LINKAGE STUDY OF TWO COMPLEMENTARY GENES FOR BRITTLE RACHIS IN BARLEY

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In the previous experiment (1959b) the writers have indicated that the complementary genes, Bt and Bt_2, for brittle rachis in barley are both located on chromosome 3: one of them, Bt, is linked with a_n, a_c and x_c with 1.24%, 13.62% and 15.31% recombination, respectively, and the other one, Bt_2, and uz are in linkage with 17.90% recombination. However, it was obscure whether Bt and Bt_2 were very closely associated or considerably distant from each other. This study was planned to disclose the linkage intensity between Bt and Bt_2 and their relation with two marker genes, x_c and al in the same linkage group.

The writers wish to express their sincere appreciation to Dr. Philip G. Miles, Associate Professor, State University of New York at Buffalo, for his kindness in reading the manuscript. Thanks are due to Mr. Isamu Moriya for his kind assistance in the preparation of experimental materials.

MATERIAL AND METHOD

A single cross between two cultivars with tough rachis, Colsess IV and Russian 82, was used as the material. The genic constitution of Colsess IV has already been known to be $\frac{x_c}{X_c} \frac{Al}{Al} \frac{bt}{bt} \frac{Bt_2}{Bt_2}$ and that of Russian 82 $\frac{X_c}{X_c} \frac{al}{al} \frac{Bt}{Bt} \frac{bt_2}{bt_2}$, where al is a gene for albino lemma and x_c for xantha seedling (lethal). Since Colsess IV was heterozygous for x_c, the cross gave two kinds of F_1 hybrids with genotype of either $\frac{x_c}{X_c} \frac{Al}{al} \frac{bt}{Bt} \frac{Bt_2}{bt_2}$ or $\frac{X_c}{X_c} \frac{Al}{al} \frac{bt}{Bt} \frac{Bt_2}{bt_2}$. For brevity, the former genotype will be called x_c-heterozygote and the latter X_c-homozygote in the following. From X_c-homozygous F_1 plants, a total of 215 F_2 plants were raised and 144 out of them were subjected to detailed analysis. For the x_c-heterozygote, 87 F_2 plants and F_3 strains were similarly studied. In order to know the genic constitution for brittleness of rachis of each of the F_2 plants, a total of 231 F_2 plants were crossed each with two kinds of genotype analysers which were already known to be of type W ($btbtBt_2Bt_2$) or of type E ($BtBtbt_2bt_2$). As the result of the test-cross, it is expected that nine possible F_2 genotypes will give the segregation of brittle and tough rachis plants in the ratios as shown in Table 1.

TABLE 1

Expected segregation ratios of brittle vs. tough rachis plants in
the F_3 and the following generation of test-cross with nine
possible F_2 genotypes

F_2 phenotype	F_2 genotype, Gametes		When crossed with		F_3 segregation
			$btBt_2$ (W)	$Btbt_2$ (E)	
			Brit.: tough	Brit.: tough	Brit.: tough
Brittle rachis	(1) $Bt\,Bt\,Bt_2\,Bt_2$	$Bt\,Bt_2$	all : none	all : none	all : none
	(2) $Bt\,Bt\,Bt_2\,bt_2$	$\begin{cases} Bt\,Bt_2 \\ Bt\,bt_2 \end{cases}$	all : none	1 : 1	3 : 1
	(3) $Bt\,bt\,Bt_2\,Bt_2$	$\begin{cases} Bt\,Bt_2 \\ bt\,Bt_2 \end{cases}$	1 : 1	all : none	3 : 1
	(4) $Bt\,bt\,Bt_2\,bt_2$	$\begin{cases} Bt\,Bt_2 \\ Bt\,bt_2 \\ bt\,Bt_2 \\ bt\,bt_2 \end{cases}$	1 : 1	1 : 1	9 (1) : 7 (1) *
Tough rachis	(5) $Bt\,Bt\,bt_2\,bt_2$	$Bt\,bt_2$	all : none	none : all	all tough
	(6) $Bt\,bt\,bt_2\,bt_2$	$\begin{cases} Bt\,bt_2 \\ bt\,bt_2 \end{cases}$	1 : 1	none : all	tough
	(7) $bt\,bt\,Bt_2\,Bt_2$	$bt\,Bt_2$	none : all	all : none	tough
	(8) $bt\,bt\,Bt_2\,bt_2$	$\begin{cases} bt\,Bt_2 \\ bt\,bt_2 \end{cases}$	none : all	1 : 1	tough
	(9) $bt\,bt\,bt_2\,bt_2$	$bt\,bt_2$	none : all	none : all	tough

* The ratio depends on linkage intensity between Bt and Bt_2.

EXPERIMENTAL RESULTS

1. X_c-Homozygote

Table 2 shows segregation of green vs. albino lemma and brittle vs.
tough-ear condition in the F_2 generation of the X_c-homozygote or the hybrid
free from the lethal gene x_c for xantha seedling. Segregation of green vs.
albino lemma character pair fitted well to an expected 3 : 1 ratio. However,
the observed phenotypes for brittle vs. tough rachis showed a slightly poor fit
to a 9:7 ratio, but a good fit to a 1:1 ratio. Further, the fit of the observed
frequencies of the four classes to the calculated on the basis of independent
assortment of the two gene pairs, $Alal$ and $BtbtBt_2bt_2$, namely 27:21:9:7 or 3:3:
1:1 segregation ratios was found to be very poor, indicating the association of
both pairs.

In Table 3 is shown the observed number of different F_2 genotypes as
determined by F_3 test and also test-crosses with two kinds of genotype analysers
of type W ($bt\,bt\,Bt_2\,Bt_2$) and of type E ($Bt\,Bt\,bt_2\,bt_2$). The most important and
remarkable fact in this result is that, with regard to the brittleness and toughness
of rachis, only three genotypes, namely, two parental and their F_1 hybrid types,
were found and no other recombination types could be found. This obviously

TABLE 2

F_2 data for the X_c-homozygote : segregation of green vs. albino lemma
and brittle vs. tough rachis and their interaction

Item	Green brittle	Green tough	Albino brittle	Albino tough	Total	χ^2
Observed count	85	67	20	43	215	
Calc. on 27 : 21 : 9 : 7	90.8	70.5	30.2	23.5	215	20.17
Calc. on 3 : 3 : 1 : 1	80.625	80.625	26.875	26.875	215	13.97

Item	χ^2	D. F.	Probability
Segregation for $Alal$ (152 : 63)	2.1225	1	0.2 - 0.1
Segregation for brittle vs. tough (105 : 110), compared with 9 : 7 ratio	4.8391	1	<0.05
Segregation for brittle vs. tough, compared with 1 : 1 ratio	0.1163	1	0.8 - 0.7

TABLE 3

F_2 genotypes from X_c- homozygous F_1 plant, as determined by F_3
test and the test-cross with genotype analyzers of
type E ($Bt\,bt_2$) and of type W ($bt\,Bt_2$)

Brittle $\dfrac{bt\,Bt_2}{Bt\,bt_2}$			Tough (type W) $\dfrac{bt\,Bt_2}{bt\,Bt_2}$			Tough (type E) $\dfrac{Bt\,bt_2}{Bt\,bt_2}$			others	Total
$Al\,Al$	$Al\,al$	$al\,al$	$Al\,Al$	$Al\,al$	$al\,al$	$Al\,Al$	$Al\,al$	$al\,al$		
7	44	18	20	12	1	0	5	37	0	144

TABLE 4

Re-arranged data shown in Table 3

A. $Alal$ - $Btbt$ (repulsion)		B. $Alal$ - Bt_2bt_2 (coupling)	
Doubly dominant group		Doubly dominant group	
$AlAl$ $BtBt$	0	$AlAl$ Bt_2Bt_2	20
$Alal$ $BtBt$	7	$Alal$ Bt_2Bt_2	7
$AlAl$ $Btbt$	5	$AlAl$ Bt_2bt_2	12
$Alal$ $Btbt$	44	$Alal$ Bt_2bt_2	44
Singly dominant : group 1		Singly dominant : group 1	
$AlAl$ $btbt$	20	$AlAl$ bt_2bt_2	0
$Alal$ $btbt$	12	$Alal$ bt_2bt_2	5
Singly dominant : group 2		Singly dominant : group 2	
$alal$ $BtBt$	37	$alal$ Bt_2Bt_2	1
$alal$ $Btbt$	18	$alal$ Bt_2bt_2	18

indicate complete linkage of $Btbt$ with Bt_2bt_2.

The data shown in Table 3 were then re-arranged and shown in Table 4.
From five out of six sets of data recombination values of $Alal$ and $Btbt$ in repulsion

phase and those of *Alal* and Bt_2bt_2 in coupling phase were first calculated separately. Then all of the recombination values obtained were put together and average, weighted recombination values were estimated as Bt and Bt_2 could be regarded to be on the same locus (Table 5). As seen in Table 5 it was 15.37 ±2.0261 (%).

TABLE 5
Calculation of weighted p value for *Alal* and *Btbt* ($=Bt_2 bt_2$)

Source of data		p	n	i	I=ni	pI
Doubly dominant	*Alal – Btbt*	0.1144	56	16.636	931.616	106.5768704
Singly dominant-1	// //	0.2308	32	7.438	238.016	54.9340928
Singly dominant-2	// //	0.1957	55	8.894	489.170	95.7305690
Doubly dominant	$Alal - Bt_2 bt_2$	0.1882	83	6.748	560.084	105.4078088
Singly dominant-2	// //	0.1000	19	6.156	116.964	11.6964000

$$SI=2435.850 \quad SpI=374.3457410$$

$$p= SpI/SI= 0.1537 \quad : \quad i_p=\sqrt{1/SI}=0.020261$$

2. x_c-Heterozygote

As stated before, this hybrid included four pairs of genes, X_cx_c, *Alal*, *Btbt* and Bt_2bt_2, in heterozygous condition. And, because of the lethal effect of x_c for xantha seedling with which three other pairs were in linkage, F_2 segregation of green vs. albino lemma and brittle vs. tough ear character pairs considerably deviated from the expected 3 : 1 and 1 : 1 ratios, respectively. This made it difficult to investigate linkage relations wih F_2 data.

TABLE 6
F_2 genotypes derived from the x_c-heterozygous F_1 plant, as determined by test-cross with two types of genotype analyzers, type W and E

	Brittle $\frac{btBt_2}{Btbt_2}$			Tough (W) $\frac{btBt_2}{btBt_2}$			Tough (E) $\frac{Btbt_2}{Btbt_2}$			Others	Total
	AlAl	*Alal*	*alal*	*AlAl*	*Alal*	*alal*	*AlAl*	*Alal*	*alal*		
X_c / X_c	0	3	8	0	1	1	0	1	18	0	32
X_c / x_c	0	41	2	3	2	0	0	7	0	0	55
Total	0	44	10	3	3	1	0	8	18	0	87

In Table 6 are shown frequencies of various genotypes among the F_2 plants derived from the x_c-heterozygous F_1 plants. These were determined by F_3 progeny test and also test-cross with two kinds of genotype analyzers for brittleness of rachis. It is noted in this table that with regards genic constitution for brittleness of rachis only parental and F_1 hybrid types have been found and no recombinants existed among the F_2 plants analysed.

Linkage intensities of X_cx_c and *Alal* and also of X_cx_c and *Btbt* (Bt_2bt_2) were

estimated from the data shown in Table 7 that were obtained by re-arranging the data in Table 6. The results are shown in Tables 8 and 9. The same kinds of results were then combined and the weighted average recombination values were calculated. The estimates obtained are 5.48 ± 1.7884 (%) for $X_c x_c$ and $Al al$ and 18.23 ± 2.6953 (%) for $X_c x_c$ and $Bt bt$ or $Bt_2 bt_2$.

TABLE 7
Re-arranged data in Table 6

A. $X_c x_c - Al al$ (Repulsion)		B. $X_c x_c - Bt bt$ (Coupling)		C. $X_c x_c - Bt_2 bt_2$ (Repulsion)	
1. Doubly dominant		1. Doubly dominant		1. Doubly dominant	
$X_c X_c$ $Al Al$	0	$X_c X_c$ $Bt Bt$	19	$X_c X_c$ $Bt_2 Bt_2$	2
$X_c x_c$ $Al Al$	3	$X_c x_c$ $Bt Bt$	7	$X_c x_c$ $Bt_2 Bt_2$	5
$X_c X_c$ $Al al$	5	$X_c X_c$ $Bt bt$	11	$X_c X_c$ $Bt_2 bt_2$	11
$X_c x_c$ $Al al$	50	$X_c x_c$ $Bt bt$	43	$X_c x_c$ $Bt_2 bt_2$	43
2. Singly dominant				2. Singly dominant	
$X_c X_c$ $al al$	27	·		$X_c X_c$ $bt_2 bt_2$	19
$X_c x_c$ $al al$	2			$X_c x_c$ $bt_2 bt_2$	7

TABLE 8
Calculation of average weighted recombination value between $X_c x_c$ and $Al al$

Source of data	p	n	i	I=ni	pI
Doubly dominant	0.0750	58	26.244	1522.152	114.1614000
Singly dominant	0.0357	29	55.314	1604.106	57.2665842

SI=3126.258 SpI=171.4279842

p=SpI/SI=0.0548 : $i_p = \sqrt{1/SI} = 0.017884$

TABLE 9
Calculation of average weighted recombination value between $X_c x_c$ and
$Bt bt$ or $Bt_2 bt_2$

Source of data		p	n	i	I=ni	pI
Doubly dominant	$X_c x_c - Bt bt$	0.1952	80	6.356	508.480	99.2552960
Doubly dominant	$X_c x_c - Bt_2 bt_2$	0.1848	61	9.366	571.326	105.5810448
Singly dominant	″ ″	0.1556	26	11.412	296.712	46.1683872

SI=1376.518 SpI=251.0047280

p=SpI/SI=0.1823 : $i_p = \sqrt{1/SI} = 0.026953$

DISCUSSION

Genetic analysis was made of 231 F_2 hybrid plants of a cross between two cultivated varieties, Colsess IV and Russian 82, differing with each other for the gene pairs, $X_c x_c$, $Al al$, $Bt bt$ and $Bt_2 bt_2$. The result was that Bt and Bt_2 were linked so completely that any kind of recombinant types was not found, though

the number of plants analysed was too small to draw such a conclusion.   In spite of this, however, the F_1 hybrid of this and similar crosses have been found to be of brittle rachis, indicating that *Btbt* and *Bt₂bt₂* are non-allelic.   So, it may be safe to consider that these two gene pairs are pseudo-allelic.

There are many examples of pseudo-allelic genes in animals and plants. Komai (1950) has suggested that these probably have different chromosomal bases as their origin.   So, it is a problem of interest to know how the pseudo-allelic loci under consideration have originated.   In any case, it may be possible to suppose that the origin of these two recessive genes, *bt* and *bt₂*, could be attributable to different mutations that have occurred independently.

Next, let us consider the distance between and order of arrangement of *Btbt* and several other genes on chromosome 3.   From the results obtained in this experiment the distance and relative position of three genes, x_c, *al* and *bt* ($=bt_2$) may be represented as shown in the upper part of Fig. 1.   Robertson (1937), Takahashi and Yamamoto (1951), Takahashi and Hayashi (1959 a, b) have estimated the recombination values between uz, a_c, x_c, *al*, a_n and *bt* ($=bt_2$) and have suggested their locations on chromosome 3 by genetical studies with different crosses.   The known distances between these genes are shown together in the lower part of Fig. 1.   There are two questions in this chromosome map. One is the relative position of x_c and a_c, of which we have previously made a brief comment (Takahashi and Hayashi 1959 a).   Robertson (1937) is of opinion that x_c is located at the right side of a_c.   However, it seems more plausible to
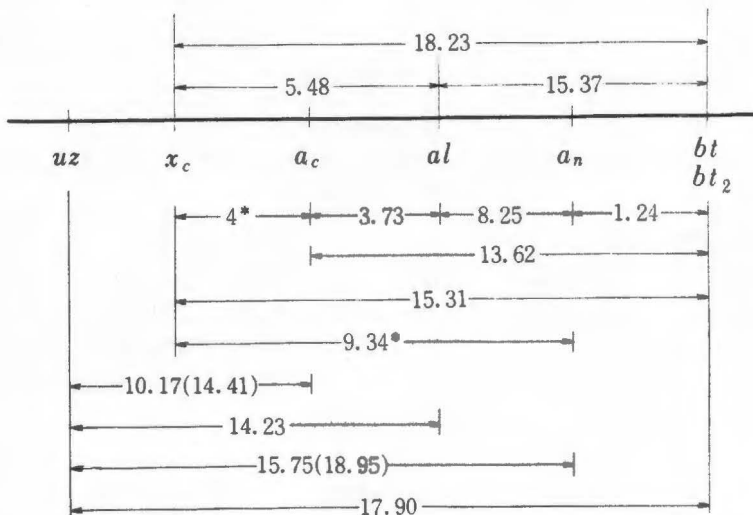

Fig. 1.   Several genes on chromosome 3 : their arrangement and distance, constructed with the data by Robertson (1937, with asterisk) and Takahashi (1951), Takahashi & Hayashi (1959a, b and this report).

consider that x_c is at the left of a_c for two reasons: the distance x_c-al (5.48%) is

1.75% longer than that for a_c-al (3.73%) and the disiance x_c-bt (15.31%) is 1.69% longer than that for a_c-bt (13.62%).   It is noted in this connection that some difficulties are encountered in ascertaining this inasmuch as x_c and a_c appear to be rather closely associated and moreover, they are the genes for lethal which reject to three point test.   Location of bt may be another problem that needs further scrutiny, although we have tentatively placed it at the right of a_n, only because of the longer distance between al and bt than that between al and a_n.

SUMMARY

A study was made of the linkage intensity between two complementary genes, $Btbt$ and Bt_2bt_2, for brittle vs. tough rachises of barley and also of their relations to X_cx_c for green vs. xantha seedling and $Alal$ for green vs. albino lemma character pairs.   The results obtained may be summed as follows:

1.   Two genes, $Btbt$ and Bt_2bt_2, were found to be pseudo-allelic: complementary action of Bt and Bt_2 in the heterozygote suggested that these two were on different loci, but no recombinants were recovered in F_2 of a cross between two different genotypes, $BtBtbt_2bt_2$ and $btbtBt_2Bt_2$.

2.   Linkages of Bt and Bt_2 with x_c and al were confirmed.   The recombination values obtained are:

$$Bt\ (=Bt_2)-al\cdots\cdots 15.37\pm 2.0261(\%)$$
$$Bt\ (=Bt_2)-x_c\cdots\cdots 18.23\pm 2.6953(\%)$$
$$x_c\ \ -\ \ al\cdots\cdots\ 5.48\pm 1.7884(\%)$$

3.   From this and some other previous results the order of arrangement of several genes on chromosome 3 was determined and shown in Fig. 1.

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