THE ASSOCIATION OF TWO GENETIC LINKAGE GROUPS IN BARLEY WITH ONE CHROMOSOME[1]

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BARLEY, with seven chromosome pairs, has had genes assigned to seven linkage groups. ROBERTSON *et al.* (1941, 1947) and SMITH (1951) have so completely summarized the linkage studies on which these groups are based that further review is unnecessary here. None of the linkage groups has yet been associated with a specific chromosome pair, as has been done in maize, nor has it been definitely shown that a different chromosome pair is associated with each linkage group. Independent assortment of genes in one linkage group from those in another is still possible, even though on the same chromosome, if there is a long unmapped region between them.

The purpose of this paper is to present evidence from a cytogenic study of nine chromosomal interchanges in barley, which indicates that two linkage groups, III and VII, may be associated with the same chromosome, and that a different chromosome is associated with each of the other five linkage groups.

WHITE and BURNHAM (1948) and BURNHAM (1951) summarized the pairing relationships in crosses among five of these interchange lines and showed that six different chromosomes, which they designated by the letters a to f, were involved. HANSON and KRAMER (1949, 1950) and HANSON (1952) presented linkage data which identified the two linkage groups associated with each of three of these five lines. HAGBERG and TJIO (1952), also working with several interchange lines furnished by BURNHAM, were able to identify the chromosomes involved in two lines by comparing idiograms of homozygous interchanges with those of normal stocks. Though these two lines have not been used in the studies presented here, their work will permit assigning several of the linkage groups to specific morphologically identifiable chromosomes.

MATERIALS AND METHODS

Of the nine interchange lines used, seven (five of which were homozygous for the interchange) were kindly supplied by C. R. BURNHAM, University of Minnesota. They were produced by X-ray treatment of seed of the variety Mars. The pedigrees of the lines, the chromosomes involved in five as summarized by BURNHAM (1951) and the linkage groups involved in three (HANSON 1952) are presented in table 1; for convenience in presentation, designations 1 to 9 as shown in the first column will be used throughout this paper.

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TABLE 1

Interchange, origin and known chromosomes and linkage groups involved.

Interchange no.	Acc. or 1950 cult.	Minnesota culture	Chromosome involved[1]	Linkage group[2]
1	A272	C1025	....	...
2	A273	C1478	....	...
3	A298	C1385	a + b	....
4	A299	C1405	c + d	....
5	A300	C1420	e + f	I + IV
6	A301	C1432	c + e	IV + VI
7	A302	C1456	a + e	II + IV
8	C156	....	....	....
9	C160	....	....	....

[1]Burnham (1951).
[2]Hanson (1952).

To obtain genetic linkage data, each of the interchange lines was crossed with each of several standard normal linkage testers differentiated by contrasting characters controlled by factor pairs in each of the linkage groups. The characters used, their symbols, the linkage group to which each is assigned, the genotype of the interchange lines and the genetic tester lines having the contrasting characters are summarized in table 2. Since some of the genetic tester stocks were heterozygous for seedling lethal marker genes, F_2

TABLE 2

Linkage groups, marker genes used, the interchange genotype and genetic tester stocks used in linkage tests with interchanges in barley.

Linkage group	Character	Gene symbol	Genotype of interchanges	Genetic tester used
I	Two vs. six-row	*V, v*	*vv*	293
II	Black vs. white pericarp + lemma	*B, b*	*bb*	293
III	Covered vs. naked caryopsis	*N, n*	*NN*	293, 7
IV	Hooded vs. awned glume	*K, k*	*kk*	293, 4, 5
	Green vs. light green seedling	Lg_3, lg_3	Lg_3Lg_3	293 seg.
	Blue vs. non-blue aleurone	*Bl, bl*	*blbl*	296
V	Long vs. short-haired rachilla	*S, s*	*SS*	294, 5, 6
	Rough vs. smooth awn	*R, r*	*rr*	294, 5, 6, 7
VI	Green vs. xantha seedling	*Xc, xc*	*XcXc*	294 seg.
VII	Green vs. virescent seedling	*Yc, yc*	*YcYc*	296 seg.
	Starchy vs. waxy endosperm	*Wx, wx*	*WxWx*	297

seeds from each F_1 plant were space-planted in separate rows to differentiate populations segregating for lethals. Such populations are presented separately only when linkage with a lethal seedling marker gene was found. F_2 plants were classified for spike fertility and for the marker genes. F_3 plant rows were grown from F_2 plants of selected phenotypes in most crosses but only F_3 data which indicate linkage or which are derived from F_2 data which indicate linkage are presented.

Tests for linkage were made using chi square tests for independence cal-

culated from formulas derived from those given in table 4 of HANSON and KRAMER (1950). For the four class segregation the formula used was

$$\chi^2 = (b + 3c - a - 3d)^2/3N, \text{ D.F.} = 1,$$

and for the two class segregations due to the elimination of recessive seedling lethals,

$$\chi^2 = (b - a)^2/N, \text{ D.F.} = 1,$$

which is in effect a test of significance for the deviation from a 1 : 1 ratio. When the deviations from a 1 : 1 ratio were significant, 2×2 contingency tables were used for the sterility and marker gene classifications and chi square for independence calculated in the usual manner.

For F_3 data χ^2 tests were calculated according to the formulae

$$\chi^2 = (2e - f)^2/2N, \text{ D.F.} = 1,$$

and

$$\chi^2 = (h - 2g)^2/2N, \text{ D.F.} = 1,$$

for the genotypes of partially sterile *A* and fully fertile *A* F_2 plants respectively.

Normal plants from non-homozygous interchange lines which gave semi-sterile F_1 plants in crosses with the genetic testers, were selected to establish the homozygous interchange lines. The nine homozygous interchange lines were then crossed in all possible combinations and sporocytes of the F_1 plants examined cytologically. Spikes were killed and fixed in Carnoy's solution and stored in a refrigerator in 80% alcohol. Aceto-carmine smears were examined at metaphase I, or when possible at diakinesis.

RESULTS OF GENETIC STUDIES

For the interchange lines not previously reported, summaries of the F_2 segregation for partially sterile vs. fully fertile spikes (S vs. F) and for each of the marker gene pairs used to test for linkage are given in tables 3–7. From table 3, linkage of partial sterility with *Nn* in linkage group III and with *Ycyc* in group VII was found. Since *Wxwx*, also in group VII might be expected to show linkage, further tests in F_3 were made of the 113 *SWx* and the 117 *FWx* plants from cross 1×297 and the results are shown in the first line of table 8. No evidence for linkage was found. If group VII is involved the point of interchange must be nearer to *Ycyc* than to *Wxwx*.

In table 4, sterility due to interchange 2 appears to be linked with *Nn* and with *Ss* and *Rr* in group V. In view of the cytological results presented later the possibility of linkage with *Wxwx* in VII also should be examined. The F_2 data failed to show significant deviation from independence of *Wxwx* and the sterility classification but is in the expected direction if linkage were present. In F_3 (line 2, table 8) the genotypes of the 93 *FWx* F_2 plants from cross 2×297 indicate linkage. Thus groups III, V, and possibly VII are involved in this interchange.

TABLE 3

Summary of F_2 segregation in crosses between barley interchange no. 1 and linkage testers.

Cross	Gene pair	Phenotype and class				Total N	χ^2 for independence
		SA a	FA b	Sa c	Fa d		
1 × 293	*Vv*	127	84	30	33	294	3.07
1 × 293	*Bb*	128	103	29	34	294	1.81
1 × 293, 7	*Nn*	258	196	39	91	584	27.13[2]
1 × 293, 4, 5	*Kk*	388	316	112	120	936	3.28
1 × 293	Lg_3lg_3	48	35	....	....	83	2.03
1 × 296	*Blbl*	135	93	39	34	301	0.81
1 × 294, 5, 6	*Ss*	396	315	121	111	943	0.92
1 × 296	*Rr*	45	50	17	16	128	0.17
1 × 296[1]	*Rr*	83	48	29	13	173	0.45
1 × 294	*Xcxc*	12	11	....	....	23	0.04
1 × 296	*Ycyc*	112	61	....	....	173	15.01[2]
1 × 297	*Wxwx*	113	117	27	33	290	0.23

[1]Segregating for *Ycyc*.
[2]P <.01.

The linkage relationships of interchange 3 are calculated in table 5. Linkage of sterility with *Nn*, *Blbl* and *Ycyc* are indicated. Linkage with *Blbl* in group IV was not substantiated by linkage with Lg_3lg_3, also in IV, nor with *Kk* in spite of a very large population. In F_3 (table 8), only one out of four populations substantiated linkage with *Blbl* and non-linkage with Lg_3lg_3 was verified.

The segregation in F_3 of the *SK* phenotype from the cross of 3 × 293 gives a significant chi square value but the deviation is in a direction opposite to that expected from linkage. Linkage with *Ycyc* was verified in one F_3 population and no linkage with *Wxwx* was found either in F_2 or F_3. On the basis of genetic data above, chromosomes carrying linkage groups III and possibly VII may be involved in interchange 3. Association with IV appears doubtful.

TABLE 4

Summary of F_2 segregation in crosses between interchange no. 2 and linkage testers.

Cross	Gene pair	Phenotype and class				Total N	χ^2 for independence
		SA a	FA b	Sa c	Fa d		
2 × 293	*Vv*	19	14	11	4	48	1.77
2 × 293	*Bb*	21	13	9	5	48	0.11
2 × 293, 7	*Nn*	140	70	33	73	316	38.08[2]
2 × 293, 4, 5	*Kk*	205	148	75	48	476	0.40
2 × 293	Lg_3lg_3	30	18	....	....	48	3.00
2 × 296	*Blbl*	52	51	15	17	135	0.12
2 × 294, 5, 6	*Ss*	265	116	52	130	563	86.85[2]
2 × 294, 5, 6	*Rr*	114	85	15	23	237	3.95[1]
2 × 297	*Wxwx*	117	93	26	32	268	2.19

[1]P <.05.
[2]P <.01.

TABLE 5

Summary of F_2 segregation in crosses between interchange no. 3 and linkage testers.

Cross	Gene pair	Phenotype and class SA a	FA b	Sa c	Fa d	Total N	χ^2 for independence
3 × 293	*Vv*	94	72	33	21	220	0.30
3 × 293	*Bb*	87	70	40	23	220	1.75
3 × 293, 7	*Nn*	190	83	12	87	372	98.77[3]
3 × 293, 4, 5	*Kk*	332	268	117	106	823	0.39
3 × 293	Lg_3lg_3	36	23	....	....	59	2.86
3 × 296	*Blbl*	76	46	14	30	166	12.22[3]
3 × 296[1]	*Blbl*	105	58	18	35	216	15.13[3]
3 × 294, 5	*Ss*	262	224	96	80	662	0.05
3 × 296	*Rr*	65	64	25	12	166	2.90
3 × 296[1]	*Rr*	95	70	28	23	216	0.11
3 × 296	*Ycyc*	123	93	....	....	216	4.17[2]
3 × 297	*Wxwx*	62	62	13	15	152	0.08

[1]Segregating for *Ycyc*.
[2]P <.05.
[3]P <.01.

Linkage of any of the marker genes and sterility due to interchange 4 was difficult to establish. Only *Xcxc* in linkage group VI showed indication of linkage (table 6) which was verified in F_3 (table 8).

Data on lines 8 and 9 was limited to the F_2 results in table 7. Sterility for line 8 shows linkage with *Nn* in III and *Wxwx* in VII while that due to line 9 shows association with *Nn*.

CYTOLOGICAL OBSERVATIONS

Cytological observations were obtained on 32 of the 36 possible F_1 hybrids between the 9 interchange lines (table 9). With one exception these could be

TABLE 6

Summary of F_2 segregation in crosses between interchange no. 4 and linkage testers.

Cross	Gene pair	Phenotype and class SA a	FA b	Sa c	Fa d	Total N	χ^2 for independence
4 × 293	*Vv*	102	99	32	25	258	0.42
4 × 293	*Bb*	108	92	26	32	258	1.49
4 × 293, 7	*Nn*	239	238	60	60	597	0.00
4 × 293, 4, 5	*Kk*	330	313	124	113	880	0.10
4 × 293	Lg_3lg_3	71	62	....	....	133	0.61
4 × 296	*Blbl*	128	116	21	28	293	1.24
4 × 294, 5	*Ss*	241	225	79	77	622	0.05
4 × 296	*Rr*	119	115	30	29	293	0.00
4 × 294	*Xcxc*	26	12	....	....	38	5.16[1]
4 × 296	*Ycyc*	109	109	....	....	218	0.00

[1]P less than .05.

TABLE 7

Summary of F_2 segregation in crosses of interchanges 8 and 9 with linkage testers.

Cross	Gene pair	Phenotype and class				Total N	χ^2 for independence
		SA a	FA b	Sa c	Fa d		
8 × 297	*Nn*	55	40	2	25	122	19.28[2]
8 × 294	*Kk*	88	59	23	26	196	2.45
8 × 294	*Ss*	81	70	30	15	196	1.97
8 × 294, 7	*Rr*	60	73	20	13	166	2.32
8 × 297	*Wxwx*	46	42	11	23	122	4.37[1]
9 × 297	*Nn*	26	11	3	13	53	12.74[2]
9 × 294, 7	*Rr*	26	21	6	5	58	0.02
9 × 297	*Wxwx*	20	17	9	7	53	0.06

[1]P <.05.
[2]P <.01.

characterized at MI either by a ring of six chromosomes and four pairs, or by two rings of four and three pairs. The ⊙6 indicates that one chromosome pair is common to both interchanges while 2⊙4 indicates that two different pairs are involved. CALDECOTT and SMITH (1952, figs. 6–8) have illustrated these configurations.

All intercrosses involving lines 3, 4 and 5 show 2⊙4 and agree with the results presented by BURNHAM (1951) showing that 6 different chromosomes are involved in interchanges in these three lines. Intercrosses between lines 5, 6 and 7 show that one chromosome involved is common to interchanges in all three lines. BURNHAM has designated this e and HANSON (1952) has shown that each of the three lines shows linkage with genes in group IV. The ⊙6 in the F_1 of 3 × 7 indicates a chromosome in common to be involved in

TABLE 8

Summary of F_2 genotypes of selected F_2 phenotypes as determined by breeding behavior in F_3.

Cross	Population[1]	Gene pair	Genotype		Total N	χ^2	Genotype		Total N	χ^2
			SAA e	SAa f			FAA g	FAa h		
1 × 297	A	*Wxwx*	37	76	113	0.02	44	73	117	0.96
2 × 297	A	*Wxwx*	44	73	117	0.96	42	51	93	5.85[2]
3 × 293	A	*Kk*	17	50	67	1.91	7	37	44	6.01[2]
3 × 295	A	"	20	62	82	2.95	21	33	54	.75
3 × 293	B	Lg_3lg_3	14	22	36	0.50	8	15	23	0.02
3 × 296	A	*Blbl*	23	53	76	0.32	16	30	46	0.04
3 × 296	B	"	23	82	105	7.17[2]	23	35	58	1.04
3 × 296	B	*Ycyc*	38	83	121	0.20	41	50	91	5.63[2]
3 × 297	A	*Wxwx*	25	37	62	1.36	22	40	62	0.13
4 × 294	B	*Xcxc*	1	25	26	10.17[3]	10	2	12	13.50[3]

[1]A = from populations not segregating for lethal seedling, B = segregating.
[2]P less than .05.
[3]P less than .01.

TABLE 9

Summary of cytological configurations observed in F_1 hybrids among 9 homozygous interchange lines in barley and the chromosomes involved.

Interchange	1	2	3	4	5	6	7	8	9
1	Tb – d								
2	1C4, 5 II[1]	Tb – d							
3	1⊙6, 4 II	1⊙6, 4 II	Ta – b						
4	1⊙6, 4 II	1⊙6, 4 II	2⊙4, 3 II	Tc – d					
5	....	2⊙4, 3 II	2⊙4, 3 II	2⊙4, 3 II	Te – f				
6	2⊙4, 3 II	2⊙4, 3 II	2⊙4, 3 II	1⊙6, 4 II	1⊙6, 4 II	Tc – e			
7	2⊙4, 3 II	2⊙4, 3 II	1⊙6, 4 II	2⊙4, 3 II	1⊙6, 4 II	1⊙6, 4 II	Ta – e		
8	1⊙6, 4 II	1⊙6, 4 II	1⊙6, 4 II	1⊙6, 4 II	2⊙4, 3 II	1⊙6, 4 II	....	Tb – c	
9	1⊙6, 4 II	....	....	2⊙4, 3 II	1⊙6, 4 II	2⊙4, 3 II	2⊙4, 3 II	1⊙6, 4 II	Tb – f

[1]Mostly chains of 4 chromosomes, occasionally a ring, infrequently 7 II.

both lines (designated a by BURNHAM). A similar conclusion may be reached regarding the F_1 of 4×6 and this chromosome has been designated c. Using these five lines as reference points the chromosomes involved in the remaining four lines are easily established and are shown in the diagonal cells in table 9. That lines 1 and 2 involve the same two chromosomes is verified by the fact that five and occasionally 7 pairs are observed in the F_1 between them.

DISCUSSION

The genetic data in tables 3–8, and the published data of HANSON and KRAMER (1949, 1950) and HANSON (1952) indicate that genes in each of the seven linkage groups have shown associations with sterility in crosses of linkage testers to one or more of the 9 interchange lines. It is noteworthy, however, that in four of the five cases in which *Nn* in group III showed association with sterility, either *Ycyc* or *Wxwx* in group VII also gave evidence of association with sterility in the same interchange.

TABLE 10

A summary of possible linkage groups involved from genetic studies and the chromosomes involved from cytological observations in 9 interchange lines.

Interchange	Possible linkage group	Chromosome involved	Suggested linkage group
1	III, VII	b – d	III, VII – V
2	III, V, VII	b – d	III, VII – V
3	III, IV, VII	a – b	II – III, VII
4	VI	c – d	VI – V
5	I, IV	e – f	IV – I
6	IV, VI	c – e	VI – IV
7	II, IV	a – e	II – IV
8	III, VII	c – b	VI – III, VII
9	III	b – f	III, VII – I

The cytological evidence indicates that only six chromosomes are involved in interchanges in the 9 lines. The genetic and cytological data are summarized for comparison in table 10. There appears to be little question that chromosome b carries linkage group III and e carries IV. Linkage of group IV appears not to be involved in line 3 in spite of a significant χ^2 test for *Blbl*. *Lg*$_3$*lg*$_3$ and *Kk* in the same group showed no linkage, F_3 data were not conclusive, and the cytological data rule out this group. Chromosome a involved in lines 3 and 6 probably carries linkage group II. Chromosome c and group VI are associated in lines 4 and 6, and in line 8, linkage with group VI was not tested. Chromosome f is involved in lines 5 and 9 but linkage data with group I are available only for line 5. Chromosome d must be associated with groups V or VII. Assigning group VII to d, however, is inconsistent with the cytological data on lines 3 and 8 and with the genetic data for line 4. Assigning group V to chromosome d causes no inconsistency either in the cytological or the genetic data though linkage was undetected in lines 1 and 4. The conclusion that both linkage groups III and VII are carried by chromo-

some b appears warranted, and the other five groups must be distributed among 5 different chromosomes. The suggested linkage groups are summarized in the last column of table 10.

HAGBERG and TJIO (1950) have presented a chromosome idiogram for barley and have numbered the five non-satellite chromosomes from I to V in order of falling total length, I being longest. Chromosome VI was the smaller satellite chromosome with a large satellite and VII was the longer chromosome with a small satellite. Later (1952), using five interchanges from BURNHAM which included lines 3, 5 and 6 studied here they attempted to determine the chromosomes involved in relation to their standard idiogram. Unfortunately the chromosomes involved in the three lines studied here could not be identified. However, on two other interchanges involving b + d and b + (g) +, they were able to show that chromosome b is III, the third longest non-satellite chromosome, d is VII and g is VI. It would appear then, that these three chromosomes carry linkage groups III and VII, V, and the last unestablished linkage group in barley, respectively.

SUMMARY AND CONCLUSIONS

Nine reciprocal chromosomal interchange lines of barley were tested for linkage of the interchange points with one or more factor pairs in each of the seven linkage groups in barley.

The nine interchanges also were intercrossed in all combinations and pairing relationships observed in the F_1 hybrids.

In correlating the genetic linkage data with the cytological observations the conclusion was reached that two linkage groups, heretofore designated III and VII are carried by the same chromosome and of the remaining five linkage groups, each is on a different chromosome.

In relating these results to the chromosome idiograms of HAGBERG and TJIO (1952) it appears probable that linkage groups III and VII are carried by the third longest non-satellite chromosome, the larger of the two satellite chromosomes probably carries linkage group V, while the smaller of the satellite chromosomes probably carries a linkage group to which no genes have as yet been assigned.

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