

LINKAGE RELATIONS IN MALTING BARLEY

By

Arden Dexter Day

AN ABSTRACT

Submitted to the School of Graduate Studies of
Michigan State College of Agriculture and
Applied Science in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Farm Crops

1954

Approved

E. E. Down

THESIS ABSTRACT

This research problem was designed to study the linkage relations between diastase activity, which is a measure of malting quality in barley, and specific visible characteristics known to be inherited on certain chromosome groups. The following crosses were made between standard barley varieties showing the opposite expression of a visible characteristic known to be inherited on a particular chromosome group:

Chromosome group I:

ALPHA	X	O.A.C.21
(2-row)		(6-row)

Chromosome group II:

DORSETT	X	O.A.C.21
(Black glumes)		(White glumes)

Chromosome group III:

O.A.C.21	X	C.I. 1370
(Covered kernels)		(Naked kernels)

Chromosome group IV:

O.A.C.21	X	MARS
(Blue aleurone)		(White aleurone)

Chromosome group V:

O.A.C.21	X	MARS
(Rough awns)		(Smooth awns)

Five chromosome groups were represented in the five crosses, and O.A.C.21 was one of the parents occurring in each cross. The pure breeding lines from the F_2 population (F_4 seed) in each cross were saved and analyzed for diastatic power using a modification of the Anderson-Sallans ferricyanide method. Progenies from twelve selections from each of the two standard varieties used as parents in each cross were also carried along in the same field with the crossed material and analyzed for diastatic power. After the chemical determinations were completed and the diastase activity of each sample was converted into degrees Lintner ($^{\circ}L$), the two parents of each cross and the two groups of pure breeding progeny from each cross were compared for significant differences by means of the t-test.

The results may be summarized as follows:

Chromosome group I:

The O.A.C.21 parent is significantly higher in diastatic power than the Alpha parent. There is no apparent linkage between high diastatic power and the 2-row characteristic. There is an apparent pleiotropic effect between high diastatic power and the 2-row characteristic.

Chromosome group II:

The O.A.C.21 parent is significantly higher in diastatic power than the Dorsett parent. There is no significant linkage between high diastatic power and the white glume or black glume characteristic.

Chromosome group III:

The O.A.C.21 parent is significantly higher in diastatic power than the C.I. 1370 parent. There is no significant linkage between high diastatic power and the covered kernel or naked kernel characteristic.

Chromosome group IV:

The O.A.C.21 parent is significantly higher in diastatic power than the Mars parent. There is no significant linkage between high diastatic power and the blue aleurone or white aleurone characteristic. However, the white aleurone group of pure breeding

progeny did tend to run a little higher in diastatic power than did the blue aleurone group.

Chromosome group V:

The O.A.C.21 parent is significantly higher in diastatic power than the Mars parent. There is an apparent linkage between high diastatic power and the rough awn characteristic.

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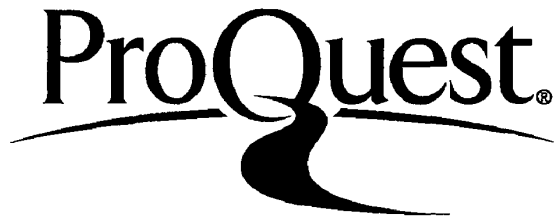
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INTRODUCTION

Malting barley (Hordeum vulgare L.) is the principal grain used in the production of malt which, in turn, is the basic material used in the brewing of beer and other alcoholic beverages. Malt is malting barley grain which has been steeped in water, germinated under specifically controlled temperature, moisture, and time conditions, and then dried by a process known as kilning.

During the germination of barley grain definite physical and chemical changes take place within the kernel. One of the major changes is an increase in the activity of the enzyme diastase which is the enzyme system that changes starch into sugar. Diastase, commonly measured in "degrees Lintner" ($^{\circ}$ L), is made up primarily of two enzymes, alpha-amylase and beta-amylase. The amount of diastase in malt is closely correlated to the amount of starch that can be digested with a given amount of malt and the rate at which the digestion is accomplished.

In this research problem an attempt was made to study the linkage relations between diastase activity,

which is a measure of malting quality in barley, and specific visible characteristics known to be inherited on certain chromosome groups.

REVIEW OF LITERATURE

The evolution of cultivated barley is tied to the past by threads of history as ancient as the origin of agriculture itself. The development of this crop has been molded in the hands of many cultures and has been accompanied by the emergence of a host of varietal forms in many environments throughout the world. Barley has always taken an important place along the agricultural frontier. The statement is often made, and probably with justification, that this cereal has a wider ecologic range than any other grain crop (25).

Malting barley is a special type of barley grain possessing certain physical and chemical qualities required by maltsters and brewers (14). The principal grain used in the production of malt, which is the basic material for brewing beer, is this type of barley (24). There are several reasons for the great popularity of barley malt in the brewing industry. First of all, barley is one of the hardiest of the small grains. It can be more easily malted for brewing purposes than any other cereal, and the solubles extracted from the malt are of a more desirable character than those extracted

from other grains. Barley malt contributes to smoother performance in the brew house by its quick conversion of starch and its production of easily strained mash. In addition, barley malt has sufficient enzymatic capacity for the conversion of the insoluble materials, both in the malt itself and in the additional adjuncts added to the malt, into soluble substances. The superior value of barley malt in the brewing of beer was recognized early in the 15th century when the original settlers came to America (26).

One of the principal measuring sticks used to determine malting quality in barley is diastase activity (14,22). Hopkins and Krause (13) verify the importance of diastatic power as a measure of malting quality in barley. Diastase activity was one of the measures of malting quality used by Lejeune et al. (15) in their study of methods of breeding new malting barley varieties. After reading the literature and visiting malt houses and breweries, it becomes very apparent that diastase activity is one characteristic of malt that deserves utmost consideration in the malting and brewing industries.

After the importance of diastatic power became firmly established, the next problem was to find an efficient and

accurate method of testing the enzymatic activity of different samples of barley grain and malt. In the early 1920's Rumsey (19) devised a long, tedious copper reduction method for measuring the diastase activity of wheat flour used for bread making, but this method was not very efficient. Later Blish et al. (8) reported a picric acid colorimetric technique for estimating diastatic power as being preferable to any of the various copper reduction methods from the standpoint of convenience without loss of accuracy.

About 1923 Hagedorn and Jensen (10) proposed a ferricyanide micro-method for the estimation of blood sugar. Hanes (11) and others found that the Hagedorn-Jensen ferricyanide method of determining reducing sugars in animal tissues and products is equally well suited to investigations with plants and plant products. Minor modifications and adaptations can usually be made to suit special purposes and requirements. In the early 1930's Blish and Sandstedt (7) modified the Hagedorn-Jensen ferricyanide method of determining reducing sugars (10) so that it could be used to estimate diastase activity in wheat flour. This method was based upon the reduction of ferricyanide to ferrocyanide by reducing sugars in

alkaline solution. After reduction was completed, the amount of sugar produced was determined by acidifying the solution with acetic acid, adding potassium iodide and soluble starch, and titrating with sodium thiosulfate.

In 1937, Anderson and Sallans (3) pointed out that the official method of the American Society of Brewing Chemists for the determination of diastatic power of malt (2), which was also tentatively adopted by the Association of Official Agricultural Chemists (6), and which was being studied by the American Association of Cereal Chemists, leaves much to be desired as a routine method. Its unsuitability was demonstrated by data given by Coleman (9) showing that seventeen laboratories obtained results varying from 103[°]L. to 158[°]L. for the same malt. Anderson and Sallans (3) modified the Blish-Sandstedt ferricyanide method for determining the reducing power of a digested starch solution (7) to permit its use in the determination of the diastatic power of malt in degrees Lintner ([°]L). Experimental data indicated, that under the conditions of the determination, the ferricyanide method was superior to any method yet reported.

As a result of investigations made by Sallans and Anderson (20, and earlier papers) it appears to be well established that the diastase activities, both free and

total, of barley grain and malt are varietal characteristics. Data accumulated by Anderson et al. (4) and Harris et al. (12) who have investigated diastase activity in barley and malt provide additional support to this hypothesis. Sallans and Anderson (20) found the proteolytic enzyme papain to be a good material to use to activate the diastase in unmalted barley grain, a fact later verified by other workers (21). Sallans and Anderson made diastase activity determinations in 144 samples of barley grain and in the malts made from them using the ferricyanide technique. The results obtained were expressed in degrees Lintner (^oL). These barley samples represented twelve common varieties grown in Canada. Varietal differences in diastase activity were demonstrated and the varieties that were high in barley diastase activity were also high in malt diastase activity. The total barley diastase activity appeared to be a good indication of what the total malt diastase activity might be for any given variety.

In 1941, Anderson et al. (5) suggested that in the early stages of a barley breeding program it should be possible to test the barley grain for diastase activity after activating it with papain, and eliminate the malting test, which requires considerable time and special equip-

ment, without any danger of discarding new lines of barley with superior malting quality. However, up to this time there was no evidence that hybrid barley strains would react the same as standard varieties to the diastase test.

In 1942, Meredith et al. (16) reported that barley diastatic power, after activation with papain, gives a good indication of malt diastatic power for hybrid lines of barley as well as for standard varieties. It was also suggested, that by using this test for his hybrid lines of barley and comparing these values with those of a good standard malting barley variety, the plant breeder may obtain useful information on his lines much earlier than it could be obtained with standard malting tests. He would then be able to concentrate on a reduced population of considerable promise and only the better material would be increased and submitted for malting tests.

Robertson et al. (17, 18) have published a summary of all of the linkage studies in barley which should be very helpful information for plant breeders who are interested in studying linkage relations in malting barley.

EXPERIMENTAL PROCEDURE

In the summer of 1950 the following crosses were made in the field between standard barley varieties showing the opposite expression of a visible characteristic known to be inherited on a particular chromosome group:

Chromosome group I:

ALPHA	X	O.A.C.21
(2-row)		(6-row)

Chromosome group II:

DORSETT	X	O.A.C.21
(Black glumes)		(White glumes)

Chromosome group III:

O.A.C.21	X	C.I. 1370
(Covered kernels)		(Naked kernels)

Chromosome group IV:

O.A.C.21	X	MARS
(Blue aleurone)		(White aleurone)

Chromosome group V:

O.A.C.21	X	MARS
(Rough awns)		(Smooth awns)

Five chromosome groups were represented in the five crosses, and O.A.C.21 was one of the parents occurring in each cross. The F_1 seed was space planted in the greenhouse in the winter of 1950-51. The F_2 seed was space planted in the field in the summer of 1951. The F_3 seed from each F_2 plant was harvested separately, and then planted in the field in the summer of 1952 as a separate line. Since this thesis problem was set up as a linkage study and did not involve segregation, the segregating population was discarded. Only the pure breeding lines from the F_2 population (F_4 seed) in each cross were saved and analyzed for diastatic power. Progenies from twelve selections from each of the two standard varieties of barley used as parents in each cross were carried along in the same field with the crossed material and analyzed for diastatic power.

The task of selecting appropriate parents for each cross, making the original crosses, carrying the hybrid material through the F_2 generation, and increasing each line until sufficient seed was obtained to grind into flour for chemical analysis, required approximately three years of field and greenhouse work and involved over 1,000 lines of barley.

The chemical method used to determine the diastase

activity of the barley samples was a modification of the Anderson-Sallans ferricyanide method (3). This method, which involves the activation of barley diastase by the proteolytic enzyme papain, is a rather long procedure, taking about 36 hours from the time the original sample is weighed out until a value for the diastatic power of the barley grain is finally obtained. After testing duplicate samples of a standard variety of barley each day for about three weeks and carefully observing the results under different environmental conditions, it was found that the temperature and timing conditions are very critical and must be carefully controlled for consistent results. This is particularly true of the steps involving enzyme activity. It was further observed that duplicate samples of the same barley, analyzed under carefully controlled conditions, should agree within 0.3 ml. of sodium thiosulfate in the final titration.

After the chemical procedure was mastered, each barley sample was analyzed in duplicate for diastatic power. If the two duplicates of each sample did not agree within 0.3 ml. of sodium thiosulfate in the final titration, they were discarded and two new duplicates were analyzed again. A standard variety was tested as

a check each day and a starch standard was used as a check on the special starch solution each week. Six barley samples (in duplicate), in addition to the checks, were the maximum number that could be carried efficiently at one time.

The entire task of obtaining the necessary equipment, learning the chemical procedure, and analyzing the barley samples for diastatic power took about one year, required \$700 worth of special equipment and chemicals, and involved about \$3,500 worth of chemical determinations.

The reagents used and the main steps involved in the chemical procedure are given on the following pages.

REAGENTS USED

1. Special Starch Reagent:

This special starch reagent should be prepared according to the specifications of the American Society of Brewing Chemists (1).

2. Acetate Buffer Reagent Used in Preparation of Special Starch:

Dissolve 68 grams of C.P. sodium acetate in 500 ml. of N acetic acid and make up to 1 liter with distilled water.

3. 0.5N Sodium Hydroxide Reagent:

Dissolve 20 grams of sodium hydroxide in 1 liter of distilled water.

4. Alkaline N/20 Ferricyanide Reagent:

Dissolve 16.5 grams of pure dry potassium ferricyanide and 22 grams of anhydrous sodium carbonate in 1 liter of distilled water. Keep in a dark glass bottle away from light.

5. Acetic Acid Reagent:

This solution should contain 200 ml. of glacial acetic acid, 70 grams of potassium chloride, and 20 grams of zinc sulfate per liter.

6. Potassium Iodide Reagent:

To a 50% solution of potassium iodide, add one drop of concentrated sodium hydroxide solution for each 100 ml. of reagent to prevent deterioration on standing. The reagent is not fit for use unless colorless.

7. Soluble Starch Reagent:

This reagent should contain 1% soluble starch in a 30% sodium chloride solution. Prepare the soluble starch suspension and pour slowly into boiling water. Boil for two minutes, add the sodium chloride, and make to volume. The reagent should be transparent and colorless.

8. N/20 Sodium Thiosulfate Reagent:

Dissolve 12.41 grams of sodium thiosulfate in 1 liter of distilled water. Select only clear crystals from the best C.P. grade.

Main Steps Involved in the Modified Anderson-
Sallans Ferricyanide Method of Determining
Diastase Activity of Barley Grain

1. Grind grain to pass through a 20-mesh screen and mix sample thoroughly.
2. Carefully weigh 2.5 grams of barley flour, 0.5 gram of papain, and place in a 200 ml. volumetric sugar flask and mix thoroughly.
3. Add 50 ml. of distilled water, mix, and place flask in a water bath at 20° C. for 21.5 hours.
4. Filter the sample through rapid flowing filter paper and collect the extract.
5. Pipette 1 ml. of barley extract into a 200 ml. volumetric flask containing 100 ml. of specially buffered starch solution (1) and mix thoroughly.
6. Exactly 30 minutes after adding the barley extract, add 10 ml. of 0.5N sodium hydroxide solution, mix, make to volume with distilled water, and mix thoroughly.

7. Pipette 5 ml. of the digested starch solution into a 100 ml., wide-mouth, hard glass, Erlenmeyer flask.
8. Add exactly 10 ml. of alkaline N/20 ferricyanide solution to the starch solution in the Erlenmeyer flask and cover with a small beaker.
9. Place the Erlenmeyer flask in a bath of vigorously boiling water for exactly 20 minutes.
10. Immediately cool the Erlenmeyer flask and its contents in a bath of cold water.
11. Add 25 ml. of acetic acid reagent to the Erlenmeyer flask and mix thoroughly.
12. Add 1 ml. of 50% KI, followed by 2 ml. of soluble starch solution to the flask, and again mix thoroughly.
13. Titrate with N/20 sodium thiosulfate to the complete disappearance of the blue color and record the number of ml. of sodium thiosulfate required.
14. Add 100 ml. of the special, buffered starch solution (1) to another 200 ml. volumetric flask as was done in step number 5 but do not add any barley extract to this flask. This is a starch standard.

15. Carry the starch standard through steps 6 - 13 inclusive.

16. Subtract the number of ml. of sodium thiosulfate required to titrate the digested starch solution (that to which the barley extract was added) from the number of ml. of sodium thiosulfate required to titrate the starch standard. The resulting number is the ferricyanide equivalent of the diastatic power of the barley grain.

17. Multiply the sodium thiosulfate equivalent by 36 to convert the results to degrees Lintner ($^{\circ}\text{L}$) which is the standard way of expressing the diastatic power of barley grain and malt.

After the chemical determinations were completed and the diastase activity of each sample was converted into degrees Lintner ($^{\circ}$ L), the two parents of each cross and the two groups of pure breeding progeny from each cross were compared for significant differences by means of the t-test.

In the comparison of the two parents of each cross the following symbols were used:

- N - The number of selections from the standard variety used as a parent.
- M - The mean.
- S.D. - The standard deviation. (single determination)
- t - The t-value of the t-test.

In the comparison of the two groups of pure breeding progeny from each cross the following symbols were used:

- N - The number of lines in the pure breeding group of progeny.
- M - The mean.
- S.D. - The standard deviation. (single determination)
- t - The t-value of the t-test.

Before running the t-test, the means of the naked kernel parent (C.I. 1370) and the naked kernel group of pure breeding progeny were adjusted for hulls using 13% as the average percentage of hulls on 6-row barleys (24).

Figure 1.

This is a picture of the special constant temperature (20°C) water bath designed for use in the determination of diastase activity in barley grain. It is constructed of stainless steel with a stainless steel shelf in two sections (each section 17" x 17") with 3/8" diameter perforations to allow water circulation. The bath itself is 18" x 36" with a depth of 12" (inside dimensions). The walls and bottom are double with a thickness of 1/2" and are insulated with glass wool. The shelves are adjustable for height by means of slotted metallic strips attached to the bath at each corner of each shelf. The shelves rest on movable triangular metal pieces fitted into the slots. This bath is equipped with an agitator, a solenoid valve, a Merc-to-Merc thermoregulator, and a relay control box. It was purchased from George T. Walker & Company, Minneapolis, Minn., and cost \$440.



Figure 1.

EXPERIMENTAL RESULTS

Chromosome group I:

Cross:

ALPHA	X	O.A.C.21
(2-row)		(6-row)

Parents:

<u>ALPHA</u>		<u>O.A.C.21</u>
N - 12		N - 12
M - 183.06		M - 215.13
S.D. - 21.79		S.D. - 24.64

t - 3.37**

Pure Breeding Progeny:

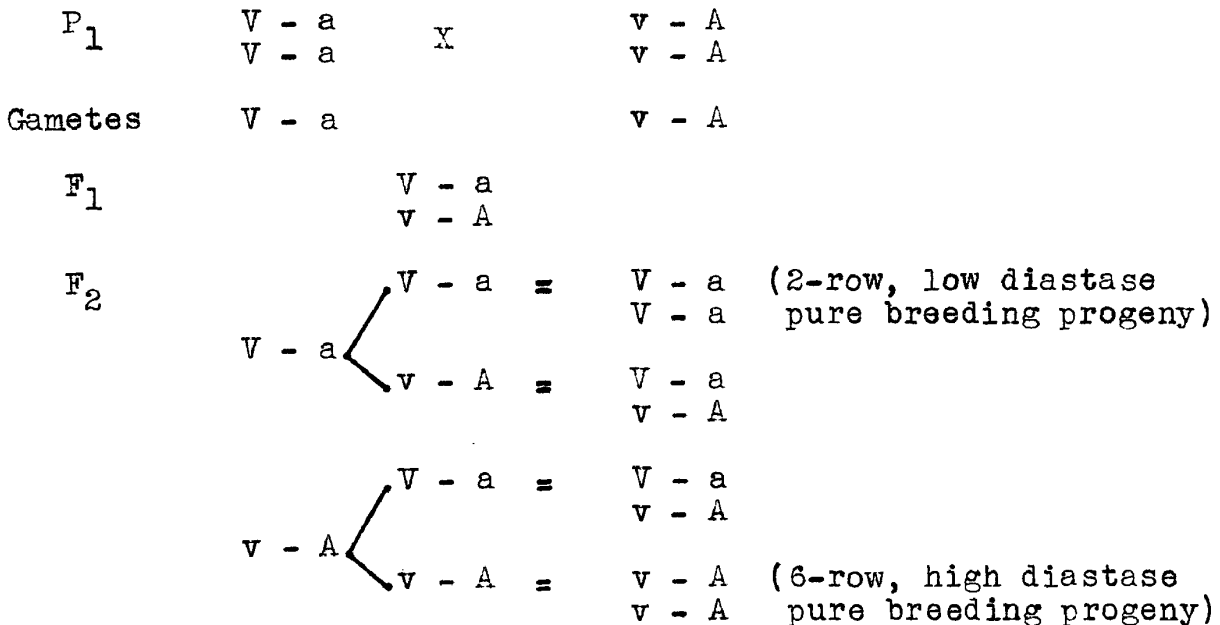
<u>2-row Group</u>		<u>6-row Group</u>
N - 30		N - 30
M - 211.09		M - 166.48
S.D. - 30.46		S.D. - 25.98

t - 6.10**

The O.A.C.21 parent is significantly higher in diastatic power than the Alpha parent. There is no apparent linkage between high diastatic power and the 2-row characteristic. There is an apparent pleiotropic effect between high diastatic power and the 2-row characteristic.

**1% level of significance

Let us set up a simple illustration to show that there is no apparent linkage between high diastatic power and the 2-row characteristic. If we assume, that head type is controlled by 1 gene ($V = 2\text{-row}$; $v = 6\text{-row}$), that diastatic power is controlled by another single gene ($A = \text{high}$; $a = \text{low}$), that there is a perfect linkage of these 2 genes on the same chromosome, and that we cross a 2-row, low diastase parent (Alpha) with a 6-row, high diastase parent (O.A.C.21), then the following situation should exist:



From the preceding illustration, it is clearly evident, if diastase activity were linked with 2-row head type, then the 2-row group of pure breeding progeny should have the lower mean, which is not the case. There is no apparent linkage between high diastase activity and the 2-row characteristic.

Let us set up a simple illustration to show that there is an apparent pleiotropic effect between diastatic power and the 2-row and 6-row characteristic. If we assume, that head type is controlled by 1 gene ($V = 2\text{-row}$; $v = 6\text{-row}$), that diastatic power is controlled by another single gene ($A = \text{high}$; $a = \text{low}$), that these 2 genes are independently inherited on different chromosomes, and that we cross a 2-row, low diastase parent (Alpha) with a 6-row, high diastase parent (O.A.C.21), then the following situation should exist:

P_1	VVaa	X	vvAA
Gametes	Va		vA
F_1		VvAa	
F_2	VVAA 1)		Pure breeding 2-row progeny saved.
	VVaa 2)		
	VVaa 1)		
	VvAA 2)		Segregating 2-row and 6-row progeny discarded.
	VvAa 4)		
	Vvaa 2)		
	vvAA 1)		Pure breeding 6-row progeny saved.
	vvAa 2)		
	vvaa 1)		

From the preceding illustration, it is evident that the pure breeding 2-row group of progeny has more genes

for high diastatic power than the 2-row parent (Alpha). Therefore, the 2-row group of progeny would be expected to have a higher diastatic power than the Alpha parent. Likewise, since the pure breeding 6-row group of progeny has fewer genes for high diastatic power than the O.A.C. 21 parent, it would be expected to have a lower diastatic power than O.A.C.21. Up to this point, diastatic power seems to be controlled by two independently inherited factors. However, if one observes that the 6-row group of progeny has a lower mean than even the lower diastase parent (Alpha), it appears that the 2-row gene is apparently giving an additional increase to the diastatic power. It is also interesting to note that the 6-row gene seems to have a greater depressing effect upon the diastatic power than the 2-row gene has an increasing effect upon the diastatic power. There is an apparent pleiotropic effect between diastatic power and the 2-row and 6-row characteristic.

Chromosome group II:

Cross:

DORSETT	X	O.A.C.21
(Black glumes)		(White glumes)

Parents:

<u>DORSETT</u>		<u>O.A.C.21</u>
N - 12		N - 12
M - 163.89		M - 215.13
S.D. - 19.82		S.D. - 24.64

t - 5.61**

Pure Breeding Progeny:

<u>Black Glume Group</u>		<u>White Glume Group</u>
N - 30		N - 30
M - 163.42		M - 169.36
S.D. - 23.42		S.D. - 30.26

t - 0.85

The O.A.C.21 parent is significantly higher in diastatic power than the Dorsett parent. There is no significant linkage between high diastatic power and the white glume or black glume characteristic.

**1% level of significance

Chromosome group III:

Cross:

O.A.C.21	X	C.I. 1370
(Covered kernels)		(Naked kernels)

Parents:

<u>O.A.C.21</u>		<u>C.I. 1370</u>
N - 12		N - 12
M - 215.13		M - 186.20
S.D. - 24.64		S.D. - 16.00
t - 3.41**		

Pure Breeding Progeny:

<u>COVERED KERNEL GROUP</u>		<u>NAKED KERNEL GROUP</u>
N - 5		N - 5
M - 145.73		M - 150.71
S.D. - 13.45		S.D. - 14.90
t - 0.55		

The O.A.C.21 parent is significantly higher in diastatic power than the C.I. 1370 parent. There is no significant linkage between high diastatic power and the covered kernel or naked kernel characteristic.

**1% level of significance

Chromosome group IV:

Cross:

O.A.C.21	X	MARS
(Blue aleurone)		(White aleurone)

Parents:

<u>O.A.C.21</u>		<u>MARS</u>
N - 12		N - 12
M - 215.13		M - 141.15
S.D. - 24.64		S.D. - 16.80
t - 8.58**		

Pure Breeding Progeny:

<u>BLUE ALEURONE GROUP</u>		<u>WHITE ALEURONE GROUP</u>
N - 33		N - 30
M - 143.17		M - 151.32
S.D. - 22.06		S.D. - 24.75
t - 1.37		

The O.A.C.21 parent is significantly higher in diastatic power than the Mars parent. There is no significant linkage between high diastatic power and the blue aleurone or white aleurone characteristic. However, the white aleurone group did tend to run a little higher in diastatic power than did the blue aleurone group.

**1% level of significance

Chromosome group V:

Cross:

O.A.C.21	X	MARS
(Rough awns)		(Smooth awns)

Parents:

<u>O.A.C.21</u>		<u>MARS</u>
N - 12		N - 12
M - 215.13		M - 141.15
S.D. - 24.64		S.D. - 16.80
t - 8.58**		

Pure Breeding Progeny:

<u>ROUGH AWN GROUP</u>		<u>SMOOTH AWN GROUP</u>
N - 32		N - 31
M - 151.29		M - 128.44
S.D. - 30.71		S.D. - 27.46
t - 3.12**		

The O.A.C.21 parent is significantly higher in diastatic power than the Mars parent. There is an apparent linkage between high diastatic power and the rough awn characteristic.

**1% level of significance

DISCUSSION

It is evident, after observing the experimental results, that diastase activity is definitely a varietal characteristic. The two parental varieties involved in every cross differed significantly from each other in diastase activity. There also appears to be definite linkage relationship between certain visible characteristics and high diastatic power, which is a measure of malting quality in barley.

The visible characteristic used in the study of chromosome I was 2-row vs. 6-row head type. The two standard varieties used in this first cross were Alpha and O.A.C.21. The O.A.C.21 parent, a 6-row variety, was significantly higher in diastase activity than Alpha. Of the pure breeding progeny from this cross, the 2-row group was significantly higher in diastatic power than the 6-row group, which indicates an apparent pleiotropic effect between high diastase activity and 2-row head type. Pleiotropism is the controlling of more than one character by a single gene. There is no apparent linkage between high diastase activity and the 2-row characteristic. If high diastase activity and 2-row head type were linked,

the 2-row group of pure breeding progeny should show the lowest mean because this is the condition that exists in the parents.

Cross number two involved Dorsett and O.A.C.21, two standard varieties of barley showing a difference in glume color. Dorsett has black glumes while O.A.C.21 has white glumes. This visible characteristic is inherited on chromosome II. The O.A.C.21 parent was significantly higher in diastatic power than Dorsett. Of the pure breeding progeny from this cross, there was no significant difference in diastatic power between the black glume group and the white glume group. This indicates that there is no apparent linkage between high diastatic power and the white or black glume characteristic.

O.A.C.21 and C.I. 1370 were the two standard varieties used as parents in the study of chromosome III. This third cross involved covered kernels vs. naked kernels. O.A.C.21 is a covered kernel variety, while C.I. 1370 has naked kernels. The O.A.C.21 parent was significantly higher in diastatic power than the C.I. 1370 parent. Of the pure breeding progeny from this cross, there was no significant difference in diastatic power between the covered kernel group and the naked kernel group. Therefore, there is no apparent linkage between high diastase activity and the covered or naked kernel characteristic.

Cross number four involved O.A.C.21 and Mars, two standard varieties of barley showing a difference in aleurone color. O.A.C.21 has blue aleurone while Mars has white aleurone. This visible characteristic is inherited on chromosome IV. The O.A.C.21 parent was significantly higher in diastatic power than Mars. The two groups of pure breeding progeny from this cross showed no significant difference in enzymatic activity, although the white aleurone group did show a higher diastatic power than the blue aleurone group. This tendency is also interesting because the white aleurone barleys seem to be preferred for malting purposes.

The visible characteristic used in the study of chromosome V was rough awns vs. smooth awns. The two standard varieties used in this case were O.A.C.21 and Mars. O.A.C.21 is a rough awn variety and Mars has smooth awns. The O.A.C.21 parent was significantly higher in diastatic power than the Mars parent. Of the pure breeding progeny from this cross, the rough awn group was significantly higher in diastatic power than the smooth awn group, which indicates an apparent linkage between high diastase activity and the rough awn characteristic. This relationship should be helpful

information to barley breeders who are interested in selecting superior malting barley lines in the early stages of their breeding program. For example, if a barley breeder should make a cross between a rough awn, high diastatic power variety such as Manchuria, Kindred, or Oderbrucker and a smooth awn, low diastatic power variety such as Wisconsin 38, Bay, or Moore and if there is a rather tight linkage between high diastatic power and rough awns, as the data in this thesis indicate, then the barley breeder should be able to go into his breeding nursery, select the most promising looking lines showing the rough awn characteristic, and be reasonably sure that he is also selecting for high diastase activity. On the other hand, if a barley breeder should make a cross between a rough awn, low diastatic power variety such as Trebi and a smooth awn, high diastatic power variety such as Montcalm, then the smooth awn characteristic should be used to select lines having high diastatic power. Therefore, if a barley breeder desires to use this apparent linkage in making selections from his breeding nursery, he must first test the parents of each cross for diastatic power so he will know which parent has the highest diastatic power and which awn type (rough or smooth) to select

for to give him high diastatic power in the selected progeny.

As was noted by Sallans and Anderson (20) in reporting their studies on the relationship between barley diastatic power, after activation by papain, and malt diastatic power, there is much to be gained in a barley breeding program, if lines can be selected at an early stage on the basis of enzyme activity. The determination of activated barley diastatic power may be made on a sample of 2.5 grams as compared to 550 grams needed for a malting test (16). It is evident that samples can be tested for barley diastatic power much earlier and more conveniently than they can be subjected to malting tests.

The ferricyanide method of determining the diastatic power of barley grain used in this problem proved to be a satisfactory method for determining the diastase activity of malting barley lines and varieties. Since barley diastatic power is positively correlated with malt diastatic power it can be used to predict the malt diastase activity of hybrid barley lines. In addition, since the ferricyanide test requires only a 2.5 gram sample of ground barley, it can be run very early in the breeding program, which would enable the barley

breeder to concentrate on a reduced population of considerable promise. He would then increase and submit only his better material for malting tests.

Now that the important factors affecting malting quality in barley have been determined (diastase activity, ^{BARLEY} malt extract percentage, protein percentage, etc.) and methods of predicting these factors in early generations have been devised, the next big step in malting barley research should be studies to determine whether or not there are additional linkage relationships between visible characteristics that the barley breeder can observe in the field and the important factors affecting malting quality. Additional studies, such as the one reported in this thesis, should be continued and expanded so that future barley breeders will know whether or not it is possible for them to select for certain visible characteristics in their barley breeding nurseries and be assured that they are also selecting for malting quality at the same time.

SUMMARY

This research problem was designed to study the linkage relations between diastase activity, which is a measure of malting quality in barley, and specific visible characteristics known to be inherited on certain chromosome groups. The following crosses were made between standard barley varieties showing the opposite expression of a visible characteristic known to be inherited on a particular chromosome group:

Chromosome group I:

ALPHA	X	O.A.C.21
(2-row)		(6-row)

Chromosome group II:

DORSETT	X	O.A.C.21
(Black glumes)		(White glumes)

Chromosome group III:

O.A.C.21	X	C.I. 1370
(Covered kernels)		(Naked kernels)

Chromosome group IV:

O.A.C.21	X	MARS
(Blue aleurone)		(White aleurone)

Chromosome group V:

O.A.C.21	X	MARS
(Rough awns)		(Smooth awns)

Five chromosome groups were represented in the five crosses, and O.A.C.21 was one of the parents occurring in each cross. The pure breeding lines from the F_2 population (F_4 seed) in each cross were saved and analyzed for diastatic power using a modification of the Anderson-Sallans ferricyanide method (3). Progenies from twelve selections from each of the two standard varieties used as parents in each cross were also carried along in the same field with the crossed material and analyzed for diastatic power. After the chemical determinations were completed and the diastase activity of each sample was converted into degrees Lintner ($^{\circ}L$), the two parents of each cross and the two groups of pure breeding progeny from each cross were compared for significant differences by means of the t-test.

The results may be summarized as follows:

Chromosome group I:

The O.A.C.21 parent is significantly higher in diastatic power than the Alpha parent. There is no apparent linkage between high diastatic power and the 2-row characteristic. There is an apparent pleiotropic effect between high diastatic power and the 2-row characteristic.

Chromosome group II:

The O.A.C.21 parent is significantly higher in diastatic power than the Dorsett parent. There is no significant linkage between high diastatic power and the white glume or black glume characteristic.

Chromosome group III:

The O.A.C.21 parent is significantly higher in diastatic power than the C.I. 1370 parent. There is no significant linkage between high diastatic power and the covered kernel or naked kernel characteristic.

Chromosome group IV:

The O.A.C.21 parent is significantly higher in diastatic power than the Mars parent. There is no significant linkage between high diastatic power and the blue aleurone or white aleurone characteristic. However, the white aleurone group of pure breeding

progeny did tend to run a little higher in diastatic power than did the blue aleurone group.

Chromosome group V:

The O.A.C.21 parent is significantly higher in diastatic power than the Mars parent. There is an apparent linkage between high diastatic power and the rough awn characteristic.

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