.4
C204	Preflowering	189	403	207	23.7	50.2	26.1
C204	Flowering	137	375	200	19.2	52.7	28.1
C196	Preflowering	137	328	215	20.2	48.2	31.6
C1G	Preflowering	172	357	243	22.3	46.2	31.5
Average		156	367	211	21.2	50.4	28.4
Grey parents							
C198	Preflowering	268	674	377	20.3	51.1	28.6
C198	Flowering	230	625	313	19.7	53.5	26.8
C157	Preflowering	291	708	413	20.6	50.2	29.2
C38	Post flowering ++	211	461	268	22.4	49.1	28.5
Average		250	617	343	20.8	50.9	28.3
Green F_2 segregates from cross of grey x green parents (C38Y x C204G)							
1307-18-1	Preflowering	140	281	196	22.7	45.5	31.8
1307-18-1	Post flowering	132	270	190	21.1	47.4	31.5
1007-80-36	Preflowering	176	339	204	24.5	47.1	28.4
1007-82-5	Preflowering	174	389	221	22.2	49.6	28.2
1007-82-5	Post flowering	159	351	213	22.0	48.5	29.5
1007-82-6	Preflowering	149	382	229	19.6	50.2	30.2
607-125-40	Preflowering	137	306	169	22.4	50.0	27.6
Average		152	335	204	22.1	48.3	29.6
Grey F_2 segregates from cross of grey x green parents (C38Y x C204G)							
1007-87-1	Preflowering	242	496	263	24.1	49.6	26.3
1007-87-3	Preflowering	245	648	342	19.8	52.5	27.7
1007-87-3	Post flowering	195	432	275	21.6	47.9	30.5
Average		227	525	293	21.9	50.0	28.1

* a, b, c, represent chlorophyll a, chlorophyll b and carotene respectively.
\+ preflowering = one week prior to flowering.
++ post flowering = one week following flowering.

plants C46G, C198Y and C204G. It appears therefore that small amounts of anthocyanin are present in the spikes and foliage of crested wheatgrass. Because of the small amounts present, they may be masked by the green pigments. The presence of anthocyanin may be the reason for the aberrant ratios of chlorophyll a : chlorophyll b obtained in this study.

Agronomic characteristics

(a) Parents

The hay yield, seed yield and height for 10 homozygous green, 15 heterozygous green and eight homozygous grey parent clones are shown in Table 17. The highest mean forage and seed yields occurred in the homozygous grey group. The S-5565 synthetic was formed in 1959 prior to any consideration of spike color. It was interesting to note that four of the 16 basic clones considered to have good agronomic value at that time, later were noted to be grey.

An analysis of variance based on strain means was used for the comparison of the three groups. No significant differences were found between the three groups for hay yield, seed yield or height ($P = .05$).

Gustafson *et al.* (7) observed an expression of heterosis when two spontaneous chlorophyll mutants were combined into a hybrid. In the crested wheatgrass material discussed here, there was no marked expression of heterosis in the heterozygous green group. The mean height of the heterozygous green material was slightly greater than the means of the other two groups, but not significantly so.

Table 17. Comparison of agronomic characteristics of homozygous green, homozygous grey, and heterozygous green parent plants.

Clone no.	Hay yield lb./plot	Seed yield gms./plot	Height inches
(a) Parent plants homozygous for green spike color			
C1G	354.6	159.3	23
C18G	303.3	156.3	23
C35G	270.0	137.7	24
C55G	272.0	194.0	24
C151G	209.3	84.0	29
C189G	317.3	113.3	28
C190G	203.3	80.0	27
C196G	238.0	146.0	26
C204G	205.0	96.0	26
C212GY	248.7	140.0	28
Mean	262.2	130.7	25.8
(b) Parent plants homozygous for grey spike color			
C5Y	331.7	161.3	24
C19Y	331.7	184.7	25
C31Y	328.6	211.3	22
C38Y	243.3	127.0	26
C152Y	235.7	139.0	26
C158Y	271.6	124.7	29
C175Y	178.3	150.0	25
C198Y	319.7	137.3	26
Mean	280.1	154.4	25.3

Cont'd ...

Table 17 continued. Comparison of agronomic characteristics of homozygous green, homozygous grey, and heterozygous green parent plants.

Clone no.	Hay yield lb./plot	Seed yield gms./plot	Height inches
(c) Parent plants heterozygous for green spike color			
C2G	294.7	168.7	24
C25G	240.3	111.3	24
C27G	263.3	163.7	26
C32G	240.7	125.0	23
C60G	276.0	146.7	31
C61G	231.3	154.3	21
C63G	271.3	164.0	23
C155G	199.3	111.0	27
C156G	325.0	158.3	31
C159G	242.3	110.7	28
C161G	244.0	105.7	30
C166G	185.0	110.0	27
C182G	275.7	111.3	26
C193G	209.0	109.7	29
C200G	222.3	122.7	25
Mean	248.0	131.5	26.3

(b) Progeny

Table 18 presents the agronomic performance of polycross progenies from homozygous green, heterozygous green and homozygous grey clones.

An analysis of variance showed no significant differences between the three groups for hay yield, seed yield, rate of recovery, heights, ungrazed residue, and lodging resistance.

The progeny of heterozygous green clones did not exhibit any heterosis, therefore did not appear to have any agronomic

Table 18. Comparison of agronomic characteristics of polycross progenies of parents that are homozygous green, homozygous grey and homozygous green for spike color.

Progenies of clone no.	Hay yield as percent of Fairway	Ungrazed residue lbs./acre dry matter	Seed yield as percent of Fairway	Height in inches	Recovery 1 = rapid 5 = slow	Lodging 1 = nil 5 = severe
A. Progenies of parents homozygous for green spike color						
C1G	107	734	109	22.6	2.6	3.0
C18G	119	767	136	23.6	1.8	3.0
C35G	102	531	107	20.6	2.6	1.8
C55G	108	565	110	22.8	2.5	2.3
C151G	120	595	128	24.9	1.6	3.2
C189G	107	557	111	22.7	2.4	2.7
C190G	111	617	106	24.0	2.1	3.3
C196G	110	569	112	21.8	1.6	4.3
C204G	113	493	130	23.3	2.2	4.2
C212GY	116	381	125	24.4	2.0	2.7
Mean	111.3	580.9	117.4	23.1	2.1	3.1

Table 18 continued. Comparison of agronomic characteristics of polycross progenies of parents that are homozygous green, homozygous grey and homozygous green for spike color.

Progenies of clone no.	Hay yield as percent of Fairway	Ungrazed residue lbs./acre dry matter	Seed yield as percent of Fairway	Height in inches	Recovery 1 = rapid 5 = slow	Lodging 1 = nil 5 = severe
B. Progenies of parents homozygous for grey spike color						
C5Y	111	690	95	23.0	1.8	3.5
C19Y	104	633	118	23.1	2.7	2.0
C31Y	106	518	102	21.4	3.4	2.3
C38Y	116	668	120	22.0	1.8	2.5
C152Y	111	636	114	23.7	2.2	3.3
C158Y	115	574	106	21.7	2.2	2.2
C175Y	111	706	111	23.2	2.4	3.5
C198Y	114	475	91	22.9	1.7	2.3
Mean	111.0	612.5	107.1	22.6	2.3	2.7

Cont'd ...

Table 18 concluded. Comparison of agronomic characteristics of polycross progenies of parents that are homozygous green, homozygous grey and homozygous green for spike color.

Progenies of clone no.	Hay yield as percent of Fairway	Ungrazed residue lbs./acre dry matter	Seed yield as percent of Fairway	Height in inches	Recovery 1 = rapid 5 = slow	Lodging 1 = nil 5 = severe
C. Progenies of parents heterozygous for green spike color						
C2G	114	564	165	23.5	2.1	3.0
C25G	109	630	143	23.8	2.7	3.5
C27G	105	533	98	22.4	3.1	2.3
C32G	108	773	92	21.5	2.2	2.3
C60G	109	509	89	22.3	2.9	2.2
C61G	107	485	150	22.6	2.8	2.5
C63G	107	572	105	22.3	2.4	2.2
C155G	108	650	108	25.2	2.2	2.5
C156G	111	536	100	22.0	3.6	3.2
C159G	111	533	110	22.0	2.1	2.5
C161G	112	494	99	22.4	2.4	2.2
C166G	110	466	88	22.0	2.5	2.7
C182G	111	477	120	23.3	2.5	4.0
C193G	112	677	93	24.2	2.7	4.3
C200G	114	599	135	23.7	2.3	2.7
Mean	109.9	566.5	113.0	22.9	2.6	2.8

DISCUSSION

In the present study, grey spike color was found to be conditioned by a single recessive gene. The various phenotypes may be designated as:

Symbol	Phenotype	Genotype
G	green	YY,Yy
GY	grey-green	Yy,YY
Y	grey	yy

In crosses between plants with grey and green spikes the F_1 progeny showed the dominant green color. F_2 progenies segregated in a ratio of 3G : 1Y spikes. These results suggest that diploid crested wheatgrass is a sexually reproducing species without apomixis. Plants with intermediate grey-green spike color appeared in the F_2 populations as well as the parental clones. It was difficult to place these in either the green or grey class. Since complete dominance was observed in the F_1, the intermediate grey-green spikes may be the result of variable expressivity of the gene for green spike color. The GY plants could not be considered heterozygous for green spike color. Clone C212 for example was classified as GY for spike color, but on test-crossing proved to be homozygous green.

The data from the chlorophyll analyses suggest that the difference between grey and green spikes may be explained on the basis of chlorophyll content. Grey spikes contain 50 to 60 percent greater amounts of chlorophyll pigments than green spikes. The results do not

agree with the established ratio of 3 chlorophyll a to 1 chlorophyll b (1). The differences in amounts of chlorophyll between grey and green spikes, however, were consistent throughout the course of the study. The grey and green spike material showed the same ratio of chlorophyll a to chlorophyll b. A difference in this ratio could cause a difference in the shade of green color expressed. Chlorophyll a is blue-green to black, while chlorophyll b alone is yellow-green to bright green (17). The amount of carotene in grey spikes parallelled the increased amounts of chlorophyll a and chlorophyll b.

It is possible that the grey allele is able to produce more pigment. von Wettstein (18) suggested that barley chlorophyll mutants arise as a result of physiological and structural changes of the photosynthetic material. The physiological change in amount of chlorophyll is parallelled by structural changes in the grana of the chloroplasts.

In the present study, a waxy "bloom" was noticed on the spikelets of some, but not all, grey plants. This material in some cases appeared to intensify the expression of the grey color. Chlorophyll extracts from grey plants may be darkened by the presence of this material and thus give higher optical density readings.

Holm (9) suggested that extraction of chlorophyll with acetone is reliable only when no water-soluble pigments such as anthocyanins are present. In the present material, spikes from grey and green plants were found to have purple tinted anthers. Also seedlings in the progeny of grey and green plants were found to be tinted with a purple pigment. This purple pigment may be an anthocyanin that is interfering with the readings taken on chlorophyll a and chlorophyll b

were homozygous for the respective spike color the genetic backgrouna

was different and hence a comparison of the grey and green phenotype is not entirely valid.

If varieties with homozygous green and homozygous grey spikes were produced, the two contrasting spike colors could be used as variety identification markers. Spike color would be a means of checking for contamination, providing seed fields were examined at the proper stage of growth.

Pollination distance studies could be carried out using populations of homozygous green and homozygous grey material. The green spike material would serve as the pollen source since it is dominant. Grey tester plots placed at varying distances from the green plot could be sampled to determine the amount of contamination of green pollen. Seed from contaminated plots would have to be grown to the heading stage, then classified for spike color. Crested wheatgrass will head out in the year of planting so that pollination distance results could be obtained in the year of seeding.

According to the Hardy-Weinburg law, the gene frequency of neutral alleles in a randomly mating population will remain constant in the following genotypic proportions:

$$q^2AA + 2q(1 - q)Aa + (1 - q)^2aa = 1$$

where AA = dominant phenotype

aa = recessive phenotype

q = frequency of dominant allele

(1 - q)= frequency of recessive alleles

According to this equation the frequency of the grey allele in the Fairway variety is .28. Theoretically, genetic equilibrium is attained in one generation of random mating and should be maintained unless

altered by selection, mutation or non-random mating.

The homozygous grey genotype had no selective advantage or disadvantage compared to the green genotype as far as the agronomic factors considered in this study were concerned. Unless there are other differences as in disease resistance or persistence, the grey type should be maintained in the Fairway variety. The frequency of grey spikes for the Fairway variety in the present study was obtained over a three year interval. It would be interesting to check the progeny of these same Fairway plots in future years to see if the grey spike frequency is maintained.

The synthetic variety S-5565 was composed of 16 clones. Test-crosses showed that four of these clones were homozygous grey, five homozygous green and seven heterozygous green. In these 16 clones, the grey allele y is represented 15 times. This gives a frequency of .468 for grey alleles in the syn. 0 generation of S-5565. The frequency of grey alleles in the syn. 1 is .422 and for syn. 2 the frequency is .464 (Table 1). This indicates that the frequency of grey spikes in S-5565 remains fairly constant. It would be of interest to observe this synthetic variety for a number of additional generations to see if any changes take place in the grey spike frequency.

Should the S-5565 strain be released as a variety the higher frequency of grey plants relative to Fairway might be used to identify this strain from Fairway.

Reports by European workers and observations on introductions of crested wheatgrass from Europe indicate that the grey spike color is quite widespread. The test-cross data from some introduced forms of crested wheatgrass suggested that the gene for grey spikes may be

the same as the one that controls grey spikes in C198Y, the grey tester parent. Other introductions indicate by their test-cross data that there is a different factor controlling spike color. Introductions from a larger number of sources should be test-crossed to determine whether there are more genetic factors controlling grey spike color in crested wheatgrass.

SUMMARY

A grey spike color was studied in the diploid form of crested wheatgrass. It was found to be controlled by a single recessive gene. The F_1 population from a cross of grey and green plants was dominant for the normal green color with the F_2 showing a good fit to a 3 : 1 ratio of green to grey spikes. The progenies of certain green plants were intermediate in color between grey and green. The allele for green spike color in certain cases may exhibit variable expressivity.

Homozygous green, heterozygous green and homozygous grey clones were evaluated for hay yield, seed yield and height. There were no significant differences between the three groups for hay yield, heights, or seed yield although the seed yield of the homozygous grey group tended to be higher.

Polycross progenies from the same groups of clones were evaluated for six agronomic characteristics. No significant differences for hay yield, seed yield, yield of ungrazed residue, heights, lodging resistance and rate of recovery were found between the three groups. From the data presented in this report neither genotype seems to have a definite agronomic advantage.

Cytological behavior at meiosis of the pollen mother cells was regular. No supernumerary chromosomes were observed.

Grey spikes showed 50 to 60 percent greater amounts of chlorophyll a, chlorophyll b and carotene than green spikes, when acetone extracts from the two were compared spectrophotometrically. Leaves from plants with grey spikes showed 13 percent more total

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