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Chromosome Mapping in Barley by Means of Telotrisomic Analysis*

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Summary. A total of 37 genetic markers located in chromosomes 2, 3, 4 and 5 were associated with specific arms by means of telotrisomic analysis in five telotrisomics (Triplo 2 L, 2 S, 3 S, 4 S, 5 L) of barley (*Hordeum vulgare* L.). The genes *v, gp* (*= gp 2*), *li, gs 5, tr* and *msg 2* showed a trisomic ratio with Triplo 2 L indicating that these genes were on the long arm of chromosome 2. A disomic ratio was obtained for genes *wst 4, gs 5,* and *v* with Triplo 2 S, confirming that these genes were on the long arm of chromosome 2(2 L). A disomic ratio was observed for genes *e, f*(*= lg*), *sk,* and *gs 6* with Triplo 2 L. Two genes, *f*(*= lg*) and *gs 6* showed a trisomic ratio with Triplo 2 S. These results indicated that genes *e, f*(*= lg*), *sk,* and *gs 6* are on the short arm of chromosome 2 (2 S). Since only one telocentric chromosome was available for chromosome 3, 4 and 5, most of the well-mapped marker genes were tested with those telocentric chromosomes. The genes *cu 2, uz, wst, als, gs 2, zb, f 2,* and *cer-zn*^{348} showed trisomic ratio with the telocentric for chromosome 3. These genes were located on the short arm of chromosome 3 (Robertson 1971). This indicated that the telocentric chromosome is for the short arm of chromosome 3(3 S). A disomic ratio was obtained for genes *yst,* x_c, *al, yst 2,* a_n, *ari-a*^{6} and x_s, indicating that these genes are on the long arm of chromosome 3. Two genes, *f 9* and *K,* showed trisomic ratio with the telocentric chromosome for 4, while genes *gl*(*= g l 2*), *br 2, yh, lg 3, lg 4* and *lk 5* showed disomic ratios. This indicated that the telocentric chromosome is for the short arm of chromosome 4. Two genes, *fs 2* and *g,* were studied with Triplo 5 L. Both showed trisomic ratio, indicating that *fs 2* and *g* are located on Triplo 5 L. The centromere position (C) on chromosome 2, 3 and 4 was thus located as (the left side of C is the short arm and the right is the long arm): chromosome 2: *f – sk – gs 6 – e –* C *– gs 5 – msg 2 – wst 4 – v – gp – li – tr;* chromosome 3: *f 2 – cer-zn*^{348} *– uz – gs 2 – als – cu 2 – wst – zb –* C *– yst –* x_c *– al – yst 2 –* a_n *– ari-a*^{6} *–* x_s; chromosome 4: *f 9 – K –* C *– lg 4 – lg 3 – gl 2 – br 2 – lk 5 – yh.* The centromere position on chromosome 5 was not precisely located.

Key words: Telotrisomics – Linkage – Chromosome mapping – Centromere

Introduction

Telocentric chromosomes have been used in the genetic linkage studies in several plant species such as tomato (Khush and Rick 1968), maize (Rhoades 1936, 1940), diploid wheat (Moseman and Smith 1954), hexaploid wheat (Love 1943; Sears 1962, 1966), cotton (Endrizzi and Kohel 1966), and oats (McGinnis et al. 1963). These chromosomes have been very useful in associating a gene with a particular chromosome arm. The theoretical basis of genetic segregation ratios in telotrisomic analysis was given by Reeves et al. (1968). In a critical combination the segregation ratio is modified from 3:1 to 5:1 or 7:1 for a total F_2 population. With the use of multiple marker stocks, centromere position and gene sequence on the linkage map can be determined (Rhoades 1936, 1940; Khush and Rick 1968; Reeves et al. 1968).

Telotrisomics have not been used in genetic studies in barley until recently (Fedak et al. 1972; Tsuchiya 1972 a, b) because of difficulty in obtaining telocentric chromosomes. In recent years seven telotrisomic lines have been isolated in barley (Tsuchiya 1971, 1972 a; Fedak et al. 1971; Singh and Tsuchiya 1977) and used in genetic linkage studies (Fedak et al. 1972; Tsuchiya 1972 a, b).

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The purpose of the present study was to use five telotrisomics (Triplo 2L, 2S, 3S, 4S, 5L) of barley to associate various genes with respective arm of a chromosome, and to delimit the centromere position on the linkage map.

Materials and Methods

Five telotrisomics (Triplo 2L, 2S, 3S, 4S, 5L) were used in the present study. All telotrisomics were of the same genetic background of 'Shin Ebisu 16' (S.E. 16), a two-rowed, spring type cultivar (Singh and Tsuchiya 1977). The origin, characteristics, designation and transmission of the telocentric chromosomes were reported by Tsuchiya (1971), Fedak et al. (1972) and Singh and Tsuchiya (1977).

Several key genetic markers located on the linkage maps (Nilan 1964; Robertson 1971) of chromosome 2, 3, 4, and 5 (Table 1) were used in the telotrisomic analysis. For more detail refer to Tsuchiya (1980). For the analysis of the hooded gene (*K*), Triplo 4S line, which is homozygous for hooded (*K*), was derived from the cross of awned (*k*) Triplo 4S and hooded (*K*) diploid. A balanced lethal stock produced by Burnham for genes a_n (albino seedling) and x_s (Xantha seedling), which

Table 1. List of genetic stocks used in the linkage studies by means of telotrisomic analysis

B. G. S. Number	Mutant name	Gene symbol	Variety or strain
Chromosome 2			
0055	Chlorina seedling	*f*	'Minn 84 – 7'
0056	White streak 4	*wst4*	'Kanyo 7'
0057	Six row	*v*	'Triple bearded club mariout'
0057	Wide outer glume	*e*	'Triple bearded club mariout'
0059	Grandpa	*gp*	'Montcalm'
0060	Liguleless	*li*	'Muyoji'
0061	Triple awned lemma	*tr*	'C.I. 6630'
0062	Subjacent hood	*sk*	'Tayeh 13'
0355	Glossy sheath 5	*gs5*	'Jotun'
0358	Male sterile 2	*msg2*	'C.I. 2330' × 'C. I. 4368'
–	Glossy sheath 6	*gs6*	'Domen'
–	Ribbon grass	*rb*	Unknown
Chromosome 3			
0101	Absent lower laterals	*als*	'Montcalm'
0102	Uzu	*uz*	'Baitori 10'
0104	Yellow streak	*yst*	'Gateway'
0105	Xantha seedling	x_c	'Colsess IV'
0107	White stripe	*wst*	'US 163' ('Streak I')
0108	Albino lemma	*al*	'Russian 82'
0109	Yellow streak 2	*yst2*	'Kuromugi 148' × 'Mensury C'
0112	Albino seedling	a_n	*H. distichum nigrinudum*
0113	Xantha seedling	x_s	'Smyrna I'
0114	Curly 2	*cu2*	'Choshiro-hen'
0117	Chlorina 2	*f2*	'28 – 3398'
0120	Zebra stripe	*zb*	'Mars'
–	Glossy sheath 2	*gs2*	'Vantage'
–	Glossy spike	*cer-zn*^{348}	'Foma'
–	Short awn	*ari-a*^{6}	'Bonus'
Chromosome 4			
0151	Chlorina 9	*f9*	'Ko A'
0152	Hooded lemma	*K*	'Colsess V'
0155	Glossy seedling	*gl* (=*gl2*)	'Himalaya'
0157	Brachytic 2	*br2*	'Svanhals'
0158	Yellow head	*yh*	'Kimugi'
0171	Light green 4	*lg4*	Unknown
0170	Light green 3	*lg3*	Unknown
0172	Short awn 5	*lk5*	'C. I. 5641'
Chromosome 5			
0208	Fragile stem 2	*fs2*	'Oshichi-hen'
–	Golden	(*g*)[a]	'R. F. Eslick'

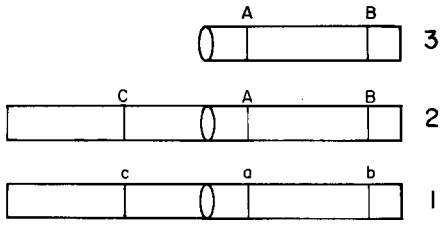
[a] Tentative symbol given to Golden mutant

were located on the long arm of chromosome 3 (Robertson 1971), were analyzed with Triplo 3S.

The mutants, as the male parent, were crossed to telotrisomics, as a female parent. Chromosomes were counted in root tip cells of F_1 hybrids. Telotrisomics together with diploid sibs were transplanted first in peat pots and later in 5″ or 6″ pots. Self-pollination was assured by applying glassine paper bags. Spikes from each plant were harvested separately and threshed individually. F_2 populations were planted in peat pots in flats. The chromosome number of each F_2 plant was counted in root tip cells which facilitated the separation of disomics ($2n=14$), telotrisomics ($2n=14+1$ telo) and other chromosomal types. Segregation ratios were calculated separately for the disomic and trisomic portions of the F_2 populations. The principle of linkage studies with telotrisomics is different from that in simple primary trisomics. Since the extra chromosome is one arm of the chromosome, the genetic ratio is modified depending upon the type of segregation and transmission rate of the extra chromosome (Reeves et al. 1968). If a gene is not located on a particular arm of a chromosome, a disomic ratio is obtained for both disomic and trisomic portions (3:1::3:1) and is known as a noncritical combination. If a gene is on the telocentric chromosome (critical combination), no recessive homozygotes will be obtained in the trisomic portion although diploid portion will show disomic ratio (3:1::all:0) (Fig. 1) provided that the gene is close to the centromere. When both disomic and trisomic portions are combined, a 7:1 ratio is expected in random chromosome segregation with a 50% female transmission rate of the telocentric chromosome. This ratio is further narrowed to 5:1 when the female transmission rate approaches 33%, and has been frequently observed in the telotrisomics of barley (Singh and Tsuchiya 1977).

For the sake of convenience in explaining telotrisomic ratios the three homologous chromosomes were designated as 1, 2 and 3 with the telocentric chromosome as 3. Assuming chromosome 1 came from the male parent and was a complete chromosome with recessive gene *a* and *b*, chromosome 2 and 3 then came from the female parent with dominant gene *A* and *B* (Fig. 1). Chromosome 2 is a complete chromosome and 3 is the telocentric chromosome.

Sometimes recessive homozygotes are obtained in the trisomic portion due to random chromatid crossing over for a gene located far from the centromere, as indicated by the gene *b* in the diagram. If the female transmission of the telocentric chromosome is 50%, a 283:5 or 56.6:1 ratio would be obtained in the trisomic portion and 119:25 or 4.76:1 ratio in the disomic portion (Fig. 1).



	C:c	A:a	B:b
2x	3:1	3:1(25%)	119:25(17.4%)or 4.76:1
2x + 1telo	3:1	a 11(4):0 (0%)	283:5 (1.74%)or56.6:1
Total	3:1	7:1	402:30(6.95%)or 13.4:1

Fig. 1. Summary of the expected segregation ratio in F_2 from telotrisomic F_1 hybrids depending upon random chromosome and random chromatid segregation (transmission rate of telocentric chromosome is 50%).
The telocentric (3) and a normal homologous chromosome (2) carry dominant genes *A* and *B*. Both chromosomes came from the telotrisomic female parent. Chromosome 1 came from the male parent with recessive gene *a* and *b*. Two normal homologous chromosomes 2 and 1 carry the genes *C* and *c*, respectively, in the arm opposite to the telocentric chromosome. The gene *Aa* is located near the centromere and *Bb* far from the centromere. *AAa* Random chromosome segregation; *Bbb* Random chromatid segregation; *Cc* Disomic segregation

Results

Chromosome 2

A total of 12 genetic markers presumed to be located on the short or long arm of chromosome 2 were studied with Triplo 2L and 2S (Table 2). Genes *v*, *gp*(=*gp 2*), *li*, *gs 5*, *tr*, and *msg 2* showed a trisomic ratio with Triplo 2L, indicating that these genes are on the long arm of chromosome 2. A disomic ratio was obtained for genes *e*, *f*(=*lg*), *sk* and *gs 6* with Triplo 2L, suggesting that these genes may be on the short arm of chromosome 2.

The gene for white streak 4 (*wst 4*) has been located on the long arm of chromosome 2 by Takahashi and Moriya (1969) by conventional linkage analysis. Although the Chi-square value suggested a trisomic ratio in the total population, nine *wst 4* homozygotes were recorded in a total of 48 telotrisomics for 2S, indicating that it was a disomic ratio (Fig. 1).

A gene for glossy sheath 6 (*gs 6*) which was located on the linkage map of chromosome 2 by primary trisomic analysis showed linkage with the gene *sk* (subjacent hood) (Takahashi and Hayashi 1966). From telotrisomic analysis with Triplo 2S, gene *gs 6* showed a trisomic ratio. The frequency of *gs 6* plants in the F_2 population was very low in the diploid portion, while a disomic ratio was obtained with Triplo 2L (Table 2).

The gene for chlorina seedling (*f*) was found allelic to the gene *lg* for light green seedling (Tsuchiya 1974). The gene was studied with both arms of chromosome 2. A telotrisomic ratio was obtained with Triplo 2S and disomic ratio with Triplo 2L, confirming its location on the short arm of chromosome 2 (Robertson 1971).

Two recessive homozygotes were obtained in the trisomic portion in Triplo 2L×*gp* (Table 2). This is interpreted as the result of random chromatid crossing over (Fig. 1), and indicates that gene *gp* (grandpa) should be far from the centromere in the long arm of chromosome 2.

Woodward (1957) located a gene for ribbon grass, *rb*, which shows a white stripe on the leaves, on chromosome 2 by conventional linkage analysis. Walker et al.

Table 2. F_2 segregation results of 16 combinations between telotrisomics for the long and short arm of chromosome 2 and various marker stocks

Telotrisomic type	Marker genes	2x			2x+1 telo			Total			Chi-Square		
		A	*a*	Total	*A*	*a*	Total	*A*	*a*	Total	3:1	5:1	7:1
Triplo 2L	*v*	122	50	172	124	0	124	246	50	296	10.37	0.00	5.21
Triplo 2L	*gp (=gp2)*	62	6	68	47	2	49	109	8	117	20.59	8.13	3.43
Triplo 2L	*li*	68	10	78	53	0	53	121	10	131	21.07	7.69	2.83
Triplo 2L	*gs5*	55	14	69	30	0	30	85	14	99	6.23	0.46	0.24
Triplo 2L	*tr*	58	9	67	43	0	43	101	9	110	16.64	5.70	1.89
Triplo 2L	*msg2*	184	42	226	122	0	122	306	42	348	31.04	4.29	0.06
Triplo 2L	*e*	116	43	159	54	23	77	170	66	236	1.11		
Triplo 2L	*f(=lg)*	62	6	68	30	11	41	92	17	109	5.13	0.09	
Triplo 2L	*sk*	58	16	74	32	5	37	90	21	111	2.19		
Triplo 2L	*gs6*	69	26	95	30	14	44	99	40	139	1.05		
Triplo 2S	*f(=lg)*	95	16	111	71	0	71	166	16	182	25.51	8.12	2.29
Triplo 2S	*gs6*	50	3	53	41	0	41	91	3	94	23.84	12.29	7.44
Triplo 2S	*wst4*	73	9	82	39	9	48	112	18	130	8.62	0.74	0.21
Triplo 2S	*gs5*	56	8	64	19	5	24	75	13	88	4.91	0.23	0.41
Triplo 2S	*v*	121	26	147	56	11	67	177	37	214	6.77	0.06	4.59
Triplo 2S	*rb*	33	16	49	23	6	29	56	22	78	0.43		

(1963) located this gene on chromosome 6 and observed loose linkage with *mul 2* with a recombination percentage of 41.1±6.5. However, a disomic ratio was observed when *rb* was analyzed with Triplo 2S, indicating that this gene was not on the short arm of chromosome 2. It may be on the long arm of chromosme 2 or on chromosome 6.

From telotrisomic analysis the gene order has been reversed on the linkage map of chromosome 2. The gene *e* (wide glume) was located near the centromere on the long arm and gene *gs5* (glossy sheath 5) on the short arm of chromosome 2 (Robertson 1971). Gene *e* showed disomic ratic with Triplo 2L and *gs5* showed a trisomic ratio, while a disomic ratio was observed for gene *gs5* with Triplo 2S (Table 2). This indicates that gene *e* should be on the short arm and *gs5* on the long arm. The centromere position on the linkage map of chromosome 2 should be between genes *e* and *gs5* (Fig. 2).

Chromosome 3

A total of 15 genetic markers located on the linkage map of chromosome 3 were studied with Triplo 3S (Table 3). Trisomic ratios were obtained for genes *cu2, uz, wst, als, gs2, zb, f2,* and *cer-zn*^{348}. This indicates that genetically the telocentric chromosome, based upon the present linkage map, is for the short arm of chromosome 3 (Nilan 1964; Robertson 1971; Konishi 1973). The occurrence of very few numbers of recessive homozygotes in the F_2 population of genes *wst* and *als* is difficult to explain. In order to study the genetic nature of *wst,* the F_2 segregation ratio was tested for *wst* in the progeny of the diploid F_1 hybrid with an obvious disomic ratio (115:40, $X^2_{(3:1)}=0.05$). However, since no recessive homozygote was obtained in the trisomic portion it was concluded that these genes showed a trisomic ratio.

Allelism testing between genes *gs2* and a Swedish mutant *cer-zn*^{348} showed that they are not allelic. This

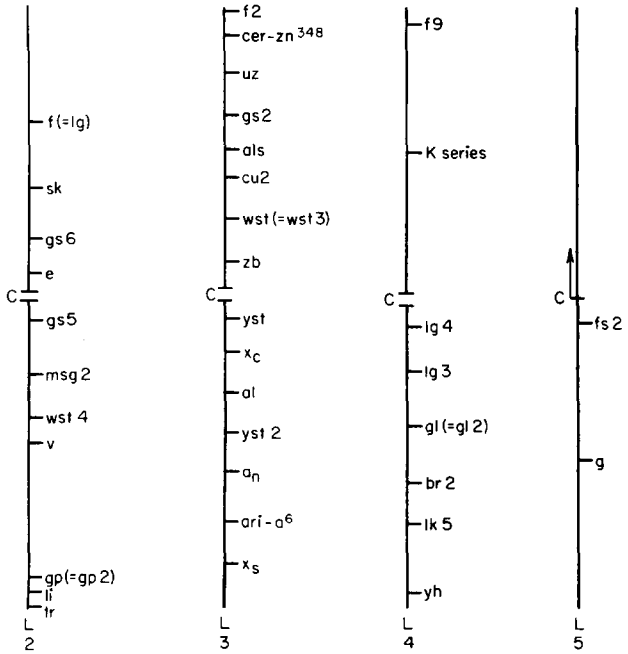


Fig. 2. Linkage maps of chromosome 2, 3, 4 and 5 based on the data obtained from the telotrisomic analysis. The gene order is mostly based on the existing linkage maps

Table 3. F_2 segregation results of 15 combinations between telotrisomic for the short arm of chromosome 3 and various marker stocks

Telotrisomic type	Marker genes	2x			2x+1 telo			Total			Chi-Square		
		A	*a*	Total	*A*	*a*	Total	*A*	*a*	Total	3:1	5:1	7:1
Triplo 3S	*cu2*	140	18	158	54	0	54	194	18	212	30.81	10.20	3.11
Triplo 3S	*uz*	148	10	158	54	0	54	202	10	212	46.52	21.79	11.74
Triplo 3S	*wst*	53	4	57	44	0	44	97	4	101	23.84	14.95	6.73
Triplo 3S	*als*	41	2	43	38	0	38	79	2	81	18.62	11.75	7.45
Triplo 3S	*gs2*	49	9	58	45	0	45	94	9	103	14.52	4.66	1.84
Triplo 3S	*zb*	99	11	110	43	0	43	142	11	153	25.88	9.90	3.94
Triplo 3S	*cer-zn*^{348}	61	12	73	52	1	53	113	13	126	14.49	3.66	0.55
Triplo 3S	*al*	61	8	69	29	6	35	90	14	104	7.39	0.77	0.08
Triplo 3S	*f2*	163	25	188	114	3	117	277	28	305	40.71	12.27	3.07
Triplo 3S	*yst*	107	27	134	50	14	64	157	41	198	1.94		
Triplo 3S	*yst2*	46	8	54	29	4	33	75	12	87	5.82	0.52	0.14
Triplo 3S	x_c	38	21	59	25	7	32	63	28	91	1.61		
Triplo 3S	x_s	65	9	74	42	3	45	107	12	119	14.12	3.71	0.63
Triplo 3S	a_n	37	9	46	17	7	24	54	16	70	0.17		
Triplo 3S	*ari-a*^{6}	Seven of 29 *ari-a*^{6} homozygotes were 2n = 14 + 1 telo						114	29	143	1.69		

test was desired because both genes showed a trisomic ratio with Triplo 3S. One recessive homozygous plant was found in the telotrisomic portion in an F_2 population from the cross Triplo 3S × *cer-zn*^{348}. This indicated that the gene *cer-zn*^{348} will be at the distal segment of the short arm of chromosome 3.

Disomic ratios were obtained for genes *al, yst, yst 2,* x_c, a_n, *ari-a*^{6} and x_s with Triplo 3S, indicating that these genes are not on the short arm of chromosome 3. The number of recessive homozygotes were much less in the F_2 population of the genes *al, yst2, f2,* and x_s (Table 3). The segregation in the diploid control was studied for these genes which showed a 3:1 ratio. The frequency of recessive homozygotes of x_s (6.7%) in the trisomic portion seems to be slightly higher than the expected trisomic ratio (1.67%) from random chromatid crossing over (Fig. 1). However, the frequency of recessive homozygotes in the diploid portion was lower than the expected (25%) disomic ratio with 12.2% for x_s.

Gene order on the linkage map can be determined if multiple marker stocks are used in the linkage studies with telotrisomic analysis. A double recessive stock for genes *cu2* and *uz* were crossed with Triplo 3S. Of the 158 F_2 plants in the diploid portion, 18 plants were *cu2* and 10 plants were *uz* (Table 3). This indicates that gene *cu2* should be closer to the centromere than *uz*.

The other example of importance of multiple marker stock in the linkage analysis is demonstrated for genes *wst* and *uz*. The gene *wst* was located far from the gene *uz* on the linkage map of chromosome 3 (Robertson 1967). Another white streak gene, *wst3,* showed close linkage with *uz* on chromosome 3 (Takahashi and Moriya 1969). Tsuchiya and Haus (1973) found that gene *wst3* was allelic to *wst*. The double recessive stock *wst-uz* established by Tsuchiya (unpublished) was used in linkage studies with Triplo 3S. Both genes showed trisomic ratio. In the F_2 population, out of 84 plants, three plants were double recessive (*wst uz*), one plant was *wst Uz* and the other one was *Wst uz*. From this observation, it appears that *wst* and *uz* genes are very closely linked. This confirms the results that *wst* and *wst3* are allelic (Tsuchiya and Haus 1973) and supports the finding of close linkage between *wst3* (= wst) and *uz* (Takahashi and Moriya 1969).

From telotrisomic analysis it was evident that the telocentric chromosome is for the short arm of chromosome 3 and the centromere should be between genes *zb* and *yst, zb* being on the short arm, *yst* being on the long arm (Fig. 2).

Chromosome 4

A total of 8 genes were studied by means of telotrisomic analysis with Triplo 4S (Table 4). Two genes *f9* (chlorina seedling 9), and *K* (hooded lemma) showed trisomic ratios. Since genes *f9* and *K* are located on the short arm of chromosome 4 (Robertson 1971), and a trisomic ratio was observed for these genes, the telocentric chromosome is designated as the short arm of chromosome 4.

Trisomic analysis for the hooded lemma (*K*) was difficult, because this trait is dominant to the awned condition (*k*). Triplo 4S was in the S.E. 16 genetic background with long awn (*k*). In this situation, the hooded lemma trait was transferred to Triplo 4S by selecting a

Table 4. F_2 segregation results of 8 combinations between Triplo 4S and various marker stocks

Telotrisomic type	Marker genes	2x			2x+1 telo			Total			Chi-Square		
		A	*a*	Total	*A*	*a*	Total	*A*	*a*	Total	3:1	5:1	7:1
Triplo 4S	*K*	51	15	66	38	0	38	89	15	104	6.20	0.37	0.34
Triplo 4S	*f9*	36	7	43	18	0	18	54	7	61	5.95	1.18	0.05
Triplo 4S	*gl* (=*gl2*)	82	28	110	34	12	46	116	40	156	0.03		
Triplo 4S	*lg3*	128	45	173	69	16	85	197	66	263	0.00		
Triplo 4S	*lg4*	65	17	82	28	9	37	93	26	119	0.63		
Triplo 4S	*br2*	60	19	79	27	9	36	87	28	115	0.02		
Triplo 4S	*yh*	51	25	76	12	11	23	63	36	99	6.81		
Triplo 4S	*lk5*	116	41	157	51	16	67	167	57	224	0.02		

homozygous dominant (*K*) line in BC_4 generation from crosses between Triplo 4S (*k*) and Colsess V (*K*). Triplo 4S with the hooded genotype (*KKK*) was crossed with S.E. 16 and the segregation which was recorded in the F_2 showed a trisomic ratio (Table 4).

A gene for yellow head (*yh*) was located on chromosome 4 by Takahashi and Hayashi (1966) from primary trisomic analysis. A disomic ratio was obtained with Triplo 4S indicating that gene *yh* is on the long arm of chromosome 4. Disomic ratio was also observed for *gl, br2, lg3, lg4* and *lk5* with Triplo 4S. Gene *gl* was found to be allelic to *gl2* (Tsuchiya and Haus 1973).

The centromere position on the linkage map of chromosome 4 was located near the genes *lg3* and *lb2* (Robertson 1971). Since *lg4, gl*(=*gl2*), *lg3, br2* and *yh* showed a disomic ratio and only *f9* and *K* showed a trisomic ratio, the centromere position should be between *K* and *lg4* (Fig. 2).

Chromosome 5

A gene for fragile stem 2 (*fs2*) showed trisomic ratio with the telotrisomic for chromosome 5 (Table 5). The linkage map of chromosome 5 was reversed, long arm to short arm and vice versa, from karyotype and genetic analysis (Tsuchiya 1972b). A new gene, golden (*g*), associated with chromosome 5 (Eslick, unpublished) showed trisomic ratio with Triplo 5L (Table 5) indicating that the locus of gene *g* (tentative symbol) for golden is on telo 5L.

Discussion

Telocentric chromosomes have been very useful in associating a gene with a particular arm of a chromosome, and to locate a centromere position on a linkage map. With the use of multiple marker stocks, a gene sequence on the linkage map can be precisely determined, as shown by Khush and Rick (1968) in tomato.

The genetic ratio is modified from 3:1 to 5:1 or 7:1 in telotrisomic analysis when a gene is located on the telocentric chromosome (critical combination). This modification depends upon the transmission rate of telocentric chromosome through the female, and the distance of the genes from the centromere. The occurrence of the recessive homozygotes in the trisomic portion of *gp* (=*gp2*), *cer-zn*^{348} and *f2* indicates that these genes may be far from the centromere (Fig. 1) and random chromatid crossing over has occurred (Reeves et al. 1968).

Linkage studies with telotrisomics for both arms of a chromosome are desirable whenever these stocks are available. This provides a definite association of a gene with a particular arm of a chromosome. Some genes such as *f*(=*lg*) and *gs6* showed a trisomic ratio with Triplo 2S and a disomic ratio with Triplo 2L. In contrast *v* and *gs5* showed trisomic ratios with Triplo 2L and disomic ratio with Triplo 2S. These results confirmed that genes *f*(=*lg*) and *gs6* are on the short arm and *v* and *gs5* on the long arm of chromosome 2.

The gene order can be reversed on the linkage map from the telotrisomic analysis. The genes *gs5* and *e* were

Table 5. F_2 segregation of the crosses between genes *fs2* and *g* with Triplo 5L

Telotrisomic type	Marker genes	2x			2x+1 telo			Total			Chi-Square		
		A	*a*	Total	*A*	*a*	Total	*A*	*a*	Total	3:1	5:1	7:1
Triplo 5L	*fs2*	46	15	61	27	0	27	73	15	88	2.97	0.01	
Triplo 5L	*g*	40	13	53	38	0	38	78	13	91	5.57	0.51	

located on the short and long arm of chromosome 2, respectively (Robertson 1971). From telotrisomic analysis, gene *gs 5* showed trisomic ratio and gene *e* disomic ratio with Triplo 2L. This indicates that the gene order should be reversed. The gene-chromosome arm relationship may be changed after definite identification of each chromosome arm is completed as has been done in chromosome 5 (Tsuchiya 1972 b).

The centromere position on the linkage maps of chromosome 2, 3 and 4 was definitely located. From translocation analysis the centromere region on chromosome 2, 3 and 4 was close to genes *msg 3* and *msg 2* on chromosome 2, *zb* and *yst* on chromosome 3, and *lg 3* and *lb 2* on chromosome 4, respectively (Robertson 1963, 1971; Nilan 1964; Robertson et al. 1965; Wiebe 1968). From telotrisomic analysis a considerable change in centromere position was made. The centromere position on chromosome 2 is between genes *e* and *gs 5*, between *zb* and *yst* on chromosome 3 and on chromosome 4 between *K* and *lg 4*. The genes *e*, *zb* and *K* are on the short arm of chromosome 2, 3 and 4, respectively.

With the use of multiple marker stocks in telotrisomic analysis the correct gene order on the linkage map can be obtained (Rhoades 1940; Khush and Rick 1968). The genes *cu 2* and *uz* were in one stock and these genes were located on the short arm of chromosome 3. From conventional linkage analysis gene *cu 2* was located further from the centromere than gene *uz* (Takahashi and Fukuyama 1977). From telotrisomic analysis with Triplo 3S, both genes showed a trisomic ratio. However, more number of recessive homozygotes were obtained for gene *cu 2* than for *uz* in diploid portion in F_2 population indicating *cu 2* is closer to the centromere than *uz* in the short arm of chromosome 3.

Sometimes a smaller number of recessive homozygotes than the expected 25% for a disomic ratio were obtained in the F_2 population even if the gene was not located on the telocentric chromosome. A wrong conclusion could be drawn if simply a Chi-square value is calculated for a total population without a chromosome count. The results obtained for genes *al*, *yst 2*, *f 2* and x_s on chromosome 3 are such cases as just mentioned above; Chi-square values for these genes fit the trisomic ratio (Table 3). However, when chromosomes were studied in the F_2, a high frequency of trisomic plants was found to be recessive homozygotes. Their frequency was too high to consider them as a trisomic ratio even if the possibilities of random chromatid crossing over was considered. From these results, it seems to be essential to count the chromosome number of every F_2 plant in telotrisomic analysis. The other example of the importance of chromosome count in F_2 population is found when the results of Fedak et al. (1972) are compared with the present results. They used the same telotrisomic line for chromosome 4 as used in this study and analyzed for only one gene *K*. Chromosomes were not counted in the F_2 population and they did not use the telotrisomic plants of the hooded (*KKK*) genotype. They found a disomic ratio for *K* and concluded that the telocentric chromosome was the long arm of chromosome 4. However, the present study showed a trisomic ratio for *K* (Table 4).

There is an alternative to test the trisomic or disomic ratios. The chromosome counts of only recessive homozygotes will give fairly accurate results regarding the segregation ratios, disomic or trisomic (cf. *ari-a*^{6} in Table 3).

It has been clearly demonstrated in this study and previous work (Fedak et al. 1972; Tsuchiya, 1972 a, b) in barley and the results reported in tomato (Khush and Rick 1968) that the telotrisomic analysis is highly efficient and very effective in linkage analysis in barley. Even though telotrisomic analysis is the only reliable technique available at present in locating the centromere position in the linkage map and to associate a gene with a particular arm of a chromosome in barley, there are still some problems.

When a univalent shift occurs, the segregation ratio would be modified in the F_2. If the telocentric chromosome for both arms are not available, it may be difficult to explain some of the abnormal results. Another problem at present is the lack of information on a definite arm designation for four barley chromosomes, 1 through 4, especially for chromosomes 3 and 4 in which the telocentric chromosome for only one arm is available. Morphologically, Triplo 3S and 4S are similar to the respective primary trisomic type, Triplo 3 and 4 (Tsuchiya 1971; Singh and Tsuchiya 1977). However, genes on the present linkage maps for 3S and 4S respectively, showed trisomic ratio in these telotrisomics (Tables 3, 4). Accurate chromosome arm designation by an improved Giemsa banding technique combined with conventional acetocarmine technique (Singh and Tsuchiya, in press) will contribute in solving these problems.

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