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Cytogenetic studies in barley chromosome 1 by means of telotrisomic, acrotrisomic and conventional analysis*

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Summary. Genetic and cytogenetic techniques were applied to linkage analysis of chromosome 1. Eight marker genes, including five on the short arm and three on the long arm, were analyzed with two telotrisomic lines, Triplo 1S and 1L, and one acrotrisomic line, Triplo $1L^{1S}$. Telotrisomic analysis confirmed the position of *a_c2, gs3, f3, br, f5,* and *f8* on the short arm, and *1k2* and *n* on the long arm of the linkage map of chromosome 1. Conventional three-point tests with two triple genetic marker stocks showed that f_c is located between *br* and *gs3,* and *n* is located in the middle of *f8* and *1k2*. Acrotrisomic for $1L^{1S}$ was used for cytogenetic linkage mapping. Giemsa N-banding technique showed that the long (1L) and short (1S) arm had deficiencies of 37.5% and 73%, respectively. Genes *f5, br,* f_c, *gs3* and *f8* in 1S and *1k2* in 1L were located in the deficient segments of $1L^{1S}$. A trisomic ratio obtained with *n* indicated an association of this gene with the long arm of the acrocentric chromosome. Cytological behavior, morphological characteristics, fertility, and transmission in the acrotrisomic $1L^{1S}$ are also reported.

Key words: Telotrisomic – Acrotrisomic – Linkage map – Cytogenetic – Conventional analysis

Introduction

The seven cytogenetic linkage groups of barley have been established by primary trisomic analysis (Tsuchiya 1961) and translocation analysis (Kramer and Blander 1961). New genes are still being assigned to the respective barley chromosomes by means of primary trisomic analysis (Gardenhire et al. 1973; Seip and Tsuchiya 1979; Shahla and Tsuchiya 1980; Tsuchiya 1983) and translocation analysis (Tuleen 1971).

The use of telotrisomics for genetic and linkage studies in barley contributed considerably to definite localization of the centromere on the genetic linkage maps of all seven chromosomes, and a number of changes were made in the arm location of marker genes (Fedak et al. 1972; Singh and Tsuchiya 1982 b; Shahla and Tsuchiya, unpublished; Tsuchiya 1972 a, b, 1984, 1986; Tsuchiya and Singh 1982). With the use of multiple marker stocks in telotrisomic analysis, centromere position and gene sequence on the linkage map could be determined (Khush and Rick 1968 a).

Physical localization of genes in the chromosome maps of barley started only recently. With the use of new trisomic types in which the extra chromosome is deficient for some segments in one arm or both arms, and marker genes with known arm location on the corresponding normal chromosome, the physical position of genes in chromosome maps have been determined (Shahla and Tsuchiya 1986; Tsuchiya et al. 1984, 1986).

The Giemsa banding technique (Linde-Laursen 1978 a, b; Noda and Kasha 1978; Singh and Tsuchiya 1982 a, b) facilitated the identification of all barley chromosomes, 1 through 7. Trisomics carrying an extra chromosome with deficient arm (telocentrics) or deficient segments (acrocentrics) may be identified by this technique because it shows the type of deficiency of the ex-

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tra chromosome (Shahla and Tsuchiya 1986; Singh and Tsuchiya 1982b; Tsuchiya et al. 1984).

This paper reports the results of cytogenetic studies on linkage mapping in chromosome 1 in barley. Telotrisomic analysis for the long and short arm (1L, 1S) with several marker genes followed by conventional linkage tests in BC_1 generations with one multiple marker stock for each arm complemented one another in providing information on the arm location, sequence, and distance in some cases of the marker genes in linkage map of chromosome 1. Acrotrisomic analysis accompanied with both of above-mentioned methods served as a model for physical linkage mapping in barley. Also included are the results of a detailed study of the acrotrisomic plants for the chromosome 1 (Triplo $1L^{1S}$).

Materials and methods

Two telotrisomics (Triplo 1L, 1S) and one acrotrisomic (Triplo $1L^{1S}$) were used in the present study. All three lines were in the genetic background of "Shin Ebisu 16" (SE 16), a two-rowed, spring type cultivar (Singh and Tsuchiya 1977; Tsuchiya 1979). Table 1 shows the list of genes known to be located on chromosome 1 and used in this study (Tsuchiya 1984). For the analysis of the albino gene (a_c2) a balanced lethal stock (Ramage and Tuleen 1964) was used. Two multiple marker stocks (*f8-1k2-n*, and *br-f_c-gs3*) were used in this experiment. Three-point tests were conducted with both multiple marker stocks.

Trisomics as the female parent were crossed with the mutant stocks, including multiple genetic markers, as the male parent. Somatic chromosome numbers were counted in root tip cells of F_1 hybrids of modified squash technique (Tsuchiya 1971). Trisomics, together with diploid sibs, were transplanted to 15 cm pots. F_2 seeds from each trisomic F_1 plant were harvested and threshed separately. The chromosome numbers of all F_2 plants were counted in root tip cells which facilitated the separation of disomics, telotrisomics, acrotrisomics, and other chromosomal types. Segregation ratios were observed and calculated for trisomic and disomic portions separately in the F_2 populations.

The theoretical segregation ratios in acrotrisomic analysis (Tsuchiya et al. 1984) are the same as those for telotrisomic analysis (Reeves et al. 1968). If a gene is not located on the extra arm of the telotrisomic or the extra acrocentric chromosome, a disomic ratio of 3 : 1 is obtained for both disomic and trisomic portions and the entire F_2. This is known as a noncritical combination. In a critial combination in which the gene under study is located on the extra arm or on the extra acrocentric chromosome, no recessive homozygotes are obtained in the trisomic portion and a trisomic ratio of 3 : 1 : 4 : 0 is observed, provided that the gene(s) is close to the centromere and no crossing over occurs between the gene(s) and centromere. The overall segregation ratio, with a 50% female transmission rate of the extra chromosome, would be 7 : 1, and 5 : 1 when the female transmission rate approaches 33%. If the gene is far enough from the centromere to allow maximum crossing over between the centromere and the gene, a 283 : 5 or 56.6 : 1 ratio would be obtained in the trisomic portion, and a 238 : 50 or 4.76 : 1 ratio in the disomic portion (Tsuchiya et al. 1984). When recessive homozygotes are obtained in the trisomic portion as a result of random chromatid crossing over, fewer homozygous recessives will be recovered in the disomic portion. By telotrisomic analysis with the use of a stock for multiple marker genes located in the same arm, the observed segregation ratio in the F_2 disomic portion will determine the sequence of the marker genes; the one with a greater number of recessive homozygotes in the disomic portion and a lower number of homozygous recessives in the telotrisomic section must, therefore, be closer to the centromere, and vice-versa.

Table 1. List of genetic stocks used in this experiment

Gene symbol	Phenotype	Arm location
f5	chlorina 5	1S
br	brachytic	1S
f_c	chlorina	1S
gs3	glossy sheath 3	1S
a_c2	albino seedling	1S
f8	chlorina 8	1S
1k2	short awn 2	1L
n	naked (hulless) caryopsis	1L

In order to identify the break points in the acrocentric chromosome, the combination of acetocarmine-Giemsa staining technique for the same cells (Singh and Tsuchiya 1982a) was used.

Meiotic behavior of the acrocentric chromosome has also been studied with the acetocarmine squash technique proposed by Tsuchiya (1971).

Morphological measurements of various plant organs were made to compare the acrotrisomic plant with other available trisomic types for chromosome 1 and diploid control. All plants were grown under the same conditions in the greenhouse. Pollen grains filled with cytoplasm with two well-developed sperm nuclei and one round vegetative nucleus were considered as normal functional pollen grains (Kihara 1937). Seed fertility and transmission of the acrocentric chromosome were also studied.

Results

Genetic studies

1 Telotrisomic analysis. A total of eight genetic markers in first linkage group were studied with Triplo 1L and 1S (Table 2). Genes *br*, *f_c*, and *gs3* showed a trisomic ratio with Triplo 1S, indicating that these genes are on the short arm of chromosome 1. The presence of two and three homozygous recessives in the trisomic portion (209 plants) for *br*, *gs3*, and *f_c*, respectively, as a result of random chromatid crossing over, suggested that all three genes are located far from the centromere. Because the three genes are very closely linked, the frequency of crossing over was very low between all three genes. However, in the diploid portion a smaller number of recessives was recovered for *br* (50) than *f_c* and *gs3* (55), indicating the locus for *br* is more distal than *f_c* and *gs3*. Also, with 209 plants in the trisomic portion, the difference in number of recessive homozygotes, three for *f_c* and two for *gs3*, would be enough to conclude that *f_c* is distal to *gs3*. These results indicate the

Table 2. Genetic segregation ratios in F_2 population of crosses between telotrisomics for the short and long arm of chromosome 1 and various marker stocks

Telotrisomic type	Marker genes	2x			2x+1 telo			Total		
		+	a	Total	+	a	Total	+	a	Total
Triplo 1S	*br*	325	50	375	207	2	209	532	52	584
Triplo 1S	*f_c*	320	55	375	206	3	209	526	58	584
Triplo 1S	*gs3*	320	55	375	207	2	209	527	57	584
Triplo 1S	*a_c2*	69	10	79	35	0	35	104	10	114
Triplo 1S	*f5*	86	20	106	56	1	57	142	21	163
Triplo 1S	*f8*	63	6	69	42	0	42	105	6	111
Triplo 1L	*f8*	93	16	109	55	9	64	148	25	173
Triplo 1L	*1k2*	97	12	109	60	1	61	157	13	170
Triplo 1L	*n*	98	11	109	61	0	61	159	11	170
Triplo 1L	*a_c2*	67	10	77	37	2	39	104	12	116

Table 3. Segregation ratios of *br, f_c, gs3* and *f8, n, 1k2* in BC_1 generation

Type of segregation	+ + + /*br f_c gs3*		+ + + /*f8 n 1k2*	
Parental type	+ + +	204	+ + +	49
	br f_c gs3	192	*f8 n 1k2*	51
Singles, region I	+ *f_c gs3*	13	+ *n 1k2*	6
	br + +	9	*f8* + +	6
Singles region II	+ + *gs3*	3	+ + *1k2*	4
	br f_c +	5	*f8 n* +	3
Doubles regions I, II	+ *f_c* +	1	+ n +	1
	br + *gs3*	0	*f8* + *1k2*	0
Total		427		120

Table 4. Genetic segregation ratios in F_2 generation of crosses between acrotrisomic $1L^{1S}$ and various genetic stocks

Gene tested	2x			2x+1 acro			Total		
	+	a	Total	+	a	Total	+	a	Total
f5 (1S)	51	17	68	27	10	37	78	27	105
f8[a] (1S)	120	37	157	86	21	107	206	58	264
1k2[a] (1L)	132	29	161	99	7	106	231	36	267
n[a] (1L)	134	27	161	100	0	100	234	27	261

[a] Includes data from Tsuchiya and Fujigaki 1981

order of these three genes, *br, f_c* and *gs3* is *gs3-f_c-br* with *gs3* closest to the centromere.

Gene *a_c2* was studied with both arms of chromosome 1. A trisomic ratio was obtained with Triplo 1S with no homozygous recessives in the trisomic portion (35 plants). Although the segregation ratio with Triplo 1L did not fit a disomic ratio, the presence of two homozygous recessives out of 39 seedlings in the trisomic portion indicated that this gene is not on the long arm (1L), but on the opposite arm (1S), and very close to the centromere.

Gene *f5* showed a trisomic ratio with Triplo 1S. The one homozygous recessive out of 57 trisomics, as a result of random chromatid crossing over, confirmed that this gene is located far from the centromere in the distal segment.

Gene *f8* was tested with both Triplo 1L and 1S. The F_2 segregation ratio with Triplo 1L did not fit a 3 : 1 disomic ratio, but nine recessive homozygotes were recovered in the trisomic portion indicating the location of this gene on the opposite arm (1S). The segregation ratio of *f8* with Triplo 1S was trisomic (Table 2) indicating its association with the short arm of chromosome 1 (1S).

When *1k2* and *n* were tested with Triplo 1L, the segregation ratios did not fit a disomic ratio. In the disomic portion of 109 plants, the number of recessive homozygotes was 12 for *1k2* and 11 for *n*, suggesting that *n* is distal to *1k2*. However, in the trisomic portion with 61 plants, one recessive homozygote was found for *1k2* (*1k2 1k2 1k2*), but *n* homozygote was not recovered. This data suggests that *1k2* is distal to *n* in the long arm, contradicting the result in the disomic portion. These two genes are probably closely linked with each other, and the total number of 170 F_2 plants was not large enough to determine the order of these two genes in the map.

2 Conventional analysis. Table 3 shows segregations in the BC_1 generation of the three-point tests performed with two triple marker stocks *br f_c gs3* for 1S and *f8 1k2 n* for 1L. The results indicated that gene *f_c* is located in the middle 5.4 and 2.1 map units from genes *br* and *gs3*, respectively in the 1S. Also, gene *n* is located in the middle 10.8 units from *f8* and 6.7 map units from *1k2* in 1L.

3 Acrotrisomic analysis. Two markers on the short arm and two on the long arm of chromosome one were analyzed with acrotrisomic $1L^{1S}$ with the results shown

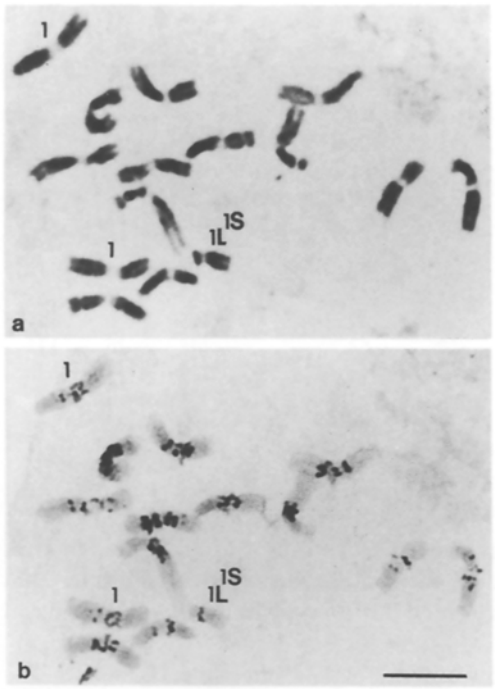

Fig. 1 a , b. Acetocarmine stained mitotic chromosomes of acrotrisomic $1L^{1S}$ (**a**) and the same cell stained by Giemsa N-banding method (**b**). Bar represents 10 μm

in Table 4. Two genes in 1S, *f5* and *f8* showed a disomic ratio, and a good number of trisomics with recessive phenotypes were obtained indicating that these genes were located in the deficient segments of the short arm of the acrocentric chromosome $1L^{1S}$.

Segregation ratio with *1k2* did not fit a disomic ratio, but the seven homozygous recessive trisomics found made it too high to be considered a trisomic ratio. Gene *n* showed trisomic ratio, and no homozygous recessive trisomic was recovered in F_2, indicating its location in the intact segment on the long arm of the acrocentric chromosome.

Cytological study of acrotrisomic plants, Triplo $1L^{1S}$

1 Identification of acrocentric chromosome. The acrocentric chromosome $1L^{1S}$ was initially tentatively identified by its origin from the primary trisomic for chromosome 1 (Tsuchiya 1979), and the morphological similarities of the acrotrisomic plant with the primary trisomic (Triplo 1) and telotrisomic for 1L (Triplo 1L). No visible similarities could be detected between the extra acrocentric chromosome and the other normal chromosomes by karyotype analysis. The total chromosome length was much shorter (47.5%) than the normal chromosome 1. Giemsa banding technique in combination with acetocarmine staining in the same cell has shown that the long arm of normal chromosome 1 (1L) has a dark centromeric band, and the short arm (1S) has a

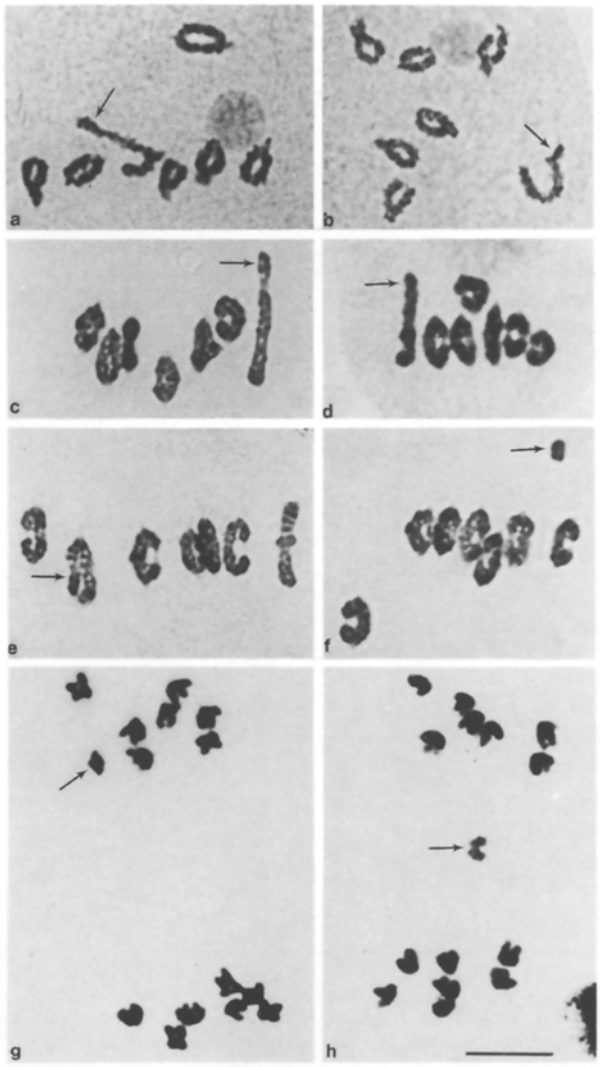

Fig. 2 a–h. Meiosis in acrotrisomic $1L^{1S}$. **a–b** Diakinesis; **c–f** Metaphase I; **g–h** Anaphase I. **a–b** $1_{III}+6_{II}$. **c–d** $1_{III}+6_{II}$ with rod-shaped trivalent. **e** $1_{III}+6_{II}$ with V-shaped trivalent. **f** $7_{II}+1_{I}$. **g** 8-7 chromosome separation. **h** 7-1-7 chromosome separation; acrocentric chromosome as a laggard (*arrow*). *Arrows* indicate acrocentric chromosomes. Bar represents 10 μm

dark centromeric band and two less dark intercalary bands (Singh and Tsuchiya 1982b). The acrocentric chromosome $1L^{1S}$ exhibits only one centromeric band in the short arm (Fig. 1). This result suggests that deficiencies occurred in the distal segment of the short arm and proximal segment of the long arm. The centromere seems to be intact. Measurements showed that the long arm (1L) and the short arm (1S) had deficiencies of 37.5% and 73%, respectively.

2 Chromosome behavior of acrosome $1L^{1S}$ in meiosis. The behavior of the acrocentric chromosome was studied at different stages of meiotic division. Overall chromosome behavior at meiosis in acrotrisomic for $1L^{1S}$

Table 5. Measurements of various plant parts of Triplo 1, Triplo $1L^{1S}$, Triplo 1L, Triplo 1S and the diploid control

	Triplo 1	Triplo $1L^{1S}$	Triplo 1L	Triplo 1S	Diploid
Culm length (cm)	52.0	55.0	46.0	74.0	80.4
Leaf length (cm)	23.0	20.2	22.0	29.0	23.1
Leaf width (mm)	10.0	10.0	11.0	15.0	14.8
Flag leaf length (cm)	32.8	31.0	34.0	33.0	33.4
Flag leaf width (mm)	9.8	9.8	9.9	16.2	18.4
Spike length (cm)	6.4	6.7	5.9	9.0	7.8
Awn length (cm)	15.0	14.0	15.0	22.0	16.8
No. spikelets/spike	20.2	21.0	19.0	29.0	28.8
Glume awn length (mm)	8.7	8.8	8.9	9.0	8.4
Rachilla length (mm)	5.3	5.7	5.4	5.5	5.2

was similar to that of Triplo 1L (Singh and Tsuchiya 1981). At diakinesis and metaphase I (MI) the acrocentric chromosome was present either in association with its normal homologues, giving $1_{III}+6_{II}$ (Fig. 2a–e), or as a univalent, forming $7_{II}+1_{I}$ configuration (Fig. 2f). The frequency of the $1_{III}+6_{II}$ was 76% at diakinesis and 71% at MI. Different trivalent types were observed at both stages. At MI, tandem V-shaped trivalents (Fig. 2e) were predominant with an average frequency of 57.1% of the sporocytes with trivalent. Ring-and-rod trivalents were next with an average of 23.7%, followed by rod-shaped (Fig. 2c, d) with the frequency of 19.2% of trivalents.

At anaphase I (AI), 73.7% of the sporocytes showed 8-7 chromosome separation (Fig. 2g). In 22.2% of sporocytes the acrocentric chromosome did not move to either pole and remained as a laggard at the equatorial plate, giving 7-1-7 chromosome separation (Fig. 2h). Sometimes at late AI, the lagging chromosome divided giving 8-8 separation; 4.1% of cells were observed with this type of chromosome separation. At telophase I (TI) and interkinesis, normal separation was observed in 75.2% of the cells, whereas one laggard (dyad) was found in 19.6% and two laggards (monads) in 5.2% of the sporocytes. At AII and TII, 72.1% of the cells showed normal separation without laggard, and 27.9% of the sporocytes showed one or two laggards in each daughter cell. At the quartet stage, the frequency of microspores with no micronucleus was 88.3%, and 11.7% had one micronucleus or two micronuclei.

Morphological characteristics of acrotrisomic plants

Morphology of acrotrisomic $1L^{1S}$ plants was compared with Triplo 1, Triplo 1L, Triplo 1S, and the diploid control (Table 5). Gross morphological features of the plants with an extra acrocentric chromosome $1L^{1S}$ resembled primary trisomic (Triplo 1), and telotrisomic for the long arm of chromosome 1 (Triplo 1L). The seedling leaves were short, narrow, dark blue-green in color, and very slightly twisted. Onion-like fused leaves were common, as in Triplo 1 and Triplo 1L. Plants were bushy, and dwarfed with many tillers. The development of the root system was very rapid with abundant roots. Culms were short and thin; the spikes were longer than Triplo 1 and Triplo 1L but shorter than Triplo 1S and diploids. Awns were shorter than Triplo 1, Triplo 1L, Triplo 1S and the diploid. Toward maturity the acrotrisomic $1L^{1S}$ plant seemed to be more vigorous than both Triplo 1 and Triplo 1L. Measurements of various organs of the acrotrisomic plants showed that most of the plant parts were smaller than those in Triplo 1S and the diploid (Table 5).

Fertility and transmission in acrotrisomic plants

In the acrotrisomic $1L^{1S}$ the frequency of normal pollen grains was 79.2%.

The average seed fertility in the selfed spikes was 92.4%. When the acrotrisomic $1L^{1S}$ plant was used as the female in crosses with the diploid, the average seed set was 71.2%.

The overall average transmission rate of the acrocentric chromosome was 32.2% in the selfed populations, and 25.4% when crossed with diploid males (female transmission). The male transmission was not studied.

Discussion

Telotrisomic analysis is useful in associating genes with chromosome arms and locating the centromere position in the genetic linkage maps as shown by Khush and Rick (1968a) in tomato and Tsuchiya and Singh (1982) in barley. With the use of multiple marker stocks gene sequence on the linkage map can be precisely determined, as shown in tomato (Khush and Rick 1968a). However, use of multiple genetic stocks in telotrisomic analysis to study the order of genes in the chromosome arm does not always give satisfactory results. When the genes under study are very tightly linked, even analyzing a large number of plants in the F_2 generation may not give conclusive result. An example is the use of the

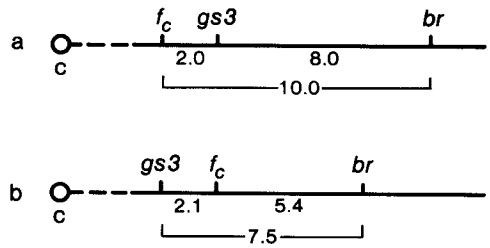

Fig. 3a, b. Comparison of a segment of linkage map in the short arm of chromosome 1. **a** Map presented by Takahashi and Fukuyama (1977). **b** Map developed by the present authors based on this experiment (Table 3), showing the change of gene orientation and map distances among three genes, *gs3*, f_c, and *br*

multiple marker stock *br*, f_c, *gs3* in telotrisomic analysis for the short arm of chromosome 1 (1S). When 252 F_2 plants were analyzed, the segregation ratio was the same in both disomic and trisomic portions for all three genes, *br*, f_c and *gs3* (Shahla 1980). However, when 584 F_2 plants were studied, the results became almost conclusive. In disomic portion with 375 plants the result is unquestionably obvious that *br* is distal to f_c and *gs3* (Table 2). In trisomic portion with 209 plants, the result seems to be conclusive that f_c is distal to *gs3*. Thus, the order of genes in the map of 1S would be centromere – *gs3*-f_c-*br*. This conclusion is in agreement with the result of conventional analysis with 427 BC_1 population for these three genes (Table 3).

However, this result is in conflict with the map presented by Takahashi and Fukuyama (1977) as shown in Figure 3. Takahashi and Fukuyama (lc) gave 8.0% crossover value between *gs3* and *br* (Fig. 3a), which is not much different from the present result of 7.5% (Table 3; Fig. 3b). The distance between f_c and *gs3* is 2.0 by Takahashi and Fukuyama (Fig. 3a) and 2.1 in the present experiment (Table 3, Fig. 3b). If f_c is moved distal to *gs3* with 2.0% crossover value in the map (Fig. 3a) of Takahashi and Fukuyama (lc), the map agrees with the present result (Fig. 3b). Takahashi and Fukuyama (lc) may not have conducted three-point test with multiple marker stock but calculated from two or more two-point tests. It is, therefore, safe to conclude that the result obtained from this experiment (Table 3, Fig. 3b) may be more reliable.

Another triple recessive *f8-1k2-n* was studied with Triplo 1L. The gene *f8* showed a trisomic ratio with Triplo 1S, indicating that this gene is located in the short arm (Table 2) of chromosome 1 in contrast with previous results (Tsuchiya 1972a) which suggest *f8* being located in 1L. Results from telotrisomic study with *1k2* and *n* confirmed the position of both genes on the long arm (1L). However, the order of *1k2* and *n* in 1L is questionable since the segregation results in disomic and trisomic portion are in conflict (Table 2). The result from a three-point test with *f8 1k2 n* showed that the distance between *n* and *1k2* is 6.7 map units.

In linkage studies with telotrisomics, the Chi-square values calculated for the total population did not always fit a disomic 3 : 1 ratio, even in a non-critical combination. However, the segregation of appreciable number of recessive homozygotes in trisomic portion is an indication of unquestionable disomic ratio. The result obtained for gene *f8* with Triplo 1L was such a case. Also, it is important to use telotrisomics for both arms of each chromosome to obtain definite information on the gene-chromosome-arm association. When a_c2 was studied with both Triplo 1L and 1S the segregation ratio with Triplo 1L was not disomic, but the 2 homozygous recessives in the trisomic portion with 39 plants was an indication that this gene is not on the long arm (1L). The result with Triplo 1S left no doubt that a_c2 is located on the short arm (1S).

It should be pointed out that telotrisomic analysis is not always useful for exact mapping of gene loci. The frequency of disomic and trisomic plants with recessive phenotype gives only an approximate indication of the crossover frequency between the centromere and the marker loci. In cytogenetic linkage mapping it is highly desirable to find the direct relationship between genes and specific chromosome segments. Use of various structural changes (such as translocations, deficiencies or inversions) are successfully applied for some organisms with readily analyzable pachytene chromosomes which provide fairly accurate information on the break points of the changed chromosome(s). Use of structural changes have been successful in *Drosophila* (Roberts 1976), maize (McClintock 1941; Rhoades 1955), and tomato (Khush and Rick 1968b), in which salivary chromosomes or pachytene chromosomes are readily analyzed. Barley chromosomes are not suitable for pachytene analysis (Sarvella et al. 1958; MacDonald 1961; Singh and Tsuchiya 1975). Therefore, because of the uncertainty of break points due to the lack of good pachytene chromosomes, the information obtained from the translocation method for linkage mapping of genes in barley is not always accurate (Persson 1969a, b; Tsuchiya 1972c, 1983, 1986).

Use of trisomic material such as acrotrisomics, in which the extra chromosome has a deficiency or deficiencies for segments of chromosome arms, appears to be useful in determining the physical position of genes and delimiting the genes to small sections in each arm. This method facilitates improvement of linkage maps of barley and also could be used for other materials in which pachytene analysis is not possible. This method is useful, however, only when the arm assignment of genes and definite centromere position in the linkage maps are known (Shahla and Tsuchiya 1986; Tsuchiya 1983, 1986; Tsuchiya et al. 1984, 1986).

One important problem associated with this method is the determination of the nature of breakage in a deficient extra chromosome. According to Muller (1940), all breakage should be considered to be two breaks with the original telomere intact, giving an intercalary deficiency. In this case, it is extremely difficult to determine the exact break points without pachytene analysis. In maize, McClintock (1941) reported that a chromosome broken at meiotic anaphase may subsequently undergo a series of fusion and breaks during mitosis in the gametophyte and endosperm. When a broken chromosome passes to sporophytic tissue, however, the broken end appears to heal. If this applies to barley chromosomes, the extra deficient chromosomes could be induced by a single break and the telomere concept could be ignored. By using the combined acetocarmine-Giemsa staining method (Singh and Tsuchiya 1982a), the extra acrocentric chromosome can be accurately identified and measured. This technique was applied to acrotrisomics $3L^{3S}$, $4L^{4S}$ (Tsuchiya et al. 1984) and $5S^{5L}$ (Shahla and Tsuchiya 1986) and others (Tsuchiya et al. 1986). For example, the data showed that $3L^{3S}$ had a deficiency only in the short arm, while $4L^{4S}$ had deficiencies in both arms whose proximal heterochromatic bands were intact and distal segments were deficient, and in $5S^{5L}$ only the long arm had a deficiency for the distal segment. However, in acrocentric chromosome $1L^{1S}$, there seems to be more than one break in the long arm.

Results from Giemsa stained chromosomes revealed that 1L has lost a total of 37.5% of its proximal and possibly part of its distal segment, and no centromeric band is visible. The short arm (1S) has 73% deficiency and the remaining segment carries only the centromeric band and some euchromatic segment (Fig. 1). Genetic analysis with this acrotrisomic may be useful in determining the break point. In this and other experiments (Tsuchiya et al. 1984, 1986) all 6 genes (*a_c2, f8, f_c, f5, br, gs3*) located on the short arm (1S) showed a disomic ratio with acrotrisomic $1L^{1S}$. Therefore, the short arm of acrocentric chromosome $1L^{1S}$ is not carrying any of them. Similarily, *1k2* on the long arm (1L) showed a disomic ratio but *n* showed a trisomic ratio (Table 4). In the linkage map of chromosome 1, *1k2* is located in the distal segment of 1L. Since *n* is located on the long arm of the acrocentric chromosome, this result may suggest that this arm has deficiencies for both proximal and distal segments of the long arm (1L), and were induced as a result of three breaks. Additional genetic information is needed, however, to support this interpretation.

It was important to study the behavior of the extra acrocentric chromosome in different stages of meiotic division. At diakinesis, the acrocentric chromosome formed trivalent with its two normal homologues in 76% of the sporocytes, which was lower than Triplo 1 (82%), Triplo 1L (80%) and Triplo 1S (80%) (Singh and Tsuchiya 1981). These results indicated that because of deficiencies on both arms, the acrocentric chromosome has less physical opportunity for association with its normal homologues.

Compared with diploid and Triplo 1S, acrotrisomic plants showed a reduction in vigor and seed fertility. Morphologically, acrotrisomic $1L^{1S}$ resembled primary trisomic (Triplo 1 or Bush) and telotrisomic for the long arm of chromosome 1 (Triplo 1L). This indicates that the acrocentric chromosome $1L^{1S}$ has almost the same effect on plant morphology as the complete chromosome. But the acrotrisomic plant seemed to be more vigorous than Triplo 1 and Triplo 1L toward maturity. Therefore, it is obvious that 62.5% of the long arm of chromosome 1 carries the genetic elements which control the morphological characteristics of primary trisomic 1 and telotrisomic 1L.

Pollen fertility of acrotrisomic $1L^{1S}$ was fairly good (79.2%), but lower than Triplo 1L (86.5%) and Triplo 1S (93.1%) (Singh and Tsuchiya 1977). The transmission rate of the acrocentric chromosome was studied only through the female and in the selfed F_2 population in which 2 plants with $2n = 16 = 14 + 2$ acro $1L^{1S}$ were recovered. Both plants showed extremely narrow leaves and bushy characters and died at an early stage of growth.

For further improvement of the linkage maps, especially for physical localization of genes in the maps, more acrotrisomic or fragment trisomic types with different break points will be necessary. Obviously, conventional linkage analysis with the use of multiple genetic marker stocks in each arm is essential for establishment of accurate gene orientation in each chromosome arm.

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