



Genetics, phenotypes, agronomic and malting performance of glossy sheath mutants in barley,
Hordeum vulgare L.
by Wayne Lucas McProud

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY in Genetics
Montana State University
© Copyright by Wayne Lucas McProud (1971)

Abstract:

Twenty four different glossy sheath mutational events, including both spontaneous and induced mutants were found to comprise six different loci. The seventeen spontaneous glossy sheath mutants occurred at random over these six loci. The tentative location of *gsl* is on chromosome 4. It has been reported that *gsl* and *cer-q* are allelic. The gene *gs2* is located on either chromosome 2 or, more probably, chromosome 3.

The location of *gs3* is on chromosome 1 with the probable gene order of *wx-gs3-br*. The gene *gs4* is located on chromosome 6. Chromosome 2 is the location of *gs5*, having the probable gene order *V-gs5-Ms2*. The gene *gs6* is located on chromosome 2.

The microscopic wax morphology of the glossy sheath mutants can be classified into four groups. Group 1, "short rods", corresponds to the locus *gsl*. Group 2, "peaks and ridges", corresponds to the loci *gs2* and *gs5*. The loci *gs3* and *gs6* comprise group 3, "flakes". Group 4, "knolls", corresponds to the locus *gs4*.

Nine barley varieties and their glossy sheath mutants were compared for yield, and for various agronomic and malting traits. Two effects may be ascribed to the glossy sheath mutants: . The glossy sheath mutant, as compared to the normal genotype, heads earlier and may have superior yielding capacity when grown under stress conditions at lower elevations.

GENETICS, PHENOTYPES, AGRONOMIC AND MALTING PERFORMANCE OF
GLOSSY SHEATH MUTANTS IN BARLEY, HORDEUM VULGARE L.

by

WAYNE LUCAS MCPROUD

A thesis submitted to the Graduate Faculty in partial
fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Genetics

Approved:

P. D. Skaar

Head, Major Department

Robert H. Eshel

Co-Chairman, Examining Committee

G. Allan Taylor

Co-Chairman, Examining Committee

Henry L. Persons

Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

August, 1971

ABSTRACT

Twenty four different glossy sheath mutational events, including both spontaneous and induced mutants were found to comprise six different loci. The seventeen spontaneous glossy sheath mutants occurred at random over these six loci. The tentative location of gs1 is on chromosome 4. It has been reported that gs1 and cer-q are allelic. The gene gs2 is located on either chromosome 2 or, more probably, chromosome 3. The location of gs3 is on chromosome 1 with the probable gene order of wx-gs3-br. The gene gs4 is located on chromosome 6. Chromosome 2 is the location of gs5, having the probable gene order V-gs5-Ms2. The gene gs6 is located on chromosome 2.

The microscopic wax morphology of the glossy sheath mutants can be classified into four groups. Group 1, "short rods", corresponds to the locus gs1. Group 2, "peaks and ridges", corresponds to the loci gs2 and gs5. The loci gs3 and gs6 comprise group 3, "flakes". Group 4, "knolls", corresponds to the locus gs4.

Nine barley varieties and their glossy sheath mutants were compared for yield, and for various agronomic and malting traits. Two effects may be ascribed to the glossy sheath mutants. The glossy sheath mutant, as compared to the normal genotype, heads earlier and may have superior yielding capacity when grown under stress conditions at lower elevations.

ACKNOWLEDGMENTS

The writer wishes to acknowledge the assistance, encouragement and constructive criticism of Professor R. F. Eslick, Dr. E. A. Hockett, and Dr. G. A. Taylor toward the completion of this thesis.

A special acknowledgment is due to Mr. R. Bronson of S. C. Johnson and Sons, Inc., Dr. S. R. Chapman, and Dr. T. Carroll for their help and suggestions in the procurement of the micrographs shown in this thesis.

The cooperation of those agronomists who grew the Western Regional Two-row Barley Nursery is gratefully acknowledged. An acknowledgment is due to the USDA Malt Laboratory for providing the malting data presented in this thesis.

The writer also wishes to acknowledge the Plant and Soil Science Department for the use of their resources and facilities.

TABLE OF CONTENTS

	<u>Page</u>
VITA-----	ii
ACKNOWLEDGMENT-----	iii
TABLE OF CONTENTS-----	iv
LIST OF TABLES-----	v
LIST OF FIGURES-----	vi
ABSTRACT-----	ix
INTRODUCTION-----	1
LITERATURE REVIEW-----	2
Early "waxless" mutants-----	2
Glossy sheath mutants-----	3
Linkages-----	4
Physical and Chemical Properties-----	6
Agronomic properties of normal, and of eceriferum mutants-----	8
Function of surface waxes-----	8
MATERIALS AND METHODS-----	10
Allelism-----	11
Linkage and inheritance studies-----	11
Morphological determination of the wax particles-----	14
Agronomic and malting characteristics-----	15
RESULTS AND DISCUSSION-----	18
Allelism-----	18
Linkage-----	22
Phenotypic Expression-----	39
Agronomic and malting characteristics-----	56
SUMMARY-----	67
LITERATURE CITED-----	70

LIST OF TABLES

	<u>Page</u>
Table 1. Source and origin of the glossy sheath mutants---	11
Table 2. F ₁ Phenotypic classification of crosses between glossy sheath (<u>gsgs</u>) x glossy sheath (<u>gsgs</u>) lines-----	19
Table 3. Previous and suggested gene designation for glossy sheath mutants-----	21
Table 4. Linkage intensities involving the six glossy sheath genes plus other barley genes-----	23
Table 5. Crosses involving the translocations T2-3g and T2-5a that deviated from theoretical ratios-----	32
Table 6. Linkage intensities of T2-3g and T2-5a break- points with <u>v</u> , <u>ms1</u> and <u>gs2</u> : calculated utili- zing the variegated position effect hypothesis---	34
Table 7. Phenotypic description of field grown glossy sheath mutants -----	40
Table 8. Average yields of normal (<u>GsGs</u>) and glossy sheath mutants (<u>gsgs</u>) for nine barley varieties-----	57
Table 9. Yield comparisons of normal (<u>GsGs</u>) and glossy sheath mutants (<u>gsgs</u>) grown at various yield levels, Mars and Chevron excluded-----	59
Table 10. Average agronomic characteristics of normal (<u>GsGs</u>) and glossy sheath mutants (<u>gsgs</u>) in nine barley varieties-----	62
Table 11. Average barley and malting quality characteristics of three normal (<u>GsGs</u>) two-row varieties and their respective glossy sheath mutants (<u>gsgs</u>)-----	65

LIST OF FIGURES

	<u>Page</u>
Figure 1. Wax particles (w) over vascular bundle of C.I. 5818, <u>gs1gs1</u> . Cell material below not focused. (Light microscope, 10x)-----	42
Figure 2. Two wax particle types of normal Betzes, heaped wax layer (h) and single granulated layer (s). (Light microscope 10x)-----	42
Figure 3. Two wax particle types of glossy Pirolina <u>gs1gs1</u> , heaped wax layer (h) and single granulated layer (s). (Light microscope, 10x)-----	43
Figure 4. Two wax particle types of glossy Barbless <u>gs6gs6</u> , heaped wax layer (h) and single granulated layer (s). (Light microscope, 40x)-----	43
Figure 5. Single granulated layer (s) of (4 Atlas x Algerian) x 2 Atlas 57 <u>gs3gs3</u> . (Light microscope, 10x)-----	44
Figure 6. Single granulated layer (s) of glossy Gateway <u>gs4gs4</u> . (Light microscope, 10x)-----	44
Figure 7. Normal amount of wax present on glaucous Betzes, (Scanning electron microscope, 100x)-----	45
Figure 8. Heaped wax layer (h) and reduced wax presence of glossy Pirolina <u>gs1gs1</u> . (Scanning electron microscope, 100x)-----	45
Figure 9. Reduced wax presence of glossy Vantage <u>gs2gs2</u> . (Scanning electron microscope, 100x)-----	46
Figure 10. Heaped wax layer (h) and reduced wax presence of glossy Mars, <u>gs3gs3</u> . (Scanning electron microscope, 100x)-----	46
Figure 11. Reduced wax presence of glossy Gateway, <u>gs4gs4</u> , (Scanning electron microscope, 100x)-----	47

LIST OF FIGURES
(Continued)

	<u>Page</u>
Figure 12. Reduced wax presence of glossy Jotun, <u>gs5gs5</u> , (Scanning electron microscope, 50x)-----	47.
Figure 13. Heaped wax layer (H) and reduced wax presence of glossy Betzes <u>gs6gs6</u> . (Scanning electron microscope, 50x)-----	48
Figure 14. Wax configuration of glaucous sheath Betzes. (Penetrating electron microscope, 500x)-----	48
Figure 15. Wax distribution on the leaf sheath of glaucous Betzes. (Scanning electron microscope, 1000x)---	50
Figure 16. Straight (s), multibranched (m) and capped (c) wax particles of Pirolina, <u>gs1gs1</u> . (Penetrating electron microscope, 1000x)-----	50
Figure 17. Capped wax particle (c) from leaf sheath of glossy Pirolina <u>gs1gs1</u> . (Scanning electron microscope, 1000x).-----	51
Figure 18. Peak (p) and ridge (r) wax particles of glossy Atlas 2, <u>gs2gs2</u> . (Penetrating electron microscope, 1000x)-----	51
Figure 19. Peak wax configuration (p) of glossy Vantage, <u>gs2gs2</u> . (Scanning electron microscope, 1000x)---	52
Figure 20. Peak wax configurations (p) of glossy Jotun <u>gs5gs5</u> . (Scanning electron microscope, 2000x)---	52
Figure 21. Flake configuration of glossy (4 Atlas x Algerian) x 2 Atlas 57, <u>gs3gs3</u> . (Penetrating electron micro- scope, 1000x)-----	53
Figure 22. Flake configuration (F) of glossy Mars, <u>gs3gs3</u> . (Scanning electron microscope, 1000x)-----	53
Figure 23. Flake configuration of glossy Betzes, <u>gs6gs6</u> . (Scanning electron microscope, 2000x)-----	54

-viii-

LIST OF FIGURES
(Continued)

	<u>Page</u>
Figure 24. Knoll configuration of glossy Gateway, <u>gs4gs4</u> . (Penetrating electron microscope, 1000x)-----	54
Figure 25. Knoll (K). configuration of glossy Gateway, <u>gs4gs4</u> . (Scanning electron microscope, 1000x)-----	55

ABSTRACT

Twenty four different glossy sheath mutational events, including both spontaneous and induced mutants were found to comprise six different loci. The seventeen spontaneous glossy sheath mutants occurred at random over these six loci. The tentative location of gs1 is on chromosome 4. It has been reported that gs1 and cer-q are allelic. The gene gs2 is located on either chromosome 2 or, more probably, chromosome 3. The location of gs3 is on chromosome 1 with the probable gene order of wx-gs3-br. The gene gs4 is located on chromosome 6. Chromosome 2 is the location of gs5, having the probable gene order V-gs5-Ms2. The gene gs6 is located on chromosome 2.

The microscopic wax morphology of the glossy sheath mutants can be classified into four groups. Group 1, "short rods", corresponds to the locus gs1. Group 2, "peaks and ridges", corresponds to the loci gs2 and gs5. The loci gs3 and gs6 comprise group 3, "flakes". Group 4, "knolls", corresponds to the locus gs4.

Nine barley varieties and their glossy sheath mutants were compared for yield, and for various agronomic and malting traits. Two effects may be ascribed to the glossy sheath mutants. The glossy sheath mutant, as compared to the normal genotype, heads earlier and may have superior yielding capacity when grown under stress conditions at lower elevations.

INTRODUCTION

Mutants with altered wax coatings are a common mutational event in barley. Lundqvist and von Wettstein (1962) report that mutants with reduced or absent wax coating on various organs are almost as readily induced as erectoides, the most frequent viable mutants in barley. The name eceriferum (from Latin: cer=wax and ferre=bear) was suggested by Lundqvist and von Wettstein (1962) to describe barley mutants having little or no wax coating on the spike, stem, leaf sheath or blades, on several organs or involving the whole plant. The Swedish source of eceriferum mutants is primarily from artificially induced mutations.

The eceriferum family of mutants contains many specific glossy mutant types. Of concern in this thesis are the combined glossy sheath, stem and spike mutants, and glossy sheath mutants with normal wax covering on the stem and spike. The sources of the glossy mutants herein reported are largely spontaneous.

The purposes of this study were to determine allelism of the combined glossy sheath, stem and spike mutants plus the two glossy sheath mutants with normal wax covering on the stem and spike, to establish linkage relationships of the various glossy sheath loci, to determine if morphological differences existed between the different glossy sheath loci on a microscopic level, and to compare agronomic and malting characteristics of normal and glossy sheath mutants.

LITERATURE REVIEW

Early "waxless" mutants

Gustafsson (1947) reported on an unpublished collection by H. Tedin of a spontaneous "waxless" mutant in the barley variety 'Primus'. Tedin found the mutant line equalled the mother line in yield. Gustafsson noted that Tedin's mutant did not have the same peculiar bright green color of the later induced waxless mutants.

The occurrence of another spontaneous eceriferum mutant was reported by A. V. Tokhlyuyev in 1935 (Gustafsson, 1947, and Smith, 1951). This mutant, described by Smith (1951) as lacking wax on the leaves, was given the gene symbol Wlw1. Smith reported on the finding of two additional waxless mutants by K. V. Ivanova. One mutant, designated Whwh, lacked waxy bloom on the spike while the second, designated Wh2wh2, lacked waxy bloom on the spike and stem. Other waxless or bloomless mutants reported on by Smith were Gege, normal vs. non-glaucous ear, and Gsgs, normal vs. non-glaucous sheath. All were reported as monofactorial recessive mutants.

Stadler (1930) reported inducing two "non-glaucous" recessive barley mutants. Gustafsson (1947) reported inducing several mutants involving wax variation. Some appeared to be extremely waxy. Other mutants induced in the varieties Maja, Ymer, and Bonus were characterized by a "bright" green color and were found to be entirely or partially waxless.

Glossy sheath mutants

The first report of a glossy sheath mutant, designated by the symbol gsgs, was made by Immer and Henderson (1943). The symbol was later changed to (cer-)gs (Robertson, et.al., 1965). The glossy sheath mutant Immer and Henderson studied, C.I. 5818, was originally obtained from Vavilov's collection at Valkie, U.S.S.R. (J. C. Craddock, Personal communication).

The identification of a second gene for the glossy sheath characteristic, (cer-)gs2, was made by R. F. Eslick (unpublished) in the barley variety Vantage. This mutant was selected from a foundation seed field at Bozeman, Montana.

Researchers at the University of Alberta, Canada (Walker, et.al., 1963) identified two additional glossy sheath genes. The first gene, induced in the variety Mars by x-radiation was designated (cer-)gs3. A second glossy sheath gene designated (cer-)gs4, was induced in the variety Gateway by treating seed with the mutagen aldrin.

Rasmusson and Lambert (1965) reported that the two glossy sheath genes in Mars and Gateway were allelic. In addition, Rasmusson and Lambert reported on two glossy sheath mutants obtained from K. Mikaelson of Norway. The mutant in Jotun was designated (cer-)gs5 while the mutant in Domen was designated (cer-)gs6.

Takahashi and Hayashi (1966) found a spontaneous glossy sheath mutant in the Japanese barley variety Sekitori. The gene symbol (cer-)gs7 was assigned to this mutant, Kogane-mugi.

A second spontaneous glossy sheath mutant reported by Takahashi et.al. (1965) (corrected by Takahashi and Hayashi, 1968) was named Okaiku No. 3. This mutant, assigned the symbol (~~cer-7~~)gs8, was selected at the Okayama Agricultural Experiment Station, Japan.

Lundqvist, von Wettstein-Knowles, and von Wettstein (1968) reported on 380 induced and 4 spontaneous eceriferum mutants. They found seven different loci producing mutants with phenotypes similar to five of the six glossy sheath mutants discussed in this thesis. The glossy sheath phenotype is an apparent absence of wax on the spike, leaf sheath, and stem. The leaf blades appear normal. The eceriferum loci associated with the glossy sheath, stem and spike phenotype are cer-a, cer-c, cer-q, cer-x, cer-z, cer-zl.

Lundqvist et.al. (1968) found three other genes, cer-n, cer-r, and cer-zm, causing an absence of wax on the spike, reduced wax on the leaf sheath and stem, and normal appearing leaf blades. One gene, cer-s, caused a reduction of wax on the spike, an absence of wax on the leaf sheath and stem, with normal appearing leaf blades.

Linkages

Five of the presently identified genes for the glossy sheath characteristic in barley have been assigned to a chromosome. Albrechtsen (1957) reported a recombination value of 23.9 between gs1 and Colorado zoned leaf z_c on chromosome 4. Anderson (1958) reported gs1 and the break point of the translocation T3-4a to be 15.0 map units apart. These

data indicate that gs1 is located on chromosome 4.

Walker, et.al. (1963), reported gs3 to be 24.1 ± 6.1 map units from brachytic, br, on chromosome 1. Using F₂ data, McProud and Eslick (1968) reported (cer-)gs3 and br to be linked, being 0.3 ± 9.0 map units apart. Rasmusson and Lambert (1965) found (cer-)gs3 linked with chlorina, fc, and waxy, wx, both on chromosome 1. The recombination values between (cer-)gs3 and fc, and between (cer-)gs3 and wx, were 2.0 ± 1.05 and 20.7 ± 5.3 , respectively.

Walker et.al. (1963) located gs4 on chromosome 6 and found it linked with orange lemma, o, and glossy leaf-4, gl4. The o gene had no recombination with gs4 while gl4 had a recombination value with gs4 of 29.4 ± 2.6 .

Rasmusson and Lambert (1965) located (cer-)gs5 on chromosome 2. They found (cer-)gs5 to be linked with long glume awn, e, and with six row, v, with the respective recombination values of 2.5 ± 0.79 and 32.0 ± 2.55 . They report a probable gene order of (cer-)gs5, e, v.

Rasmusson and Lambert (1965) reported (cer-)gs6 to segregate independently from the following marker genes on chromosome 1; brachytic, br, naked caryopsis, n and chlorina, fc. In addition, they found (cer-)gs6 independent of six row, v, on chromosome 2; white seedling, ac, and xantha seedling, xc, on chromosome 3; hooded, K, glossy seedling, gl, and blue alcurone, Bl, on chromosome 4; black lemma and pericarp, B, albino seedling, at, on chromosome 5; and orange lemma, o, on chromosome

6. No marker genes on chromosome 7 were tested with (cer-)gs6 by Rasmusson and Lambert.

Takahashi and Hayashi (1966) located (cer-)gs7 on chromosome 2 through the use of trisomic analysis.

Takhashi, et.al. (1965) (corrected by Takahashi and Hayashi, 1968) located (cer-)gs8 on chromosome 2 by the use of trisomics. They found (cer-)gs8 to be linked with purple stem, Pr, and with six row, v, with a recombination value of 36.6 and 25.1, respectively. The probable gene order is Pr, v, (cer-)gs8.

Physical and Chemical Properties

Lundqvist and von Wettstein (1962) and Eglinton and Hamilton (1967) have reported on DeBary's observations regarding wax structures. Using a light microscope, DeBary identified four structural configurations of surface waxes. Two of these structural types he identified as being present on barley surfaces were heaped wax layer and single granulated layer. Lundqvist and von Wettstein (1962) found that in barley "the spike, and the central and lateral florets are covered with a heaped layer of needles, whereas the awns are covered with relatively few isolated granules. The stem nodes and internodes as well as the leaf sheaths are coated with a thick heaped layer of needles. The leaf blades have only a granular coating either in the form of isolated wax granules or of a continuous granules layer". It was determined by von Wettstein-Knowles (1971) that the average length of the rods or needles on the

barley sheath is five microns. She points out that the rods may be single or branched, and that their entire length may lay flat along the cuticle or may stand erect.

Lundqvist and von Wettstein (1962) stated no wax could be detected microscopically on the concerned organs of eceriferum mutants. It was found by von Wettstein-Knowles (1971) that there were changes in structure density and/or distribution of the epidermal waxes on four eceriferum mutants. A glossy sheath mutant, cer-c, was found to have a 34% reduction in the amount of wax present on the internodes. The internodes of Bonus barley had 51 mg/cm² of wax while the mutant cer-c had 17 mg/cm² of wax.

Chemical analyses of the normal and the glossy sheath, an eceriferum mutant, have been conducted by Jackson (1971) and von Wettstein-Knowles (1971). Jackson (1971) investigated the chemical composition of surface lipid extracts from the internode region of both normal and glossy sheath barley plants. In both normal and glossy sheath mutant plants hydrocarbons made up 5-7% of the surface lipids while alcohols (as acetates) accounted for 15-20% of the surface lipid extract. From normal barley 25-30% of the surface lipids were made up of the B-diketones. hentriacontane-14-16-dione; plus 0-1% hydroxy-B-diketones. The glossy sheath mutants lack these compounds in their epidermal waxes.

It has been noted by von Wettstein-Knowles that the internode waxes from normal Bonus contain paraffins, esters, primary alcohols,

aldehydes, lactones, B-diketones and hydroxy-B-diketones (1971). Wax bodies which contain B-diketones and hydroxy-B-diketones are long thin rods. When these two B-diketones classes are missing, as in the waxes on wild type leaves, no such rods are formed.

Agronomic properties of normal and of eceriferum mutants.

X-ray induced eceriferum mutants have been tested by the Swedish Seed Association for yield and other agronomic characteristics (Froier, 1954). Froier characterized bright green 2 by "less waxiness and lighter green-leaf colour". Nine years data show that bright green 2 yielded 98.1% of its mother line, Maja. Date ripe, hectoliter weight, 1000-kernel weight, sifting percentages (percent plump barley) and percent crude protein were about the same as Maja. Eight years data show that the glossy mutant bright green 3 yielded 96.5% of its mother line, Bonus. The mutant was similar to Bonus for hectoliter weight and 1000-kernel weight, but somewhat superior in kernel shape and development and ripened at least one day earlier (Froier, 1954).

Lundqvist et.al. (1968) reported "rather unspecific pleiotropic effects caused by the eceriferum loci on viability, plant height, or fertility. In some cases these effects could signify deletions, including pieces of adjacent genes".

Function of surface waxes.

Eglinton and Hamilton (1967) suggested various functions for the surface waxes. Such functions may be to protect the plant from

mechanical damage, from insect, fungal and bacterial attack, and possibly protect the plant from frost damage. Epidermal waxes may help preserve the plants water balance or alter the ability of substances to penetrate through the leaf. Surface waxes may scatter light or other radiations striking the plants surface. Jackson (1971) noted that "the B-diketones, nucleic acids and amino acids absorb ultra-violet light in about the same region of the spectrum and the B-diketone may serve a dual role in preventing ultraviolet damage and desecration in the plant".

Lundqvist, et.al. (1968) and Jackson (1971) pointed out that the glossy appearance of plant organs is due to the wax's chemical and physical properties and not to the actual absence of wax.

MATERIALS AND METHODS

Allelism

A collection of 24 different glossy sheath mutants was assembled at Bozeman, Montana. The glossy sheath mutants of Ymer, Mars, Jotun, Domen, and C.I. 9132 are believed to have originated by radiation treatments while the glossy sheath mutant of Gateway arose from a seed treatment with the mutagen aldrin. The glossy sheath mutants of the varieties Pirolina, Velvon, Vantage, Atlas 1, Atlas 2, Compana 1, Compana 2, Compana3, Compana 4, Betzes, and Heines Hanna were found as spontaneous mutants in the Rocky Mountain region of the northern United States and probably represent separate mutational events. The remaining spontaneous glossy sheath mutants are from various parts of the world. A complete resume of the various sources of the glossy sheath mutants, as far as they are known to the author, is included as Table 1. Letters following the locus symbol represent, presumably, separate mutational events. The letters were assigned arbitrarily to the stocks available.

Tests for allelisms were made by inter-crossing the glossy sheath mutants. Most crosses were made using male sterile glossy sheath plants as females; otherwise, crosses were made utilizing hand emasculated plants. The resulting F_1 's were grown in the greenhouse and selected F_2 populations were grown.

Linkage and Inheritance Studies

Crosses were made between the glossy sheath, genes and lines carrying known marker genes. A translocation tester set was crossed

Table 1. Source and origin of the glossy sheath mutants.

Variety	Suggested gene symbol locus and event	C.I. No. of parental variety	Origin	History and/or source
Piroline	<u>gs1b</u>	9558 11330*	Spontaneous	Selected from a head row at Tetonia, Idaho
Glossy Tester	<u>gs1a</u>	5818*	Vavilov's collection	Supplied by D. W. Robertson Fort Collins, Colorado
Velvon	<u>gs1c</u>	6109	Spontaneous	Supplied by R. W. Woodward Logan, Utah
Vantage	<u>gs2d</u>	7324	Spontaneous	Selected from a foundation seed field at Bozeman, Montana, in 1958
Atlas 1	<u>gs2f</u>	4118	Spontaneous	Selected as 2 sources from a commercial field at Dayton, Washington, in 1964 and 1966
Atlas 2	<u>gs2g</u>	4118	Spontaneous	
Ymer	<u>gs2e</u>	7275	Irradiation	Mutation from Ymer (P.I.184886) received from the Institute of Genetics at Lund, Sweden via USDA, Beltsville, Maryland
Klargrin	<u>gs2h</u>		Believed irradiation induced	Supplied by D. C. Rasmusson, St. Paul, Minnesota (originally from A. Gustaffson Lund, Sweden).

Table 1. Continued

Variety	Suggested gene symbol locus and event	C.I. No. of parental variety	Origin	History and/or source
Mars	<u>gs3i</u>	7015	Irradiation	Supplied by J. W. Lambert, St. Paul, Minnesota. Additional sources G. W. Walker, Edmonton, Alberta, Canada, and D. C. Rasmusson, St. Paul, Minnesota
Chevron	<u>gs3j</u>	1111	Spontaneous	Selected as a single plant in the 1953 Beltsville Greenhouse. Supplied by G. A. Weibe, USDA
<u>Atlas⁴ x Algerian x Atlas 57²</u>	<u>gs3k</u>		Spontaneous	Supplied by C. O. Qualset, Davis, California
Gateway	<u>gs4l</u>	10072	Chemical mutagen aldrin	Supplied by G. W. Walker, Edmonton, Alberta, Canada
Jotun	<u>gs5m</u>		Irradiation	Supplied by D. C. Rasmusson, St. Paul, Minnesota (supplied to Minnesota by K. Mikaelson, Norway).
Okaiku No. 3	<u>gs5n</u>		Spontaneous	Supplied by R. Takahashi, Ohara Institute, Japan
Domen	<u>gs6o</u>	9562	Irradiation	Supplied by D. C. Rasmusson, St. Paul, Minnesota (supplied to Minnesota by K. Mikaelson, Norway)

Table 1. Continued.

Variety	Suggested gene symbol locus and event	C.I. No. of parental variety	Origin	History and/or source
Sekitori	<u>gs6q</u>		Spontaneous	Spontaneous mutant Kogane- mugi. Supplied by R. Takahashi, Ohara Institute, Japan
Barbless	<u>gs6r</u>	5105	Spontaneous	Selected from a head row at Madison, Wisconsin
Compana 1	<u>gs6u</u>	5438	Spontaneous	Selected from four separate commercial sources in Montana
Compana 2	<u>gs6v</u>	5438	Spontaneous	
Compana 3	<u>gs6w</u>	5438	Spontaneous	
Compana 4	<u>gs6x</u>	5438	Spontaneous	
Betzes	<u>gs6s</u>	6398 10863*	Spontaneous	Selected from a commercial field in Pondera County, Montana
Heines Hanna	<u>gs6t</u>	8060 10862*	Spontaneous	Selected from a malting barley nursery plot at Manhattan, Montana. This seed source was imported as a commercial lot from Idaho
C.I. 9132	<u>gs6p</u>	9132*	Irradiation	Supplied from the USDA world collections

(* = C.I. number of the glossy sheath mutant).

to gs1 and gs2. F₁ plants were grown in the greenhouse while F₂ rows and F₃ head hills were field grown. Allard's tables (1956) were used to facilitate linkage calculations.

Morphological determination of the wax particles

Field-grown plants representing the six identified glossy sheath genes were used in producing micrographs utilizing the penetrating and scanning electron microscopes. Those plants used for the light microscopic study were grown in the greenhouse. For the penetrating electron microscope, epidermal surfaces were prepared according to the techniques of Juniper and Bradley (1958). Plant parts were mounted on a glass slide and placed in a shadow cast unit under high vacuum. Palladium was deposited onto the leaf surface at an angle of 45° and carbon was evaporated on the specimen at an angle of 60°. The 100-200 Å thick carbon film thus produced was backed with Formvar from a 2% solution in chloroform, then with Bedacryl from a 5% solution in acetone, and finally with "sellotape". The replica was stripped from the specimen, fastened to a glass slide and immersed in acetone. After the Bedacryl had dissolved, EM-specimen grids were inserted between the tape and the Formvar film. The Formvar was removed with chloroform, preparing the grid for examination.

Scanning electron micrographs of normal and glossy sheath barleys were prepared by R. Bronson of S. C. Johnson and Son, Inc., Racine, Wisconsin. Samples of the six glossy sheath genes plus normal Betzes

were prepared for mailing by suspending six inch segments of the upper most leaf sheath by nylon line through the holes of a peg board partition into moistened cotton. These samples were shipped to Racine by parcel post.

Samples for examination by light microscopy were prepared by stripping away the lower sections of sheath tissue leaving the upper cuticle. The upper cuticle was stained with a saturated solution Sudan III in 70% ethyl alcohol-water for one to two minutes. The sample was then washed with 0.2% phenol in 50% glycerol after which the specimen was mounted for examination.

Agronomic and Malting Characteristics

Nine pairs of normal and glossy sheath mutant barleys were tested for yield. Comparative yield trials were conducted between the normal and the glossy sheath mutants of the varieties Mars and Barbless at Bozeman, Montana, for four and three years, respectively. Three years of trials comparing two glossy sheath mutants and the normal genotype of the variety Compana were conducted at Bozeman. In addition, two years of yield trials at Bozeman compared the normal and one glossy sheath mutant of the variety Compana. Further yield trials comparing the normal and glossy sheath mutants of the varieties Mars, Barbless and Compana were conducted for two years at Moccasin, Montana, and for one year at Havre, Montana.

Comparative yield trials were conducted between the normal and

glossy sheath mutants of the varieties Chevron (3 years), Velvon (3 years), Vantage (1 year), Heines Hanna (3 years), Betzes (2 years), and Pirolina (1 year) at Bozeman. In 1959, yield trials compared the normal and glossy sheath mutants of the varieties Betzes, Compana, Pirolina, Heines Hanna, and Vantage under irrigated and dryland conditions at Sidney, Huntley, and Creston, Montana; dryland conditions at Moccasin and Havre; and irrigated conditions at Bozeman.

Montana yield trials were grown in 3 or 4 row plots with three meters of the center row (s) harvested. Plots were grown in randomized block or split plot designs with four replications. Agronomic data were obtained from six Montana Agricultural Research Centers. Percent protein data were obtained from Kjeldahl analysis by the Montana Cereal Quality Laboratory.

Data from the 1960 Western 2-Row Barley Nursery, grown in the northwestern portion of the United States, includes a comparison of the normal and glossy sheath mutants of the varieties Betzes and Heines Hanna for yield and agronomic characteristics. Yield and agronomic data for the normal and glossy sheath mutant of the variety Pirolina were obtained from the 1961 Western 2-Row Barley Nursery. These data are presented with the permission of the many agronomists who cooperated in growing the Western 2-Row Barley Nurseries.

Malting quality data were summarized from the "Malting Quality of Barley Varieties and Selections Grown in Rocky Mountain and West-

tern Stations in 1960" and "in 1961" for Betzes, Heines Hanna and Piro-line and their respective glossy sheath mutants. The data were obtained by the United States Department of Agriculture Barley and Malt Laboratory, Madison, Wisconsin and is presented with their permission.

The range of elevations used in calculating correlations between elevation of the trial site and yields of the glossy sheath mutants expressed as a percent of their normal genotype were from 61.0 to 2337.1 meters.

Differences between normal and mutant types for agronomic and malting characteristics were evaluated by the paired t-test. Approximate significance of a difference for clarity of wort was determined by an interaction chi-square test.

RESULTS AND DISCUSSION

Allelism

From crosses between the different glossy sheath mutants, F_1 plants were classified as being either normal or glossy. If the F_1 plants were glossy, the two parental sources were considered allelic, if normal, they were considered non-allelic. All F_2 populations from glossy sheath F_1 plants were glossy sheathed while F_2 populations from F_1 normal plants segregated in the ratio of 9 normal plants to 7 glossy sheathed plants. The homozygous double recessive was indistinguishable from those plants carrying only one of the recessive glossy sheath genes in a homozygous condition.

The F_1 plant phenotypes from crosses made between different glossy sheath mutants are recorded as Table 2. The 24 different glossy sheath mutational events were assigned to six different loci, gs1 through gs6. It was found that the reported glossy sheath genes (cer-)gs5 (Rasmusson and Lambert, 1965) and (cer-)gs8 (Takahashi, et.al., 1965; corrected by Takahashi and Hayashi, 1968) were allelic. The glossy sheath mutant Okaiku No. 3 (cer-)gs8, has been reassigned to the locus gs5. The reported glossy sheath genes (cer-)gs6 (Rasmusson and Lambert, 1965) and (cer-)gs7 (Takahashi and Hayashi, 1966) were found to be allelic. The glossy sheath mutant Kogane-mugi, (cer-)gs7, has been reassigned to the locus gs6.

The suggested gene symbolization is to use gs to identify the mutants phenotype, (glossy sheathed), to use numbers one through six

Table 2. F₁ Phenotypic classification of crosses between glossy sheath (gsgs) x glossy sheath (gsgs) lines.

Parent A	Genotype and suggested gene symbol ^{1/}	Genotype of Parent B									
		<u>gs1</u>	<u>gs1</u>	<u>gs2</u>	<u>gs2</u>	<u>gs3</u>	<u>gs3</u>	<u>gs4</u>	<u>gs4</u>	<u>gs5</u>	<u>gs5</u>
		Number of F ₁ plants and phenotype*									
Piroline	<u>gs1</u> <u>gs1</u>	25	gs	32	Gs	41	Gs	20	Gs	20	Gs
C.I. 5818	<u>gs1</u> <u>gs1</u>	24	gs	14	Gs	18	Gs	-----	-----	-----	-----
Velvon	<u>gs1</u> <u>gs1</u>	21	gs	17	Gs	8	Gs	-----	-----	-----	-----
Vantage	<u>gs2</u> <u>gs2</u>	32	Gs	48	gs	45	Gs	20	Gs	20	Gs
Atlas 1	<u>gs2</u> <u>gs2</u>	55	Gs	7	gs	17	Gs	-----	-----	-----	-----
Atlas 2	<u>gs2</u> <u>gs2</u>	10	Gs	10	gs	10	Gs	-----	-----	-----	-----
Ymer	<u>gs2</u> <u>gs2</u>	6	Gs	29	gs	8	Gs	-----	-----	-----	-----
Klargrin	<u>gs2</u> <u>gs2</u>	10	Gs	10	gs	10	Gs	-----	-----	-----	-----
Mars	<u>gs3</u> <u>gs3</u>	43	Gs	69	Gs	16	gs	20	Gs	20	Gs
Chevron	<u>gs3</u> <u>gs3</u>	11	Gs	11	Gs	6	gs	-----	-----	-----	-----
Atlas ⁴ x Algerian	<u>gs3</u> <u>gs3</u>	13	Gs	10	Gs	10	gs	-----	-----	-----	-----
Atlas 57 ²											
Gateway	<u>gs4</u> <u>gs4</u>	20	Gs	20	Gs	20	Gs	-----	-----	9	Gs
Jotun	<u>gs5</u> <u>gs5</u>	10	Gs	10	Gs	10	Gs	5	Gs	10	gs
Okaiku No. 3	<u>gs5</u> <u>gs5</u>	10	Gs	10	Gs	10	Gs	4	Gs	10	gs
Domen	<u>gs6</u> <u>gs6</u>	10	Gs	10	Gs	10	Gs	-----	-----	-----	-----
Kogane-mugi	<u>gs6</u> <u>gs6</u>	2	Gs	3	Gs	5	Gs	-----	-----	10	Gs
Barbless	<u>gs6</u> <u>gs6</u>	10	Gs	46	Gs	39	Gs	20	Gs	20	Gs
Heines Hanna	<u>gs6</u> <u>gs6</u>	3	Gs	8	Gs	12	Gs	-----	-----	-----	-----
C.I. 9132	<u>gs6</u> <u>gs6</u>	7	Gs	5	Gs	5	Gs	-----	-----	-----	-----
Betzes	<u>gs6</u> <u>gs6</u>	7	Gs	8	Gs**	6	Gs	-----	-----	-----	-----
Compana 1	<u>gs6</u> <u>gs6</u>	9	Gs	7	Gs	24	Gs	-----	-----	-----	-----
Compana 2	<u>gs6</u> <u>gs6</u>	-----	-----	-----	-----	7	Gs	-----	-----	-----	-----
Compana 3	<u>gs6</u> <u>gs6</u>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Compana 4	<u>gs6</u> <u>gs6</u>	1	Gs	5	Gs	-----	-----	4	Gs	2	Gs**

* Gs = normal, gs = glossy

** 5 glossy plants noted

^{1/} See Table 3 for gene symbol changes from gene designations by previous authors

to identify the loci, and to use letters 'a' through 'x' to represent the separate mutational events. Previously assigned gene symbols and the newly suggested gene designations of reported glossy sheath mutants are given in Table 3.

All three glossy sheath mutants found at the locus gs1 were spontaneous while three of the five glossy sheath mutants at the locus gs2 were spontaneous. At the locus gs3, two of the three different events studied arose spontaneously. The only mutational event at the locus gs4 was artificially induced. The locus gs5 had two glossy sheath mutations, one occurring spontaneously while eight of the ten glossy sheath mutational events occurring at the locus gs6, arose spontaneously.

The frequency of spontaneous glossy sheath mutants for the loci 1 through 6 is 3:3:2:0:1:8, respectively. To test for the random occurrence of spontaneous glossy sheath mutations at each of the six loci, a chi-square test, corrected for continuity, was used. A chi-square value of 10.353, ($p=0.10-0.05$) was found, indicating that the 17 spontaneous glossy sheath mutants occurred at random over the six loci.

Lundqvist, et.al. (1968) assigned induced *eceriferum* mutants with glossy sheath phenotypic descriptions to seven different loci. Spontaneous glossy sheath mutants have been found at the loci gs1, gs2, gs3, and gs6. It is probable then, that three additional glossy

Table 3. Previous and suggested gene designation for glossy sheath mutants.

Variety	Suggested gene symbol, locus and event	Previous gene symbol	Authority for previous gene symbol
C.I. 5815	<u>gs1a</u> <u>gs1a</u>	<u>gsgs</u> <u>(cer-) gs (cer-) gs</u>	Immer and Henderson (1943) Robertson et.al. (1965)
Velvon	<u>gs1c</u> <u>gs1c</u>	<u>gsgs</u>	Woodward (1957)
Mars	<u>gs3i</u> <u>gs3i</u>	<u>gs3 gs3</u> <u>(cer-) gs3 (cer-) gs3</u>	Walker et.al. (1963) Rasmusson and Lambert (1965)
Gateway	<u>gs41</u> <u>gs41</u>	<u>gs4 gs4</u> <u>(cer-) gs3 (cer-) gs3</u>	Walker et.al. (1963) Rasmusson and Lambert (1965)
Jotun	<u>gs5m</u> <u>gs5m</u>	<u>(cer-) gs5 (cer-) gs5</u>	Rasmusson and Lambert (1965)
Okaiku No. 3	<u>gs5n</u> <u>gs5n</u>	<u>(cer-) gs8 (cer-) gs8</u>	Takahashi and Hayashi (1968)
Domen	<u>gs6o</u> <u>gs6o</u>	<u>(cer-) gs6 (cer-) gs6</u>	Rasmusson and Lambert (1965)
Kogane-mugi	<u>gs6q</u> <u>gs6q</u>	<u>(cer-) gs7 (cer-) gs7</u>	Takahashi and Hayashi (1966)

sheath loci, one of them being gs4, will be found arising spontaneously.

Four additional loci have been found within the European induced eceriferum mutants that cause a close phenotypic approximation of the glossy sheath mutations. One type of near glossy sheath mutant, described as having reduced wax on the spike, glossy leaf sheath and stem with normal leaf blades, is reported on in this thesis. This locus, gs5, contains a spontaneous event and may be allelic with the near glossy sheath mutant described by Lundqvist et.al. (1968).

A second type of near glossy sheath mutant described as having glossy spike, reduced wax on the leaf sheath and stem with normal leaf blades, was found at three loci. No spontaneous mutants fitting this description have been reported.

Linkage

Linkage data involving the six glossy sheath loci, plus other barley genes, are shown as Table 4.

Glossy sheath 1, gs1.

Glossy sheath 1, gs1, segregated independently from marker genes and translocation breakpoints located on the seven barley chromosomes. Albrechtsen (1957) found gs1 to be linked with Colorado zoned leaf, zb_c, on chromosome 4 (recombination value 23.9). Anderson (1958) found gs1 to be linked with the translocation breakpoint of T3-4a (recombination value 15.0). Woodward (1957) reported gs1 showed a tendency to be linked with genes in group IV (chromosome 4). Using F₂ data, it was

Table 4. Linkage intensities involving the six glossy sheath genes plus other barley genes.

Cross	or	Number of plants									Linkage x ²	Proba- bility	% Recombi- nation
		AA	Aa	AA	Aa	aa	aa	aaBB	aaBb	aabb			
		Fert	Fert	Semi	Semi	Fert	Semi						
Expected ratio													
aaBB x AAbb	F ₃	1	2	2	4	1	2	1	2	1			
Expected ratio													
aa x Translocation													
Crosses with <u>gslgsl</u>	F ₃	1	2	1	2	1	1						
<u>Chromosome 1</u>													
<u>gslgsl</u> NN													
x <u>GslGsl</u> nn	F ₃	8	6	12	14	0	0	4	13	0	5.811	(.25-.1)	Ind.
<u>gslgsl</u> LK2Lk2													
x <u>GslGsl</u> lk2lk2	F ₃	8	6	10	16	0	0	4	13	0	5.641	(.25-.1)	Ind.
<u>gslgsl</u> msl0msl0													
x <u>GslGsl</u> msl0msl0	F ₃	9	5	11	15	--	--	6	11	--	2.958	(.25-.1)	Ind.
<u>gslgsl</u>													
x T1-6d <u>GslGsl</u>	F ₂	<u>73</u>		<u>87</u>		19	31				0.765	(.5-.25)	Ind.
<u>gslgsl</u>													
x T1-3c <u>GslGsl</u>	F ₂	<u>68</u>		<u>51</u>		37	28				0.181	(.75-.5)	Ind.
<u>Chromosome 2</u>													
<u>gslgsl</u> IVV													
x <u>GslGsl</u> vv	F ₃	20	14	16	20	10	14	9	14	0	7.620	(.25-.1)	Ind.
<u>gslgsl</u>													
x T2-3g <u>GslGsl</u>	F ₂	<u>97</u>		<u>100</u>		35	30				0.412	(.75-.5)	Ind.
<u>gslgsl</u>													
x T2-5a <u>GslGsl</u>	F ₂	<u>79</u>		<u>72</u>		25	37				2.893	(.1-.05)	Ind.

Table 4. Continued

Cross	or	Number of plants								Linkage x ²	Proba- bility	% Recombi- nation
		AA	Aa	AA	Aa	aa	aa					
		Fert	Fert	Semi	Semi	Fert	Semi					
Chromosome 3												
<u>gslgsl</u>												
x T2-3g <u>GslGsl</u>	F ₂	97		100		35	30			0.412	(.75-.5) Ind.	
<u>gslgsl</u>												
x T1-3c <u>GslGsl</u>	F ₂	68		51		37	28			0.181	(.75-.5) Ind.	
Chromosome 4												
<u>gslgslkk</u>												
x <u>GslGslKK</u>	F ₂		100			45		49	20	2.300	(.25-.1) 43.2+4.7	
<u>gslgsl</u>												
x T4-5a <u>GslGsl</u>	F ₂	30		40		12	9			1.322	(.25-.1) Ind.	
<u>gslgsl</u>												
x T4-7b <u>GslGsl</u>	F ₂	94		112		34	29			1.350	(.25-.1) Ind.	
Chromosome 5												
<u>gslgslMslMsl</u>												
x <u>GslGslmslmsl</u>	F ₃	15	29	20	30	--	--	11	18	--	0.174 (.95-.9) Ind.	
<u>gslgsl</u>												
x T2-5a <u>GslGsl</u>	F ₂	79		72		25	37			2.893 (11+.05) Ind.		
<u>gslgsl</u>												
x T4-5a <u>GslGsl</u>	F ₂	30		40		12	9			1.322 (.25-.1) Ind.		
Chromosome 6												
<u>gslgsl100</u>												
x <u>GslGsl100</u>	F ₃	17	15	10	27	12	13	8	13	8	7.385 (.25-.1) Ind.	

-24-

Table 4. Continued

Cross	or	Number of plants									Linkage χ^2	Proba- bility	% Recombi- nation
		AA	Aa	AA	Aa	aa	aa						
		Fert	Fert	Semi	Semi	Fert	Semi						
		AABB	AABb	AaBB	AaBb	AAbb	Aabb	aaBB	aaBb	aabb			
<u>gslgsl</u>													
x T1-6d <u>GslGsl</u>	F ₂	73		87		19	31				0.765	(.5-.25)	Ind.
<u>gslgsl</u>													
x T6-7b <u>GslGsl</u>	F ₂	26		29		10	6				1.0581	(.5-.25)	Ind.
<u>Chromosome 7</u>													
<u>gslgslMs16Ms16</u>													
x <u>GslGslms16ms16</u>	F ₃	23	21	53	73	--	--	22	25	--	0.443	(.9-.75)	Ind.
<u>gslgslMs19Ms19</u>													
x <u>GslGslms19ms19</u>	F ₃	17	51	39	93	--	--	30	50	--	3.239	(.25-.1)	Ind.
<u>gslgsl</u>													
x T4-7b <u>GslGsl</u>	F ₂	94		112		34	29				1.350	(.25-.1)	Ind.
<u>gslgsl</u>													
x T6-7b <u>GslGsl</u>	F ₂	26		29		10	6				1.058	(.5-.25)	Ind.
<u>Crosses with <u>gs2gs2</u></u>													
<u>Chromosome 1</u>													
<u>gs2gs2</u>													
x T1-6d <u>Gs2Gs2</u>	F ₃	24	32	17	28						0.066	(.975-.95)	Ind.
<u>gs2gs2</u>													
x T1-3c <u>Gs2Gs2</u>	F ₃	19	41	19	40						1.143	(.75-.5)	Ind.
<u>Chromosome 2</u>													
<u>gs2gs2vv</u>													
x <u>Gs2Gs2VV</u>	F ₃	20	19	19	30	4	8				5.256	(.1-.05)	Ind.

Table 4. Continued

Cross	or	Number of plants									Linkage χ^2	Proba- bility	% Recombi- nation
		AA	Aa	AA	Aa	aa	aa						
		Fert	Fert	Semi	Semi	Fert	Semi	aaBB	aaBa	aabb			
<u>gs2gs2vv</u> x <u>Gs2Gs2VV</u>	F ₃	8	12	16	29	4	6	6	16	3	1.200	(.9-.75)	Ind.
<u>Chromosome 3</u>													
<u>gs2gs2</u> x T1-3c <u>Gs2Gs2</u>	F ₃	19	41	19	40						1.143	(.75-.5)	Ind.
<u>Chromosome 4</u>													
<u>gs2gs2</u> x T4-7b <u>Gs2Gs2</u>	F ₂	49		53		23	20				0.388	(.75-.5)	Ind.
<u>gs2gs2</u> x T4-5a <u>Gs2Gs2</u>	F ₃	27	46	20	36						0.062	(.9-.75)	Ind.
<u>Chromosome 5</u>													
<u>gs2gs2</u> x T4-5a <u>Gs2Gs2</u>	F ₃	27	46	20	36						0.062	(.9-.75)	Ind.
<u>gs2gs2ms1ms1</u> x <u>Gs2Gs2Ms1Ms1</u>	F ₃	12	29	21	39						0.438	(.9-.75)	Ind.
<u>gs2gs2ms1ms1</u> x <u>Gs2Gs2Ms1Ms1</u>	F ₃	18	20	29	52						1.420	(.25-.1)	Ind.
<u>gs2gs2ms1ms1</u> x <u>Gs2Gs2Ms1Ms1</u>	F ₃	16	31	36	46						1.302	(.5-.25)	Ind.
<u>gs2gs2ms1ms1</u> x <u>Gs2Gs2Ms1Ms1</u>	F ₃	18	25	20	37						0.389	(.75-.5)	Ind.

Table 4. Continued

Cross	or	Number of plants									Linkage χ^2	Proba- bility	% Recombi- nation
		AA	Aa	AA	Aa	aa	aa						
		Fert	Fert	Semi	Semi	Fert	Semi	aaBB	aaBb	aabb			
<u>gs2gs2mslms1</u> x <u>Gs2Gs2MslMs1</u>	F ₃	10	14	17	34				6	19	1.690	(.5-.25)	Ind.
<u>Chromosome 6</u>													
<u>gs2gs2</u> x T1-6d <u>Gs2Gs2</u>	F ₃	24	32	17	28						0.066	(.975-.95)	Ind.
<u>gs2gs2</u> x T6-7b <u>Gs2Gs2</u>	F ₃	10	20	14	31	15	11				2.199	(.5-.25)	Ind.
<u>Chromosome 7</u>													
<u>gs2gs2</u> x T4-7b <u>Gs2Gs2</u>	F ₂	49		53		23	20				0.388	(.75-.5)	Ind.
<u>gs2gs2</u> x T6-7b <u>Gs2Gs2</u>	F ₃	10	20	14	31	15	11				2.199	(.5-.25)	Ind.
<u>gs2gs2rrr</u> x <u>Gs2Gs2RRR</u>	F ₃	9	21	11	36	13	10	--	--	--	2.254	(.5-.25)	Ind.
<u>gs2gs2rr</u> x <u>Gs2Gs2RRR</u>	F ₃	8	9	9	25	7	17	3	16	6	4.780	(.5-.25)	Ind.
<u>Crosses with gs3gs3</u>													
<u>Chromosome 1</u>													
<u>gs3gs3BrBr</u> x <u>Gs3Gs3brbr</u>	F ₃	0	3	7	29	22	4	18	5	0	11.558	(.025-.01)	11.3+10.6
<u>gs3gs3MsbMsb</u> x <u>Gs3Gs3msbmsb</u>	F ₃	14	23	19	54	--	--	13	22	--	2.181	(.5-.25)	Ind.
<u>gs3gs3NN</u> x <u>Gs3Gs3nn</u>	F ₃	1	4	8	5	0	0	2	4	1	5.158	(.5-.25)	Ind.

Table 4. Continued

Cross	or	Number of plants									Linkage χ^2	Proba- bility	% Recombi- nation	
		AA	Aa	AA	Aa	aa	aa	Fert	Semi					
		Fert	Fert	Semi	Semi	Fert	Semi		aaBB	aaBb				aabb
<u>gs3gs3LK2LK2</u>														
x <u>Gs3Gs3lk2lk2</u>	F ₃	2	3	8	4	0	1	1	5	1	5.844	(.25-.1)	Ind.	
<u>gs3gs3Ms10Ms10</u>														
x <u>Gs3Gs3ms10ms10</u>	F ₃	21	29	39	51	--	--	23	32	--	0.006	(.99)	Ind.	
<u>gs3gs3</u>														
x T1-7s <u>Gs3Gs3</u>	F ₃	8	10	4	6	6	3				0.298	(.9-.75)	Ind.	
<u>gs3gs3</u>														
x T1-7c <u>Gs3Gs3</u>	F ₃	28	39	13	26	30	14				2.166	(.5-.25)	Ind.	
<u>gs3gs3</u>														
x T1-6d <u>Gs3Gs3</u>	F ₃	14	30	6	14	8	6				1.846	(.5-.25)	Ind.	
<u>Chromosome 2</u>														
<u>gs3gs3Ms2Ms2</u>														
x <u>Gs3Gs3ms2ms2</u>	F ₂	66				24		22		11	0.533	(.5-.25)	Ind.	
<u>gs3gs3VV</u>														
x <u>Gs3Gs3vv</u>	F ₂	62				29		17		15	3.144	(.1-.05)	Ind.	
<u>Crosses with gs4gs4</u>														
<u>gs4gs4Ms11Ms11</u>														
x <u>Gs4Gs4ms11ms11</u>	F ₃	2	3	8	13	--	--	3	6	--	0.112	(.95-.9)	Ind.	
<u>Crosses with gs5gs5</u>														
<u>Chromosome 2</u>														
<u>gs5gs5Ms2Ms2</u>														
x <u>Gs5Gs5ms2ms2</u>	F ₃	0	18	8	108	--	--	43	3	--	124.962	($\geq$.005)	8.4 $\pm$ 1.8	
<u>gs5gs5Ms2Ms2</u>														
x <u>Gs5Gs5ms2ms2</u>	F ₃	0	4	0	52	--	--	29	2	--	103.539	($\geq$.005)	4.6 $\pm$ 1.8	

Table 4. Continued

Cross	or	Number of plants									Linkage χ^2	Proba- bility	% Recombi- nation
		AA	Aa	AA	Aa	aa	aa						
		Fert	Fert	Semi	Semi	Fert	Semi	aaBB	aaBb	aabb			
<u>gs5gs5Ms2Ms2</u>													
x <u>Gs5Gs5ms2ms2</u>	F ₃	1	13	10	90	--	--	47	17	--	98.465	(>.005)	12.8 $\pm$ 2.3
<u>gs5gs5vv</u>													
x <u>Gs5Gs5VV</u>	F ₃	14	4	37	63	0	16	2	15	29	57.799	(>.005)	24.4 $\pm$ 2.6
<u>gs5gs5vv</u>													
x <u>Gs5Gs5VV</u>	F ₃	6	8	23	63	0	14	4	29	31	40.358	(>.005)	27.3 $\pm$ 3.7
<u>Crosses with gs6gs6</u>													
<u>Chromosome 2</u>													
<u>gs6gs6VV</u>													
x <u>Gs6Gs6vv</u>	F ₃	16	13	28	34	5	12	15	14	5	0.888	(.975-.95)	Ind.
<u>gs6gs6VV</u>													
x <u>Gs6Gs6vv</u>	F ₃	13	25	28	52	5	6	17	31	9	0.623	(.975-.95)	Ind.
<u>gs6gs6Ms2Ms2</u>													
x <u>Gs6Gs6ms2ms2</u>	F ₃	14	32	29	69	--	--	23	38	--	1.248	(.75-.5)	Ind.
<u>gs6gs6Ms2Ms2</u>													
x <u>Gs6Gs6ms2ms2</u>	F ₃	12	31	31	55	--	--	17	40		1.081	(.75-.5)	Ind.
<u>Chromosome 7</u>													
<u>gs6gs6Ms16Ms16</u>													
x <u>Gs6Gs6ms16ms16</u>	F ₃	17	28	40	45	--	--	23	42	--	1.624	(.5-.25)	Ind.
<u>gs6gs6Ms19Ms19</u>													
x <u>Gs6Gs6ms19ms19</u>	F ₃	14	40	37	68	--	--	23	37	--	2.199	(.25-.1)	Ind.
<u>Crosses with other barley marker genes</u>													
<u>Chromosome 2</u>													
<u>vvMs2Ms2</u>													

Table 4. Continued

Cross	or	Number of plants									Linkage χ^2	Proba- bility	% Recombi- nation
		AA	Aa	AA	Aa	aa	aa						
		Fert	Fert	Semi	Semi	Fert	Semi						
		AABB	AABb	AaBB	AaBb	AAbb	Aabb	aaBB	aaBb	aabb			
<u>x VVms2ms2</u>	F ₃	5	48	17	65	--	--	29	16	--	17.179	(>.005)	32.5 $\pm$ 4.1
<u>vvMs2Ms2</u>													
<u>x VVms2ms2</u>	F ₃	9	24	27	73	--	--	22	23	--	7.342	(.05-.025)	35.2 $\pm$ 4.4
<u>vvms2ms2</u>													
<u>x VVMs2Ms2</u>	F ₃	43	41	21	69	--	--	2	29	--	29.291	(>.005)	27.5 $\pm$ 3.4
<u>vvms2ms2</u>													
<u>x VVMs2Ms2</u>	F ₃	33	25	22	86	--	--	5	15	--	27.732	(>.005)	23.3 $\pm$ 3.2
<u>Chromosome 7</u>													
<u>rr x T4-7bRR</u>	F ₃	7	27	14	32	11	12				0.787	(.75-.5)	Ind.
<u>rr x T6-7bRR</u>	F ₃	10	21	10	29	15	15				0.640	(.75-.5)	Ind.
<u>Chromosome 5</u>													
<u>mslms1</u>													
<u>x T2-5aMs1Ms1</u>	F ₂	104		109							0.118	(.75-.5)	Ind.
<u>mslms1</u>													
<u>x T4-5aMs1Ms1</u>	F ₂	42		49							0.538	(.5-.25)	Ind.

determined that the recombination value between hooded, K, on chromosome 4, and gs1 was 42.2 ± 4.7 , though linkage chi-square for this cross was not significant. F_3 data may provide further insight into the relationship of the genes K and gs1. One would have to agree with Woodward that there is a tendency for gs1 to be linked with genes on chromosome 4. L ndqvist and von Wettstein (1962) reported gs1 and cer-q to be allelic.

Glossy sheath 2, gs2

Vantage gs2gs2 mslmsl vv was crossed to a translocation tester set in an attempt to locate gs2 on a chromosome. It was determined in F_3 that gs2 segregated independently from the breakpoints of T1-6d, T1-3c, T2-5a, T4-5a, T4-7b and T6-7b (Table 4). Recombination between gs2 and the breakpoint of T2-3g did not coincide with any theoretical ratio (Table 5). Six-row, v, on chromosome 2 (Nilan, 1964) and male sterile 1, msl, on chromosome 5 (Nilan, 1964) segregated in the same unusual manner with the breakpoints of T2-3g and T2-5a as did gs2 with the breakpoint of T2-3g (Table 5).

An explanation for these ratios postulates a gametic lethal gene in or around the centromere region of chromosome 2 that is subject to a variegated position effect. According to this hypothesis this lethal gene cannot function in a homozygous or heterozygous translocation. The only genotype in which the lethal gene can function is in a homozygous non-translocation whose parent was a heter-

Table 5. From the crosses involving the translocations T2-3g and T2-5a that deviated from the theoretical ratios.

Cross	Number of plants						Linkage χ^2	Proba- bility	Percent recombi- nation
	AA	Aa	AA	Aa	aa	aa			
	Fert	Fert	Semi	Semi	Fert	Semi			
<u>Theoretical ratios:</u>									
Expected ratio when gene and translocation breakpoint linked:									
	F ₃	1	0	0	2	1	0		
Expected ratio when gene and translocation breakpoint independent:									
	F ₃	1	2	1	2	1	1		
<u>Observed ratios:</u>									
Vantage <u>gs2gs2</u> x	F ₂	53		81		0	26	5.210	(.025-.01) 56.2 $\pm$ 7.9
T2-3g <u>Gs2Gs2</u>	F ₃	29	28	29	44	4	25	14.384	($\geq$.005) Semi 38.0 $\pm$ 13.2
Vantage <u>mslmsl</u> x	F ₂	53		107				18.224	($\geq$.005) 0.5 $\pm$.8
T2-3g <u>MslMsl</u>	F ₃	56	5	12	83			88.321	($\geq$.005) Semi 13.0 $\pm$ 3.6 Fert 2.2 $\pm$ 0.1
Vantage <u>vv</u> x	F ₂	51		100		2	7	2.648	(.5-2.5)
T2-3g <u>VV</u>	F ₃	50	9	15	75	2	5	80.077	($\geq$.005) Semi 17.2 $\pm$ 4.4 Fert 4.3 $\pm$ 1.4
Vantage <u>gs2gs2</u> x	F ₂	48		78		14	16	1.214	(.5-.25) Independent
T2-5a <u>Gs2Gs2</u>	F ₃	18	32	26	45	--	--	0.038	(.9-.75) Independent
Vantage <u>mslmsl</u> x	F ₂	62		94		--	--	6.564	(.025-.01) 11.2 $\pm$ 7.5
T2-5a <u>MslMsl</u>	F ₃	48	2	19	54	--	--	49.187	($\geq$.005) Semi 29.1 $\pm$ 7.7 Fert 1.0 $\pm$ 0.7
Vantage <u>vv</u> x	F ₂	61		75		1	19	3.264	(.1-.05)
T2-5a <u>VV</u>	F ₃	33	17	26	29	0	18	10.090	(.01-.005) Semi 57+ Fert 11.6 $\pm$ 3.1

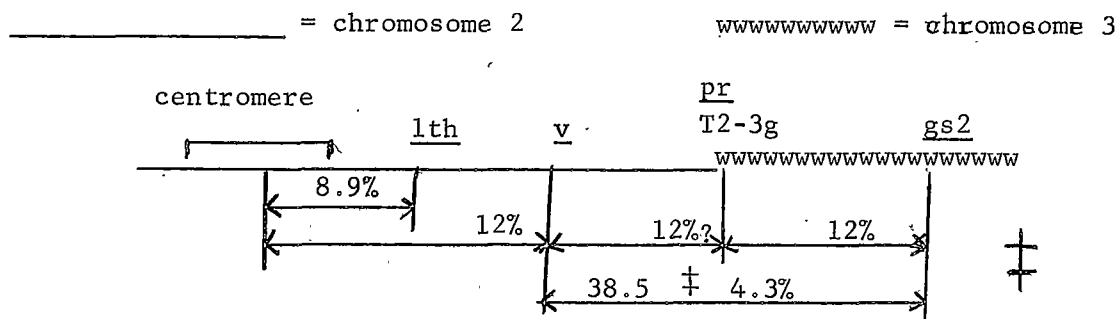
ozygous translocation.

Assuming varying degrees of crossing over between the lethal gene, *lth*, and the observable gene (*gs2 msl* or *v*), but no crossing over between the lethal gene and the translocation breakpoint, most F_2 ratios and T2-3g F_3 ratios could be explained. The gene *msl* shows close linkage (9%) with T2-3g, suggesting that this male sterile gene may actually be *ms2*, or some other male sterile gene located on chromosome 2. The postulated recombination values between the observable gene (*gs2*, *msl*, or *v*) and the lethal gene, *lth*, using the lethal variegated position effect hypothesis, along with a chi-square for goodness of fit, are shown as Table 6.

The gene *gs2* appears to be linked with the gametic lethal when T2-3g is the involved translocation but does not appear to be linked with the lethal when T2-5a is the involved translocation. This suggests that *gs2* is on chromosome 3. The gene *v*, on chromosome 2 (Nilan, 1964), segregated independently from *gs2* in crosses other than Vantage x T2-3g. From linkage data derived from the cross Vantage *gs2gs2 vv mslmsl* x T2-3g, the following map can be constructed. The location of T2-3g and purple stem, *Pr*, is taken from Nilan (1964).

Table 6. Linkage intensities of T2-3g and T2-5a breakpoints with v, msl and gs2; position effect hypothesis.

Cross		Postulated % recombination	χ^2	Probability
Vantage <u>vv</u> x T2-5a <u>VV</u>	F ₂	20	5.148	(.25-.1)
	F ₃	Not determined		
Vantage <u>mslmsl</u> x T2-5a <u>MslMsl</u>	F ₂	10	1.219	(.5-.25)
	F ₃	Not determined		
Vantage <u>vv</u> x T2-3g <u>VV</u>	F ₂	12	3.707	(.5-.25)
	F ₃	12	10.438	(.1-.05)
Vantage <u>mslmsl</u> x T2-3g <u>MslMsl</u>	F ₂	5	0.111	(.75-.5)
	F ₃	9	6.307	(.1-.05)
Vantage <u>gs2gs2</u> x T2-3g <u>Gs2Gs2</u>	F ₂	25	5.571	(.25-.1)
	F ₃	34	9.851	(.1-.05)



To test the variegated position effect hypothesis, crosses were made onto homozygous male sterile, non-translocated barley. The male parents were taken from Vantage gs2gs2 mslmsl VV x T2-3g fertile F_3 rows. According to the variegated position effect hypothesis, all F_3 fertile plants, or the male parents, should be translocation homozygotes if there is no crossing over between the lethal, lth, and the translocation breakpoint or centromere. When balanced tertiary trisomic ms2, BTTms2, was used as the female parent there were 24 semi-sterile offspring (probably translocation heterozygotes) and 2 fertile offspring (probably homozygous for non-translocated chromosome). This is a 7.7% recombination between the lethal gene, lth, and the centromere of chromosome 2.

When balanced tertiary trisomic ms1, Bttms1, was used as the female parent there were 27 semi-sterile offspring and 3 fertile offspring. This is a 10.0% recombination between the lethal gene, lth, and the centromere of chromosome 2. Combined data gave an 8.9% recombination between lth and the centromere of chromosome 2.

An unexpected degree of lethality of F_1 plants was encountered

with the BTTms2 and BTTms1 crosses (55.9%). This high degree lethality is yet unexplained, but could be due to the postulated lethal gene.

Glossy sheath 2 appears to be independent of translocation breakpoints on chromosomes 1, 4, 5, 6, and 7. Utilizing a possible variegated position effect, gs2 has been linked with translocation breakpoints on chromosome 2 or, more probably, chromosome 3. Loci from the European eceriferum series that could be allelic with gs2 are cer-zm and cer-r.

Glossy sheath 3, gs3

Glossy sheath 3, gs3, has been reported on chromosome 1 (Walker et.al., 1963, Rasmusson and Lambert, 1965, McProud and Eslick, 1968). Our F₃ data show gs3 to be linked (11.3+10.6) with brachytic, br, on chromosome 1. Independent segregation was noted between gs3 and male sterile 10, msl0, male sterile b, ms-b, naked caryopsis, n, and short awn, lk2, all on chromosome 1. T1-7a, T1-7c, and T1-6d breakpoints segregated independently from gs3.

Bodil Sogaard(1971) has reported linkages involving various eceriferum mutants. The gene cer-a, a glossy sheath mutant, was found in F₂ to be linked to brachytic, br, with no recombination and with dense spike, ert-a, with 36+ 3% recombination. These data suggest cer-a and gs3 are allelic.

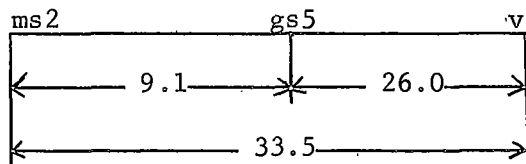
Glossy sheath 4, *gs4*

The mutant *gs4* is linked with orange lemma, *o*, (no recombination) and with glossy leaf-4, *gl4*, (recombination 29.4 ± 2.6) both on chromosome 6, (Walker et.al., 1963). To date, none of the European eceriferum mutants have been reported as located on chromosome 6.

Glossy sheath 5, *gs5*

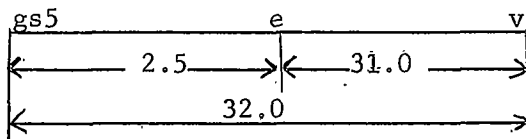
Crosses were made with *gs5* and three different sources of male sterile 2, *ms2*, on chromosome 2, giving recombination values of 8.4 ± 1.8 , 4.6 ± 1.8 , and 12.8 ± 2.3 . Two different sources of six-row, *v*, on chromosome 2, were crossed to *gs5* with the resulting recombination value of 24.4 ± 2.6 and 27.3 ± 3.7 . In the above crosses *ms2* and *v* had recombination values of 32.5 ± 4.1 and 35.2 ± 4.4 .

Combined data gave the following recombination values: between *gs5* and *ms2*, 9.1 ± 0.7 ; between *gs5* and *v*, 26.0 ± 1.4 ; and between *ms2* and *v*, 33.5 ± 2.1 . The probable gene order for these three genes is

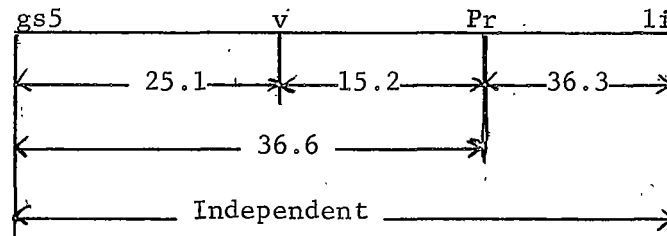


in the *v* to centromere region of the long arm of chromosome 2.

Rasmusson and Lambert (1965) reported the following gene order with *gs5*, *v*, and long-awned glume, *e*;



Takahashi and Hayashi (1968) reported the following gene order with the five genes (cer-) gs8 [gs5], v, e, purple stem, Pr, and liguleless li;



R. T. Ramage (personal communication) found Ms2 and E to be very tightly linked; thus the gene order found in our study is probably correct.

The only gene from the European eceriferum stocks that fits the general phenotypic description of gs5 is cer-s (U. Lundqvist et.al., 1968). Bodil Sogaard(1971) reports 2.1+1.1 and no recombination of cer-s with the breakpoints of T2-7d and T2-3f, respectively. Recombination of cer-s and v was reported as 24+4.0. These results are in general agreement with our, and other published, gs5 results and it is quite probable that gs5 is allelic to cer-s.

Glossy sheath 6, gs6

The gene gs6 has been located on chromosome 2 by trisomic analyses (Takahashi and Hayashi, 1966). Data here presented show gs6 to segregate independently from ms2 and v, both on chromosome 2. Of the seven loci reported by Lundqvist et.al., (1968) that give the same general phenotypic description as gs6, four are probably not

allelic with gs6. Lundqvist and von Wettstein (1962) reported that cer-q and gs1 are allelic. From linkage data presented by Sogaard (1971) cer-a is located on chromosome 1 (and possibly allelic with gs3) while cer-b and cer-x are located on chromosome 7. This means that cer-c, cer-z and cer-z1 would be the most apt to be allelic with gs6.

Phenotypic Expression

Phenotypically all of the glossy sheath mutants are very similar with the exception of the mutant forms of Jotun and Okaiku No. 3. These two glossy sheath mutants, which are allelic with one another, display a normal wax appearance on the stems and on the heads. In addition, the leaf sheaths, though glossy, appear to have scattered wax particles over the surface.

The other glossy sheath mutants, with two exceptions, appear to have glossy stems, spikes, and leaf sheaths. The basal leaf sheaths of the glossy sheath mutant of Gateway show the top half to be waxy while the lower half, adjacent to the node, is glossy. The upper leaf sheaths are all glossy. C.I. 9132 becomes progressively normal in appearance as the plant matures. A summary of 1970 field phenotypic readings is shown as Table 7.

Photographs of the surface of the leaf sheaths were obtained by the use of a light microscope, a scanning electron microscope and a penetrating electron microscope. To show that the photographed

Table 7. Phenotypic description of field grown glossy sheath mutants.

Gene, locus and event	Variety	Stem	Spike	Leaves	Lower leaf sheaths	Upper leaf sheaths
<u>gslb</u>	Pirolina	gs	gs	Gs	gs	gs
<u>gsla</u>	C.I. 5818	gs	gs	Gs	gs	gs
<u>gslc</u>	Velvon	gs	gs	Gs	gs	gs
<u>gs2d</u>	Vantage	gs	gs	Gs	gs	gs
<u>gs2e</u>	Ymer	gs	gs	Gs	gs	gs
<u>gs2f</u>	Atlas 1	gs	gs	Gs	gs	gs
<u>gs2g</u>	Atlas 2	gs	gs	Gs	gs	gs
<u>gs2h</u>	Klargrin	gs	gs	Gs	gs	gs
<u>gs3i</u>	Mars	gs	gs	Gs	gs	gs
<u>gs3j</u>	Chevron	gs	gs	Gs	gs	gs
<u>gs3k</u>	(Atlas ⁴ x Algerian) x Atlas 57 ²	gs	gs	Gs	gs	gs
<u>gs4l</u>	Gateway	gs	gs	Gs	+	gs
<u>gs5m</u>	Jotun	Gs	Gs	Gs	‡	‡
<u>gs5n</u>	Okaiku No. 3	Gs	Gs	Gs	‡	‡
<u>gs6r</u>	Barbless	gs	gs	Gs	gs	gs
<u>gs6s</u>	Betzes	gs	gs	Gs	gs	gs
<u>gs6t</u>	Heines Hanna	gs	gs	Gs	gs	gs
<u>gs6p</u>	C.I. 9132		gs	Gs	⤿	⤿
<u>gs6o</u>	Domen	gs	gs	Gs	gs	gs
<u>gs6u</u>	Compana 1	gs	gs	Gs	gs	gs
<u>gs6v</u>	Compana 2	gs	gs	Gs	gs	gs
<u>gs6x</u>	Compana 4	gs	gs	Gs	gs	gs
<u>gs6q</u>	Compana <u>ms10ms10</u> x					

* Gs = normal, gs = glossy

+ Upper ½ waxy, lower ½ glossy

‡ Glossy with scattered wax particles

⤿ Becomes progressively glaucous as matures

objects were on the sheath's surface, a picture was taken of unscraped sheath tissue (Figure 1). In this picture the chlorophyll and cell walls are not focused while the wax particles, as seen over the vascular bundle, are focused. This demonstrates that these particles are on the surface.

DeBary (reported by Lundqvist and von Wettstein 1962, Eglinton and Hamilton, 1967) and Lundqvist and von Wettstein (1962) described two wax types that they observed on the sheath's surface, one being a heaped wax layer (Figures 2, 3, 4) and the other a single granulated layer (Figures 1, 2, 3, 4, 5, and 6). Both the normal genotype and glossy sheath mutants displayed these two wax types.

It has been noted by von Wettstein-Knowles (1971) that the *eceriferum* mutant cer-c, a glossy sheath mutant, has a 34% reduction in the amount of surface internodal wax. When scanning electron micrographs of the six glossy sheath loci (Figures 8-13) are compared to the scanning electron micrograph of normal Betzes (Figure 7), this reduction of epidermal wax becomes apparent.

The stem nodes and internodes of normal barley have been described by Lundqvist and von Wettstein (1962) as being coated with a thick heaped layer of needles. It was noted by von Wettstein Knowles (1971) that the entire length of the wax rods may lay flat along the cuticle or the rods may stand erect.

As shown in Figure 14, long thin rods or needles of wax are highly

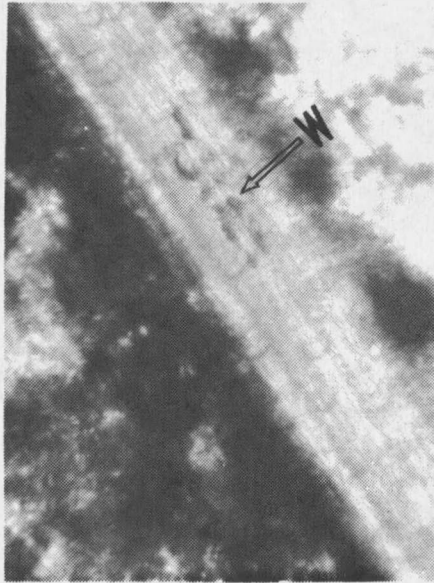


Figure 1: Wax particles (W) over vascular bundle of C.I.5818, gslgsl. Cell material below not focused. (Light microscope, 10x)

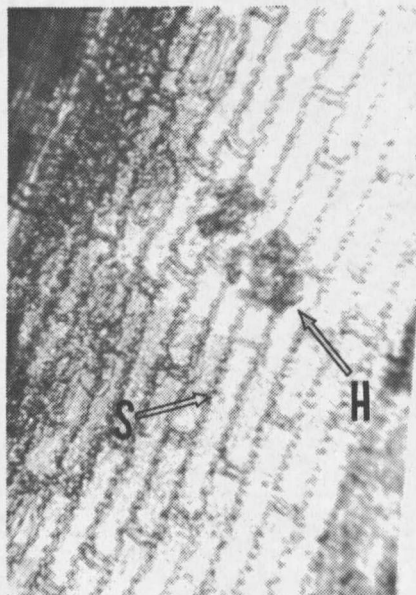


Figure 2: Two wax partical types of normal Betzes, heaped wax layer (H) and single granulated layer (S). (Light microscope, 10x)

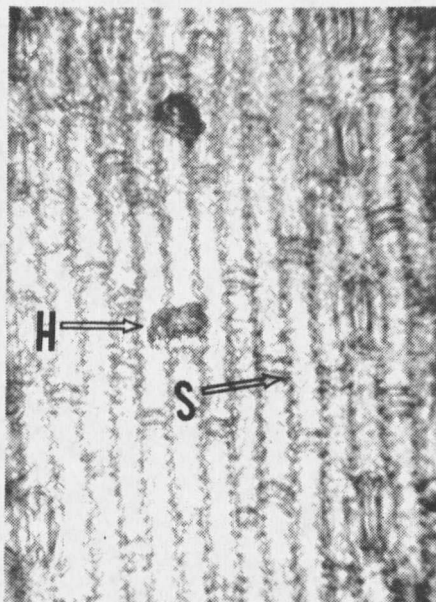


Figure 3: Two wax types of glossy Pirolina gslgsl, heaped wax layer (H) and single granulated layer (S). (Light microscope, 10x)

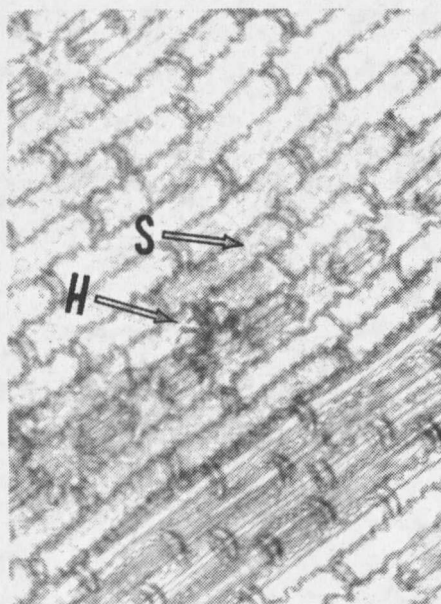


Figure 4: Two wax types of glossy Barbless gs6gs6, heaped wax layer (H) and single granulated layer (S). (Light microscope, 40x)

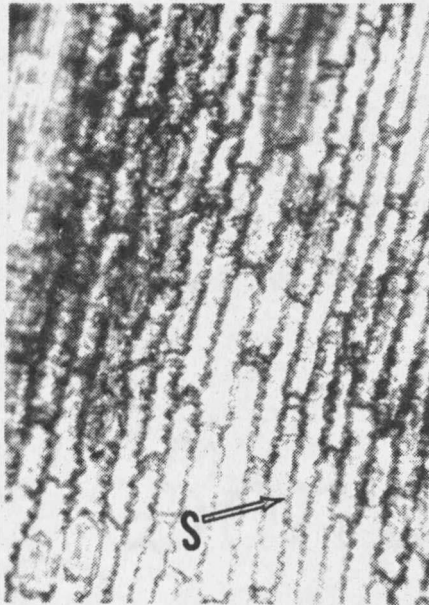


Figure 5. Single granulated layer (S) of (4 Atlas x Algerian) x 2 Atlas 57 gs3gs3. (Light microscope, 10x)

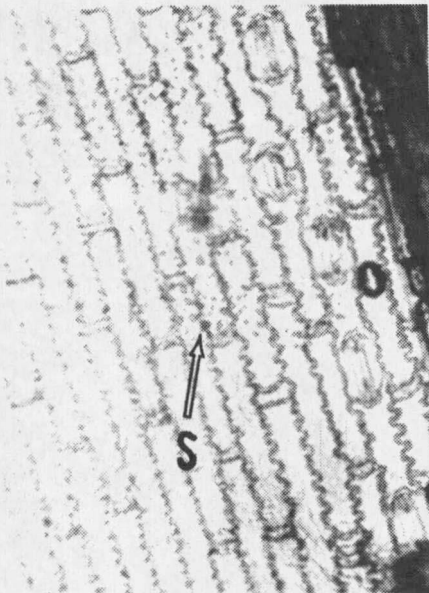


Figure 6: Single granulated layer (S) of glossy Gateway gs4gs4 (Light microscope, 10x)

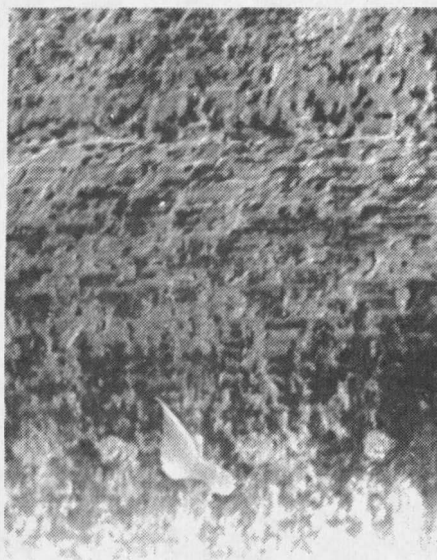


Figure 7: Normal amount of wax present on glaucous Betzes. (Scanning electron microscope, 100x)



Figure 8: Heaped wax layer (H) and reduced wax presence of glossy Pirolina gslgsl (Scanning electron microscope, 100x)

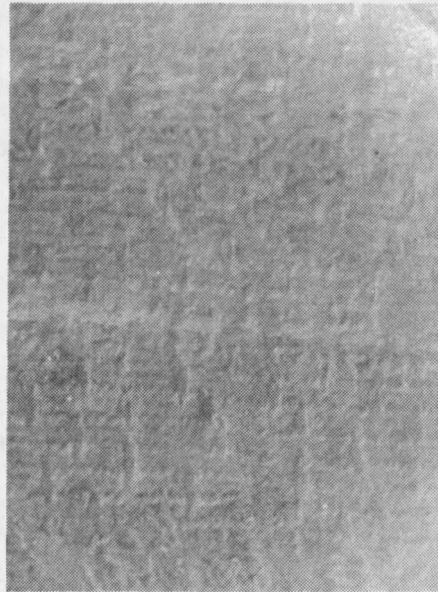


Figure 9: Reduced wax presence of glossy Vantage gs2gs2. (Scanning electron microscope, 100x)



Figure 10: Heaped wax layer (H) and reduced wax presence of glossy Mars, gs3gs3. (Scanning electron microscope, 100x)

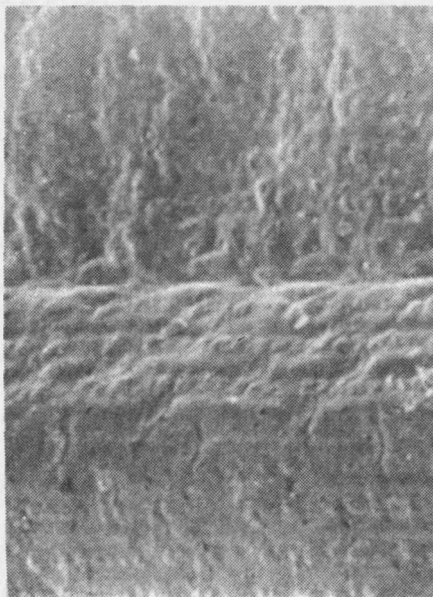


Figure 11: Reduced wax presence of glossy Gateway, gs4gs4. (Scanning electron microscope, 100x)

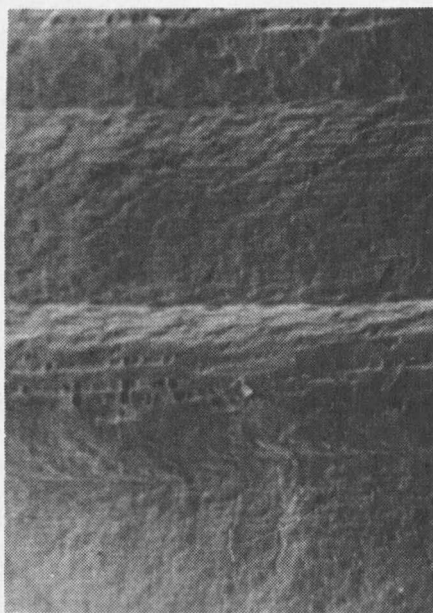


Figure 12: A reduced wax presence of glossy Jotun, gs5gs5. (Scanning microscope, 50x)

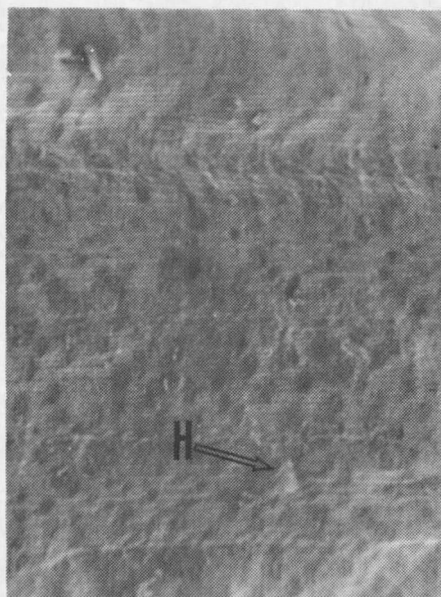


Figure 13: Heaped wax layer (H) and reduced wax presence of glossy Betzes gs6gs6. (Scanning electron microscope 50x)



Figure 14: Wax configuration of glaucous sheath Betzes. (Penetrating electron microscope, 500x)

intertwined, leading to the matted appearance of Figure 15.

The classification of the various glossy sheath genes into groups on the basis of their microscopic epidermal wax morphology is possible. The locus gs1, representing group 1, appears as greatly shortened rods. These rods can be straight or bent, they may be non-branched, singly or multiple branched or they may be "capped". Examples of group 1 are shown in Figures 16 and 17.

Group 2, including the loci gs2 and gs5, are composed of microscopic wax particles resembling peaks and ridges. Figures 18, 19 and 20 are of group 2 microscopic wax morphologies.

The loci gs3 and gs6 compose group 3. These wax particles are reminiscent of flakes. Microscopic wax particles of group 3 are shown in Figures 21, 22, and 23.

The final group found, group 4, appears as knolls. Pictures of this group, represented by the locus gs4, appear in Figures 24 and 25.

There is no apparent relationship between the microscope morphology and the macroscopic morphology of those glossy sheath loci that comprise a microscopic morphology group, or between the microscopic morphology and the chromosomal location of the loci comprising a microscopic morphology group. Group 2, "peaks and ridges", is composed of gs2 and gs5. The gene gs2 expresses a glossy spike, stem and leaf sheath while the gene gs5 shows a normal wax appearance on the spike and stem with a glossy sheath on which scattered wax particles are



Figure 15: Wax distribution on the leaf sheath of glaucous Betzes
(Scanning electron microscope, 1000x)

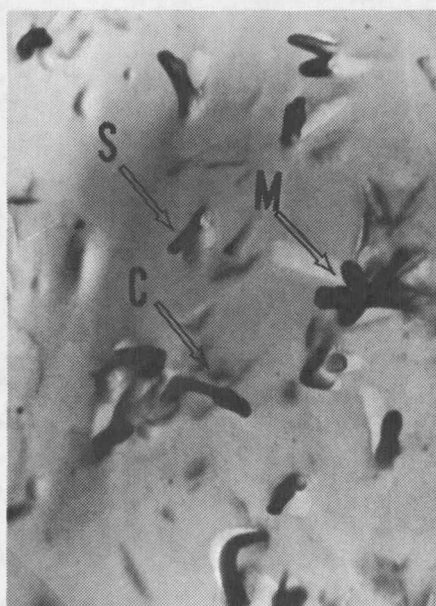


Figure 16: Straight (S), multibranched (M) and capped (C) wax particles
of Pirolina, gslgsl (Penetrating electron microscope, 1000x)



Figure 17: Capped wax particle (C) from leaf sheath of glossy *Pirolina* *gslgs1* (Scanning electron microscope, 1000x)

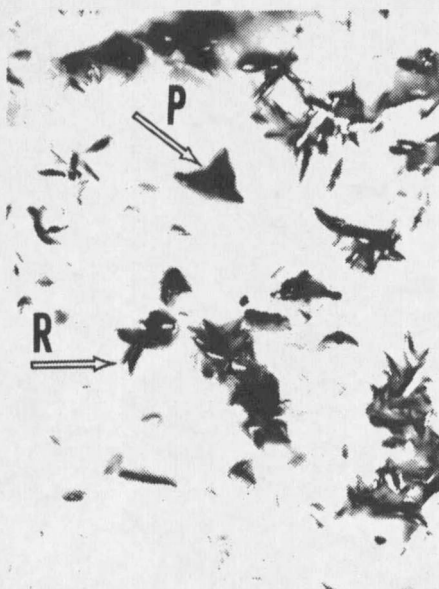


Figure 18: Peak (P) and ridge (R) wax particles of glossy *Atlas 2*, *gs2gs2* (Penetrating electron microscope, 1000x)

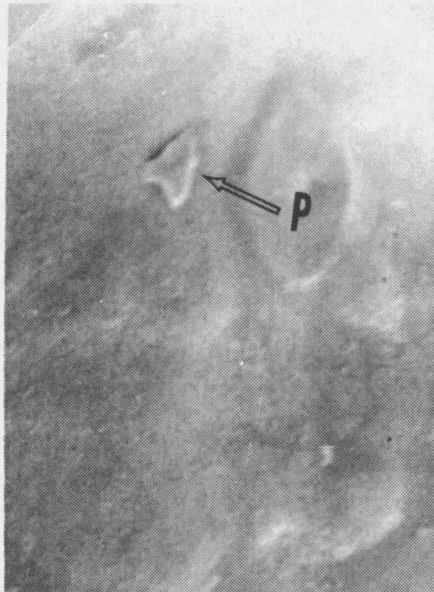


Figure 19: Peak wax configuration (P) of glossy Vantage, gs2gs2
(Scanning electron microscope, 1000x)



Figure 20: Peak wax configurations (P) of glossy Jotun gs5gs5
(Scanning electron microscope, 2000x)

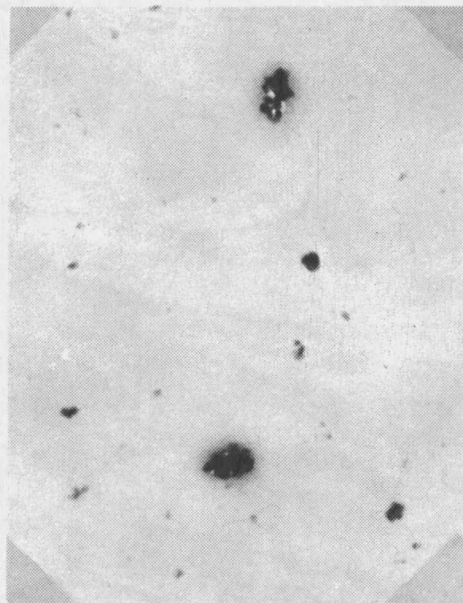


Figure 21: Flake configuration of glossy (4 Atlas x Algerian) x 2 Atlas 57, gs3gs3. (Penetrating electron microscope, 1000x)

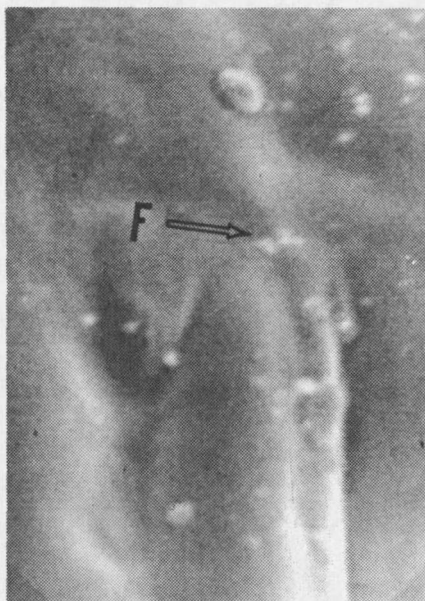


Figure 22: Flake configuration (F) of glossy Mars, gs3gs3 (Scanning electron microscope, 1000x)



Figure 23: Flake configuration of glossy Betzes gs6gs6 (Scanning electron microscope, 2000x)

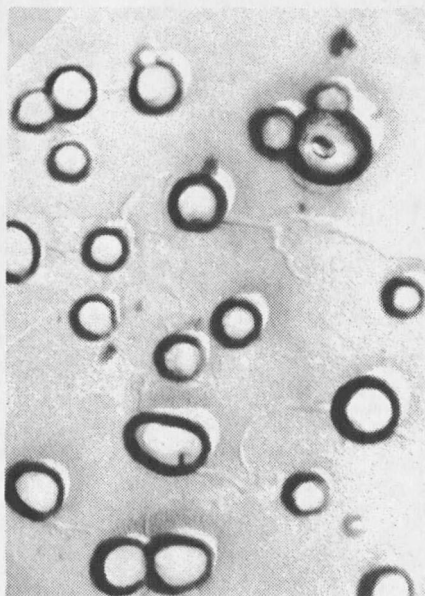


Figure 24: Knoll configuration of glossy Gateway, gs4gs4. (Penetrating electron microscope, 1000x)

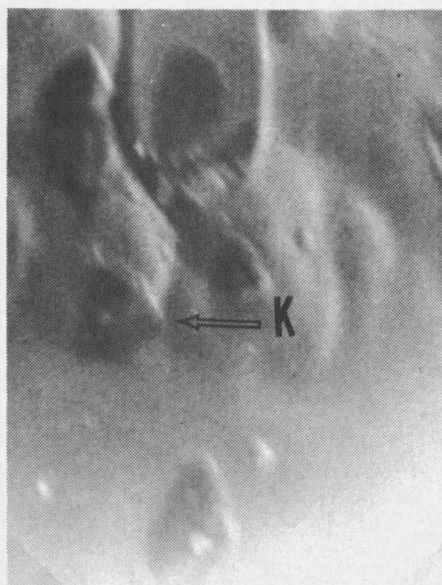


Figure 25. Knoll (K) configuration of glossy Gateway gs4gs4 (Scanning electron microscope, 1000x)

visible. The gene gs5, located on chromosome 2, is linked with male sterile 2, ms2, and six row, v. The gene gs2 is on either chromosome 2 or, more probably, chromosome 3 and is independent of ms2 and v. Group 3, "flakes", is composed of gs3, located on chromosome 1, and gs6, located on chromosome 2.

Agronomic and Malting Characteristics

In seven out of the nine comparisons made between a normal variety and its glossy sheath mutant there were no significant differences in yield (Table 8). The two varieties with significant yield differences between the normal parent and the glossy sheath mutant were Heines Hanna and Mars. The glossy sheath mutant in Mars arose by x-ray mutation. The radiation which induced this glossy sheath mutation could have caused additional mutations which were responsible for the reduction in yield.

The significant yield reduction in the spontaneous glossy sheath mutant of Heines Hanna could be due to : (1) the inclusion of other deleterious mutations, (2) the mutation in Heines Hanna occurred in an "inferior" genotype present in the general population, or (3) an inferior yield response resulting from the glossy sheath character in the Heines Hanna genotype.

Yield comparisons between the normal genotype and the glossy sheath mutants were made for various yield levels (Table 9). The yield level intervals of 1600 kilograms per hectare were chosen because they

Table 8. Average yields of normal (GsGs) and glossy sheath mutants (gsgs) for nine barley varieties.

Variety	C.I. No. of <u>GsGs</u> genotype	Gene symbol of mutant	No. of trials	Average yield- Kg/Ha		Glossy as % of normal
				<u>GsGs</u>	<u>gsgs</u>	
Piroline	9558	<u>gs1b</u> ^{1/}	24	2834	2860	100.9
Velvon	6109	<u>gs1c</u>	3	4073	4342	106.9
Vantage	7324	<u>gs2d</u>	10	2229	2234	100.2
Chevron	1111	<u>gs3j</u>	3	1636	1614	98.7
Mars	7015	<u>gs3i</u>	7	2857	2265	79.3***
Heines Hanna	9532	<u>gs6t</u> ^{2/}	29	2923	2783	95.2**
Barbless	5105	<u>gs6r</u>	6	3277	3169	96.7
Betzes	6398	<u>gs6s</u> ^{3/}	30	3112	3044	97.8
Compana	5438	<u>gs6u</u>	23	2068	3109	101.3

*, **, *** Significantly different from normal at 0.10, 0.05 and 0.01 probability levels respectively, by paired t-test.

^{1/} ^{2/} ^{3/} C.I. number of glossy sheath mutants respectively, C.I. 11330, C.I. 10862, C.I. 10863.

correspond to yield classes frequently used for summary purposes at the Bozeman Station. The yields of the glossy sheath mutants tended to be higher than the normal genotypes at yield levels less than 1600 kilograms per hectare. At yield levels between 1600 and 3200 kilograms per hectare the normal genotypes tended to out-yield the glossy sheath mutants. A correlation coefficient was obtained for the yields of those normal genotypes yielding below 3200 kilograms per hectare and with the yields of their respective glossy sheath mutants expressed as percent of the normal genotype. A correlation of $r = -0.362$, significant at the .01 level, was found. This indicated that as the yields of normal genotypes decreased, the glossy sheath mutant of that variety increased in yield over its normal genotype.

Further correlations were computed between elevation of the trial site and yields of the glossy sheath mutants expressed as a percent of the yield of their respective normal genotypes. A negative correlation, $r = -0.385$ ($P=.05$), was found when the yield of the normal genotype was between 0 to 1600 kilograms per hectare. For this low yield level, the mean elevation was 1067.4 meters with a range from 61.0 to 1847.1 meters. The mean yield was 1,049.6 kg/ha, with a range for the normal genotype of 414.0 to 1533.6 kg/ha. A significant negative correlation was not found when all of the data was used nor the other yield level classes were used (Table 9). At lower elevations and under low yield conditions, the glossy sheath mutants tend to out-yield their respective

Table 9. Yield comparisons of normal (GsGs) and spontaneous glossy sheath mutants (gsgs) grown at various yield levels, Mars and Chevron excluded.

Mean yield level class of the normal genotype - Kg/ha	Total trials	Number of times normal yielded more than glossy	Kg/ha		Correlation coefficient +
			<u>GsGs</u>	<u>gsgs</u>	
0-1600	36	14	1035	1097*	-.385**
1600-3200	32	22	2296	2225*	.008
3200-4800	42	23	4076	3987	-.211
4800-6400	11	5	5390	5317	-.261
6400 and up	4	3	7011	6915	.694

*, **, *** Significantly different from normal at 0.10, 0.05, and 0.01 probability levels respectively.

+ Elevation of trial location correlated with yield of glossy sheath mutant expressed as percentage of normal genotype.

normal genotypes.

Unpublished data of the author suggests that the superior yield performance of the glossy sheath mutants at low yield levels is due to increased water use efficiency by the glossy sheath plants. This advantage in water use efficiency by the glossy sheath plants could be negated at higher elevations by the increased penetration of ultraviolet light into the glossy plants. Jackson (1971) and Lundqvist et.al., (1968) have reported that the glossy sheath mutants lack B-diketones in the sheath epidermal waxes. Jackson noted that the B-diketones, nucleic acids and some amino acids absorb ultraviolet light in about the same region of the spectrum. This would mean more ultraviolet light can penetrate the glossy sheath mutants causing greater ultraviolet light damage in those plants. Since there is less ultraviolet light at lower elevations than at higher elevations, this inability of the glossy sheath mutants to absorb ultraviolet light in the surface waxes may adversely affect the glossy sheath plants ability to yield at higher elevations. (It is recognized that the correlations reported here could be an artifact of the data.)

The summary presented as Table 9 does not include data for the varieties Mars and Chevron. The glossy sheath mutant of Mars, the only artificially induced mutant, was significantly out-yielded by normal Mars (0.01 probability level). The unadapted variety Chevron

was excluded due to excessive spike drop prior to ripening. As shown in Table 9 there is a trend for yield differences favoring the glossy sheath mutants over parental types under low yield conditions and at lower elevations. More critical tests are needed.

A comparison of agronomic characteristics of the glossy sheath mutants and the normal genotypes is made in Table 10. The glossy sheath mutants of Pirolina, Betzes and Heines Hanna varied significantly from their normal genotype for one of six, for two of seven, and for three of six traits tested, respectively. No significant differences were found between the glossy sheath mutants and the normal genotypes of Compana (5 traits tested), Vantage (5 traits tested), Chevron (2 traits tested), Velvon (2 traits tested), Mars (2 traits tested) or Barbless (2 traits tested).

The normal genotypes and their respective glossy sheath mutants were compared for each trait over all varieties (Table 10). Mars was excluded from these comparisons because it was the only artificially induced mutant. Heines Hanna was excluded because the high number of significant differences between the normal genotype and the glossy sheath mutant lead to the conclusion that the normal and glossy sheath mutant were not isogenic. A highly significant difference between the normal genotype and the glossy sheath mutant was found for date headed with the glossy sheath mutant heading earlier. The six remaining traits showed no significant differences when tested

Table 10. Average agronomic characteristics of normal (GsGs) and glossy sheath mutants (gsgs) in nine barley varieties.

Characters measured		Height	Date	Hecto-	Lodging	Barley	Protein	Shatter-
Varieties		-cm	headed	liter	wt-gm	on 6/64	- %	ing -%
			m/day		% %	sieve -%		
Piroline	No. trials	14	17	17	4	29	9	
	Normal mean	65.8	6/25	64.5	32.6	67.2	15.8	
	Normal - glossy	-1.5	-1	-0.4**	-1.6	-1.1	+0.4*	
Betzes	No. trials	18	20	23	8	25	11	3
	Normal mean	71.6	6/30	63.7	21.8	60.0	15.6	31.9
	Normal - glossy	+1.9**	-2	+0.1	-13.9	+2.3**	0	+11.5
Heines								62
Hanna	No. trials	15	20	21	6	25	11	
	Normal mean	76.5	6/30	64.5	67.5	67.0	15.7	
	Normal - glossy	-2.5***	+2	+0.9**	-11.5	+5.8***	-0.3	
Compana	No. trials	4	8	7		17	17	
	Normal mean	50.8	6/30	61.4		48.5	15.5	
	Normal - glossy	-1.3	-1*	0		-2.5	+0.1	
Vantage	No. trials	4	7	6		9	7	
	Normal mean	64.0	7.2	58.7		45.3	15.7	
	Normal - glossy	+2.5	-1	-0.4		+4.4	-0.1	
Chevron	No. trials					2	2	
	Normal mean					19.8	17.7	
	Normal - glossy					+13.4	-0.5	

Table 10. (Continued)

Characters measured		Height	Date	Hecto-	Lodging	Barley	Protein	Shatter-
Varieties		-cm	mo/day	liter wt-gm	-%	on 6/64 sieve -%	- %	ing - %
Velvon	No. trials					2	2	
	Normal mean					67.4	13.9	
	Normal - glossy					+20.3*	+0.1	
Mars	No. trials					3	3	
	Normal mean					27.7	13.8	
	Normal - glossy					+11.6	+0.2	
Barbless	No. trials					3	3	
	Normal mean					41.2	13.4	
	Normal - glossy					+1.1	+0.2	
Over all	No. trials	40	54	55	12	87	51	3
Varieties ↓	Normal mean	66.7	6/29	63.1	28.1	57.2	15.5	31.9
	Normal - glossy	+0.4	-1***	-0.1	-9.9	+1.0	+1.0	+11.5

*, **, *** Significance of difference at 0.10, 0.05 and 0.01 probability levels respectively



Mars and Heines Hanna excluded, see text.

over all varieties.

Comparative malting tests involving fifteen characteristics were determined by the U.S. Department of Agriculture Malt Laboratory at Madison, Wisconsin, on Pirolina, Betzes and Heines Hanna and their respective glossy sheath mutants (Table 11). Neither of the glossy sheath mutants of Betzes or Pirolina were significantly different from their normal genotype for any of the fifteen malting characters tested. Heines Hanna and its glossy sheath mutant varied significantly for five of the fifteen tested malting traits.

In comparing the normal genotypes and the glossy sheath mutants for each trait across Betzes and Pirolina, no significant differences for malting quality were found. Heines Hanna was excluded from this comparison because the normal genotype and its glossy sheath mutant are probably not isogenic.

The data presented indicate that isogenic lines of normal and glossy sheath plants show no appreciable difference in yield, agronomic or malting characteristics with the exception of data headed,

In the case of Pirolina, only one of the 22 characteristics tested showed significant variations between the glossy sheath mutant and the parental type. The glossy sheath mutant of Betzes differed significantly from normal Betzes for only two of the 23 traits evaluated. No significant differences were observed between the parental types and the glossy sheath mutants of Compana, Vantage,

Table 11. Average barley and malting quality characteristics of three normal (GsGs) two-row varieties and their respective glossy sheath mutants (gsgs) +.

Characteristics	<u>Piroline-9 trials</u>		<u>Betzes-11 trials</u>		<u>Heines Hanna-10 trials</u>	
	Normal mean	Differ- ence Gs-gs	Normal mean	Differ- ence Gs-gs	Normal mean	Differ- ence Gs-gs
Barley nitrogen	2.15	+0.05*	2.04	-0.05	2.56	+0.44
Barley kernel weight, mg.	38.4	+0.1	37.7	+0.7	40.4	-0.4
Malt kernel weight, mg.	33.9	-0.4	34.3	+0.5	36.8	-0.4
Barley color-agtron unit	60.0	0	68.2	+1.1	58.5	+1.6
Malt moisture, %	5.4	+0.1	5.3	0	5.3	0
Malt extract, dry %	77.5	+0.5	78.6	+0.4	77.8	+0.8**
Wort color, °lov 52	1.4	-0.1*	1.4	+0.1	1.3	+0.1
Clarity of wort, no. of samples clear	3	-4*	10	0	10	+1
Wort nitrogen, %	0.633	+0.004	0.615	-0.020	0.627	-0.001
Malt nitrogen, %	2.11	+0.03	1.98	-0.06	2.20	-0.06
Wort N/malt N, %	30.3	-0.2	31.6	0	31.6	+1.1
Diastatic power, °	193.0	+7.0	148.5	-3.8	165.3	+29.2***
Beta amylase, maltose equiv.	610.0	+20.0	462.7	-8.2	530	+127.4***
Alpha amylase, 20° dext. units	48.0	-0.1	39.2	-2.4	44.0	+6.6***
Ratio, beta/alpha amylase	12.5	-0.1	11.9	+0.4	12.2	+1.5**

*, **, *** Significance of difference at 0.10, 0.5 and 0.01 probability levels respectively.

+ Data in this table were obtained by the United States Department of Agriculture Barley and Malt Laboratory, Madison, Wisconsin, and is presented with their permission.

Chevron, Velvon, or Barbless for any of the comparisons.

The glossy sheath mutant of Mars, the only artificially induced mutant tested, varied significantly from the normal genotype for one of three traits tested, that being yield. Heines Hanna and its glossy sheath mutant varied significantly for nine of 21 traits tested, indicating that the glossy sheath mutant and the normal genotype of Heines Hanna are not isogenic.

SUMMARY

Mutants with a reduced wax coating on the various organs are almost as readily induced as erectoides, the most frequent viable barley mutant. The eceriferum family of mutants, of which the glossy sheath mutants are a member, is characterized by a glossy appearance on the spike, stem, leaf sheath or blade, on several organs or over the entire plant.

A collection of 24 different glossy sheath mutational events, of which 17 occurred spontaneously, was tested for allelism. Six different loci were identified as imparting the glossy sheath character in barley. This study has shown that the glossy sheath mutants of Mars and Gateway are non-allelic, though they had previously been reported as allelic. The numbered loci (cer-)gs5 and (cer-)gs8 were found to be allelic as were the numbered loci (cer-)gs6 and (cer-)gs7. It was found that the 17 spontaneous mutants occurred at random over the six loci.

Attempts were made to locate each of the glossy sheath loci and to propose possible allelisms with the European eceriferum series. The gene gs1, allelic with cer-q, has been tentatively located on chromosome 4. The gene gs2 is located on either chromosome 2 or, more probably, chromosome 3. The gene gs3, has been located on chromosome 1 with the probable gene order wx-gs3-br. Glossy sheath 4 is located on chromosome 6. The gene gs5, has been located on chromosome 2 with probable gene order of v-gs5-ms2, while the gene gs6 has been located

on chromosome 2.

Both the normal genotype and the glossy sheath mutants were shown to have two wax configurations on the leaf sheath surface, one being heaped wax layer and the other single granulated layer. The reported reduction of the amount of wax present on the leaf sheaths of glossy sheath mutants was demonstrated. Based on microscopic wax morphology, the glossy sheath mutants can be classified into four distinct groups. These are group 1, "short rods"; gs1 locus, group 2 "peaks and ridges", gs2 and gs5, loci, group 3, "flakes", gs3 and gs6 loci, and group 4, "knolls", gs4 locus.

Nine barley varieties and their glossy sheath mutants were compared for yield and for various agronomic and malting traits. Seven of the nine varieties showed no significant differences in yield over numerous environments. Pirolina and Betzes were significantly different from their glossy sheath mutants for only one of 22 and for two of 23 tested characters, respectively. Normal Heines Hanna and the glossy sheath mutant of Heines Hanna were significantly different for nine of 22 characteristics tested, including yield. No significant differences were observed between the normal and glossy sheath mutants of Compana, Vantage, Chevron, or Barbless for any of the traits tested. Mars and its glossy sheath mutant, the only artificially induced mutant tested, varied significantly for one of three traits tested, that being yield.

A significant negative correlation was found between the yield of those normal genotypes grown in trials yielding less than 3200 kg/ha and with the yields of their respective glossy sheath mutants expressed as percent yield of the normal genotype. As the yields of the normal genotypes decreased, the glossy sheath mutants tended to yield more than their normal genotype.

A significant negative correlation between elevation of the trial site and yields of the glossy sheath mutants expressed as a percent of the yield of their respective normal genotypes when the normal genotypes yielded less than 1600 kg/ha was found. This indicates that at lower elevations and under low yield conditions, the glossy sheath mutant outyielded its respective normal genotype.

Two possible effects may be ascribed to the glossy sheath gene in barley. The glossy sheath mutant heads earlier than its normal genotype and the glossy sheath mutant may have a superior yielding capacity over its normal genotype under stress conditions at lower elevations.

LITERATURE CITED

1. Allard, R. W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. *Hilgardia* 24:235-278.
2. Albrechtson, R. S. 1957. Hood elevation and awn length inheritance studies in barley. M.Sc. Thesis, Utah State University. Pp. 74 typed.
3. Anderson, W. R. 1958. Linkage relationships of located and unlocated genetic testers in certain normal and translocation stocks of barley. M.Sc. Thesis, Utah State University, Pp. 36 typed.
4. Eglinton, G. and R. J. Hamilton. 1967. Leaf epicuticular waxes. *Science* 156:1322-1335.
5. Froier, K. 1954. Aspects of the agricultural value of certain barley x-ray mutations produced and tested at the Swedish Seed Association, Savlof, and its branch stations. *Acta Agriculturae Scandinavica* 4:515-542.
6. Gustafsson, A. 1947. Mutations in agricultural plants. *Hereditas* 33:1-100.
7. Immer, F. R., and M. T. Henderson. 1943. Linkage studies in barley. *Genetics*. 28:419-440.
8. Jackson, L. L. 1971. Beta diketone, alcohol and hydrocarbons of barley surface lipids. *Phytochemistry*. 10:487-490.
9. Juniper, B. E., and D. E. Bradley. 1958. The carbon replica tech-

- ... unique in the study of the ultrastructure of leaf surfaces. ...
Journal of Ultrastructure Research. 2:16.
10. Lundqvist, U. and D. von Wettstein. 1962. Induction of eceriferum mutants in barley by ionizing radiations and chemical mutagens. Hereditas. 48:342-362.
 11. Lundqvist, U., P. von Wettstein-Knowles and D. von Wettstein. 1968. Induction of eceriferum mutants in barley by ionizing radiations and chemical mutagens II. Hereditas 59:473-504.
 12. McProud, W. L. and R. F. Eslick. 1968. Linkage data for two eceriferum mutants. Barley Newsletter 12:16.
 13. Nilan, R. A. 1964. The cytology and genetics of barley. Monographic Supplement No. 3. Research Studies. Washington State University.
 14. Rasmusson, D. C. and J. W. Lambert. 1965. Inheritance of the glossy-sheath character in barley, Hordeum vulgare L. Crop Science 5:251-253.
 15. Robertson, D. W., G. A. Wiebe, R. G. Shands, and A. Hagberg. 1965. A summary of linkage studies in cultivated barley, Hordeum spp. Supplement III. 1954-1963. Crop Science 5:33-43.
 16. Smith, L. 1951. Cytology and genetics of barley. Botanical Review. 17:1-51, 133-202, 285-355.
 17. Sogaard, B. 1971. Linkage studies on eceriferum mutants in barley. Barley Genetics Newsletter. 1:41-47.

18. Stadler, L. J. 1930. Some genetic effects of x-rays in plants.
Journal of Heredity. 21:3-19.
19. Takahashi, R. and J. Hayashi. 1966. Inheritance and linkage studies in barley, II. Assignment of several new mutants to their respective linkage groups by trisomic method of analysis. Berichte des Ohara Instituts fur Landwirtschaftliche. Biologie Bd XIII, Heft 4. pp. 185-198.
20. Takahashi, R. and J. Hayashi. 1968. Linkage studies. Barley Newsletter 12:58-59.
21. Takahashi, R., J. Hayashi, Y. Moriya, and Ch. Hisao. 1965. Linkage Studies. Barley Newsletter. 9:68-69.
22. von Wettstein-Knowles, P. 1971. The molecular phenotypes of the eceriferum mutants. Barley Genetics II, Proceedings of Second International Barley Genetics Symposium. Washington State University Press.
23. Walker, G. W., R. J. Deitrich, R. Miller and K. Kahsa. 1963. Recent barley mutants and their linkages, II. Genetic data for further mutants. Canadian Journal of Genetics and Cytology. 5:200-219.
24. Woodward, R. W. 1957. Linkage in barley. 1957.. Journal of the American Society of Agronomy. 49:28-32.

MONTANA STATE UNIVERSITY LIBRARIES



3 1762 10010981 6

1242

cop.2

Genetics, phenotypes,
agronomic and malting
performance ...

[illegible]

13.21.2

2000 - 2001 P5

12.21-72

Ly. 121

10-9-73

42a Uthman 7D

D378

M242

Cap 2