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Identification and Designation of Telocentric Chromosomes in Barley by Means of Giemsa N-banding Technique*

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Summary. Seven complete chromosomes and nine telocentric chromosomes in telotrisomics of barley (*Hordeum vulgare* L.) were identified and designated by an improved Giemsa N-banding technique. Karyotype analysis and Giemsa N-banding patterns of complete and telocentric chromosomes at somatic late prophase, prometaphase and metaphase have shown the following results: Chromosome 1 is a median chromosome with a long arm (Telo 1L) carrying a centromeric band, while short arm (Telo 1S) has a centromeric band and two intercalary bands. Chromosome 2 is the longest in the barley chromosome complement. Both arms show a centromeric band, an intercalary band and two faint dots on each chromatid at middle to distal regions. The banding pattern of Telo 2L (a centromeric and an intercalary band) and Telo 2S (a centromeric, two intercalary and a terminal band) corresponded to the banding pattern of the long and short arm of chromosome 2. Chromosome 3 is a submedian chromosome and its long arm is the second longest in the barley chromosome complement. Telo 3L has a centromeric (fainter than Telo 3S) and an intercalary band. It also shows a faint dot on each chromatid at distal region. Telo 3S shows a dark centromeric band only. Chromosome 4 is the most heavily banded one in barley chromosome complement. Both arms showed a dark centromeric band. Three dark intercalary bands and faint telomeric dot were observed in the long arm (4L), while two dark intercalary bands in the short arm (4S) were arranged very close to each other and appeared as a single large band in metaphase chromosomes. A faint dot was observed in each chromatid at the distal region in the 4S. Chromosome 5 is the smallest chromosome, which carries a centromeric band and an intercalary band on the long arm. Telo 5L, with a faint centromeric band and an intercalary band, is similar to the long arm. Chromosomes 6 and 7 are satellited chromosomes showing mainly centromeric bands. Telo 6S is identical to the short arm of chromosome 6 with a centromeric band. Telo 3L and Telo 4L were previously designated as Telo 3S and Telo 4S based on the genetic/linkage analysis. However, from the Giemsa banding pattern it is evident that these telocentric chromosomes are not correctly identified and the linkage map for chromosome 3 and 4 should be reversed. One out of ten triplo 2S plants studied showed about 50% deficiency in the distal portion of the short arm. Telo 4L also showed a deletion of the distal euchromatic region of the long arm. This deletion (32%) may complicate genetic analysis, as genes located on the deficient segment would show a disomic ratio. It has been clearly demonstrated that the telocentric chromosomes of barley carry half of the centromere. Banding pattern polymorphism was attributed, at least partly, to the mitotic stages and differences in techniques.

Key words: Telotrisomics – Linkage groups – Centromere – Heterochromatin – *Hordeum vulgare* L.

Introduction

Seven linkage groups corresponding to the haploid chromosome number (n = 7) have been established in barley (*Hordeum vulgare* L.) by conventional and cytogenetic studies (Burnham and Hagberg 1956; Nilan 1964; Robertson 1971; Tsuchiya 1959, 1960, 1964). The chromosome-linkage group relationship established by Kramer and Blander (1961) and Tsuchiya (1961) was

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well accepted until Tuleen (1973) questioned the identity of chromosomes 1, 2 and 3. Tuleen (1973) and Künzel (1976) stated that chromosome 2 was the longest chromosome in the barley chromosome complement based on the results of multiple translocation analysis. However, it had been difficult to definitely identify chromosomes 1 through 4 of barley by conventional staining techniques (Singh 1974; Tjio and Hagberg 1951; Tsuchiya 1960). The recent application of the Giemsa banding technique to barley chromosomes facilitated the identification of individual chromosomes based on their characteristic heterochromatic banding pattern (Islam 1980; Linde-Laursen 1975, 1978a, b; Noda and Kasha 1978; Singh and Tsuchiya 1981a; Vosa 1976).

The centromere position on the linkage maps and gene-chromosome arm relationship has not been precisely established in barley. The extra telocentric chromosome in telotrisomics (1L, 1S, 2L, 2S, 3L, 3S, 4L, 5L and 6S) of barley were tentatively identified and designated based upon their morphological effect on telotrisomic plants (Seip 1980; Singh and Tsuchiya 1977, 1981c) and association with marker genes located on their respective linkage maps previously established (Singh 1974; Tsuchiya and Singh 1982). However, it is necessary to cytologically identify the telocentric chromosome for the establishment of gene-chromosome arm relationships in barley.

The telocentric chromosomes designated previously as 1L, 1S, 2L, 2S, 3S and 4S of barley could not be identified from the conventional staining methods (Singh 1974; Tsuchiya 1971, 1972a). Triplo 5L was originally identified as Triplo 5S by Fedak (1969) and Fedak et al. (1971, 1972) because it showed association with the genes of the short arm of the linkage map for chromosome 5 (Robertson 1971). However, Tsuchiya (1972b) found from karyotype analysis that the telocentric chromosome was for the long arm and the linkage map of chromosome 5 was reversed. The present study was initiated to identify cytologically the telocentric chromosomes in telotrisomics of barley by an improved N-banding technique (Singh and Tsuchiya 1982) which will provide a definite relationship between a chromosome arm and its respective linkage group.

Materials and Methods

Nine telotrisomics of barley used in the present study were all obtained in the progenies of triploid or primary trisomics except Triplo 3S which was obtained in the progeny of a novel compensating diploid (Singh and Tsuchiya 1981c). The original sources of these materials are presented in Table 1. All the telotrisomics were in the same genetic background of a spring type, two-rowed cultivar, Shin Ebisu 16 (Seip 1980; Singh and Tsuchiya 1977, 1981b).

An improved Giemsa N-banding technique (Singh and Tsuchiya 1982) was used. Conventional staining and Giemsa N-banding techniques were applied to the same cells (Nakata et al. 1977). Ten cells from each of 5 to 10 telotrisomic plants were stained first with acetocarmine and later the same cells were stained by the N-banding method to obtain quantitative and qualitative information on each chromosome arm (Singh and Tsuchiya 1981a, 1982). Chromosomes were measured in μm from the enlarged photomicrographs (×2,500). Relative length (%) was used to avoid the differences in the degree of contraction of each chromosome at different metaphase stages (Tjio and Hagberg 1951). To compare the present data with published results, arm ratio (short/long) and reltive length (based on chromosome 6 as 100 units without satellite) were calculated (Burnham and Hagberg 1956). The combination of chromosome measurements and banding patterns of telocentric and complete chromosomes was useful in constructing a precise idiogram of barley chromosomes.

Table 1. Proposed designation and sources of telotrisomics of barley

Proposed designation of telotrisomics	Original variety	Source	Authority
Triplo 1L	Shin Ebisu 16 (SE 16)	Autotriploid	Tsuchiya 1971a, 1972a
Triplo 1S[a]	Herta × Wong	Triploid hybrid	Tsuchiya 1971a
Triplo 2L	Shin Ebisu 16 (SE 16)	Primary trisomic, Slender	Tsuchiya 1971a, b
Triplo 2S	Shin Ebisu 16 (SE 16)	Primary trisomic, Slender	Tsuchiya 1971a, b
Triplo 3L[a] (3S)[b]	Herta × Wong	Primary trisomic, Pale (F_1 hybrid)	Tsuchiya 1971b
Triplo 3S	Shin Ebisu 16 (SE 16) × Kuromugi 148 × Mensury C	Novel compensating diploid (13 + 1 acro $3L^{3S}$ + 1 telo 3S)	Singh and Tsuchiya 1981c
Triplo 4L (4S)[b]	Shin Ebisu 16 (SE 16)	Primary trisomic, Robust	Tsuchiya 1971a, b
Triplo 5L[a] (5S)[b]	OAC 21 × Montcalm	Triploid hybrid	Tsuchiya 1971a, b, 1972b
Triplo 6S[a]	Shin Ebisu 16 (SE 16) × Angustifolia	Primary trisomic, Purple (F_2)	Seip 1980

[a] These types were back crossed six times with cultivar, Shin Ebisu 16
[b] Previous designation

Results

Each of the barley chromosomes was identified and chromosome arms were designated based upon the information obtained from karyotype analysis and the banding pattern of the complete and telocentric chromosomes. It is fortunate that telotrisomics are available for the four longest, non-satellited chromosomes, both arms of chromosomes 1, 2 and 3, and one arm of chromosome 4. Identification of these telocentric chromosomes was difficult by the conventional staining methods (Fedak et al. 1971; Singh 1974; Tsuchiya 1971, 1972 a).

Chromosome 1

This is the third longest non-satellited chromosome and carries a median centromere (Table 2). In four (cells 1, 3, 7, 9) out of ten cells measured, the long and short arms of chromosome 1 were equal in length. In three cases (cells 4, 5, 10) the long arm was longer than the short arm and in three other instances (cells 2, 6, 8) the short arm was longer (Table 2). Since both arms were almost equal in measurement, their designation in the idiogram depended on the morphological effect on telotrisomic plants, gene-chromosome arm relationships and also on the Giemsa N-banding patterns.

The Giemsa N-banding technique revealed that the telocentric chromosome in Triplo 1L had only a centromeric band, while Telo 1S had a centrometric and two intercalary bands. The band proximal to the centromere was darker than the one toward the telomere (Figs. 1 and 2).

Chromosome 2

This is the longest chromosome among the five non-satellited chromosomes of the barley complement and has a submedian centromere (Table 2). The long arm was the third longest chromosome arm in the barley karyotype. The Giemsa N-banding pattern of Telo 2L indicated that this telocentric chromosome carried a

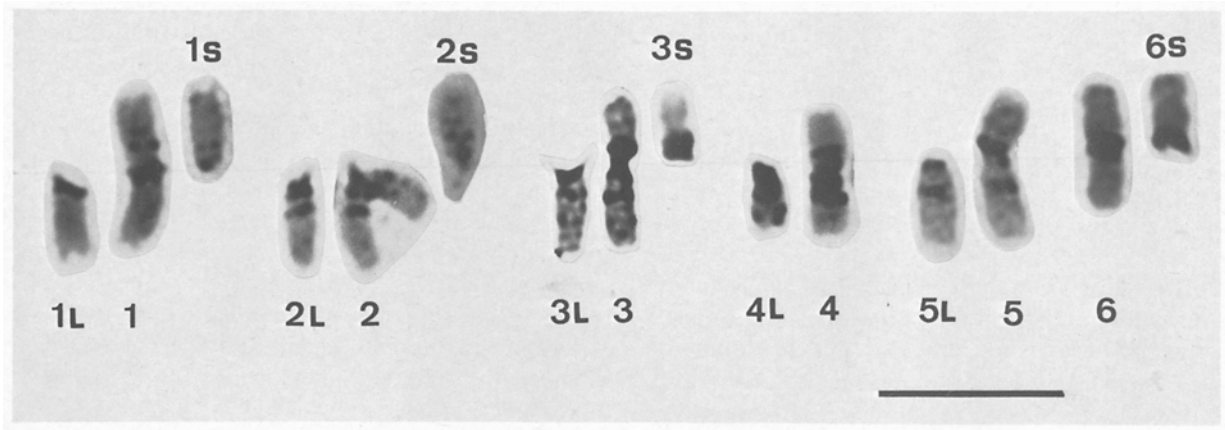

Fig. 1. Giemsa N-banding pattern of telocentric chromosomes of barley. Bar represents 10 μm

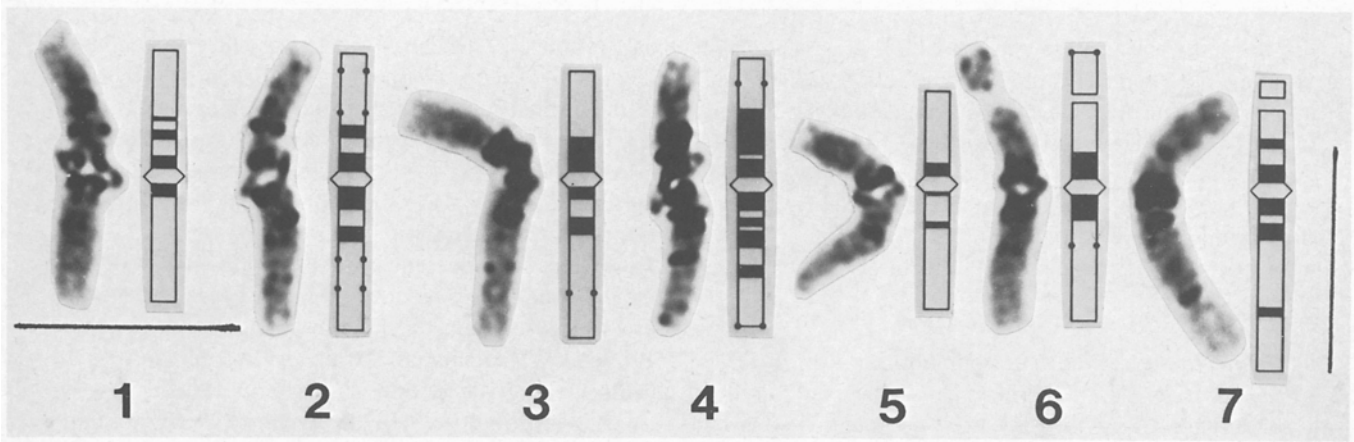

Fig. 2. Idiogram of Giemsa N-banded chromosomes of barley. The somatic chromosomes were taken from Singh and Tsuchiya 1982, J. Hered. 73:227–229 (Figs. 2A and 3) with permission

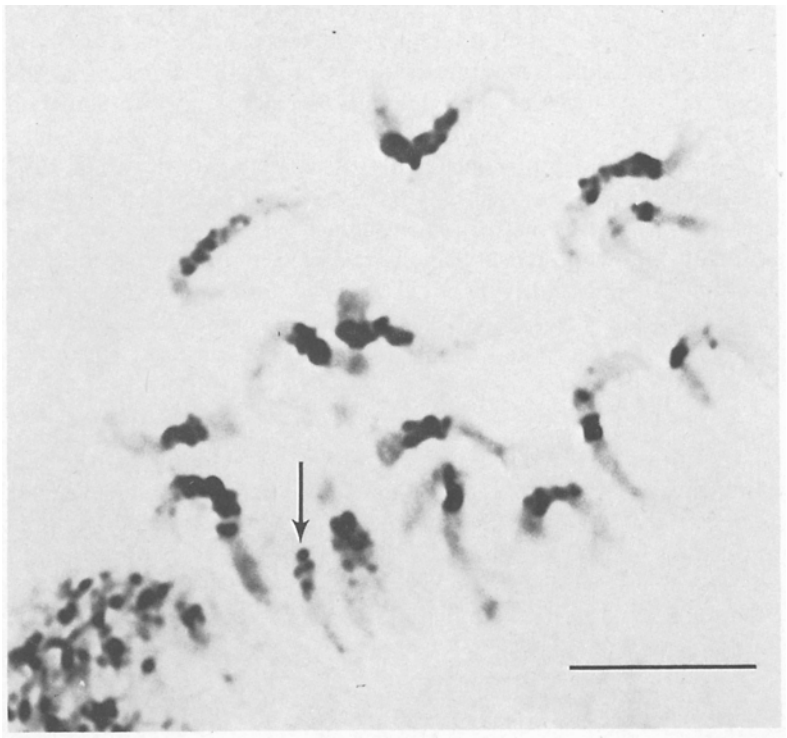

Fig. 3. Giemsa N-banded somatic prophase chromosomes of Triplo 2S. Arrow indicates Telo 2S. Bar represents 10 μm

centromeric and an intercalary band that is similar to the banding of the long arm of complete chromosome 2 (Fig. 1). However, two faint intercalary dots on each chromatid are also observed on the long arm at prometaphase (Fig. 2).

Telo 2S had a centromeric band, two intercalary bands and a terminal band (Figs. 1, 2, 3). These bands were observed on the short arm of complete chromosome 2. The band proximal to the centromere was darker than the other two and the terminal band was rather faint (Figs. 1, 2, 3).

While working with the Giemsa N-banding pattern of Triplo 2S, it was found that in one plant (Telo 80-32-7) the telocentric chromosome was smaller than the normal short arm of chromosome 2 (Fig. 4a). The banding pattern and karyotype analysis revealed that about 50% of the short arm had been deleted (Fig. 4b). If such types of telotrisomic plants were used in the genetic/linkage analysis, a wrong conclusion would be drawn because the genes located on the deficient segment would show a disomic ratio.

Chromosome 3

This is a submedian chromosome with an arm ratio of 0.71 (Table 2). The long arm was observed as the second longest arm in the barley complement. Chromosome 3 showed a dark centromeric band. The band on the short arm appeared as a large dark block at metaphase (Fig. 1), although it consisted of three close bands which were observed in prometaphase chromosomes under the light microscope. The long arm had a dark interstitial band (close to the centromere) and a faint dot on each chromatid in the middle of the long arm (Figs. 1 and 2). The morphological appearance of the telotrisomic plants and the banding pattern of telocentric chromosome 3L and 3S indicated that they belong to the long and short arm of chromosome 3, respectively (Fig. 1).

Chromosome 4

This is a submedian chromosome with an arm ratio of 0.83 (Table 2). This chromosome was correctly identified in previous studies (Table 3). Chromosome 4 was easily distinguished from the rest of the chromosomes because it was the most heavily banded in the barley complement (Fig. 2). About 48% of the chromosome was heterochromatic. Sometimes it was difficult to locate the centromere position in condensed metaphase chromosomes (Fig. 1). However, the appearance of a diamond-shaped centromere (Fig. 2) and use of the acetocarmine stained Giemsa N-banding technique (Singh and Tsuchiya 1981c, 1982) facilitated the precise localization of the centromere. At least one centromeric and three intercalary bands and a dot at the distal end were observed on the long arm, while one large centromeric and two intercalary bands and a dot at the distal portion were

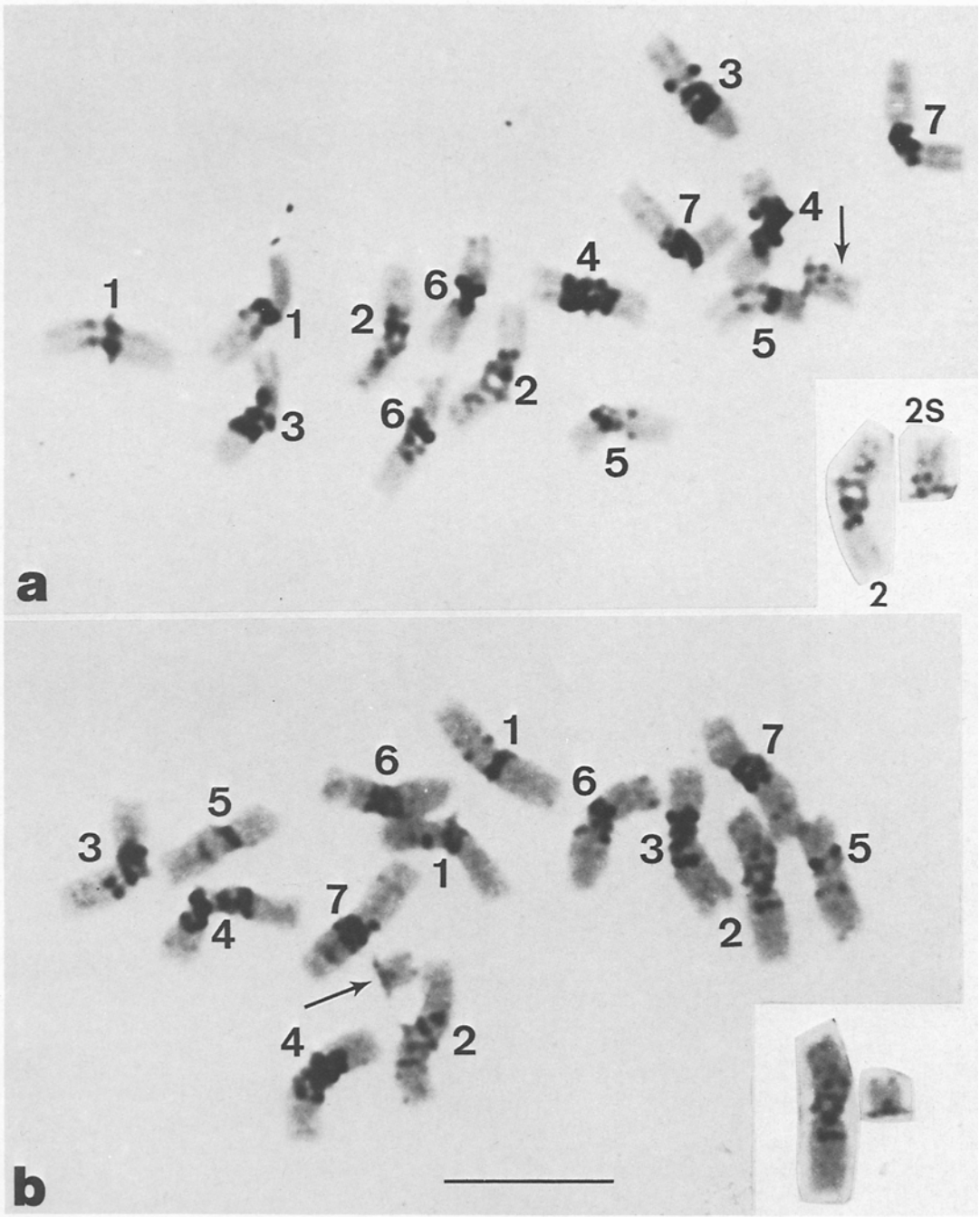

Fig. 4a and b. Giemsa N-banded somatic metaphase chromosomes of Triplo 2S of barley. **a** 14+1 telo 2S, arrow indicates normal Telo 2S. Compare complete chromosome 2 and normal telocentric chromosome 2S (bracket), **b** 14+1 telo 2S. Arrow indicates Telo 2S. Compare the size of Telo 2S, half of the short arm is deleted (bracket). Bar represents 10 μm

observed on the short arm (Fig. 2). The faint bands or dots in both the long and short arms were not observed in condensed metaphase chromosomes (Fig. 1).

The banding pattern of the telocentric chromosome at first revealed that this did not correspond to either the long or short arm (Figs. 1 and 5). The critical observations of karyotype and banding pattern suggested that the telocentric chromosome resembled the long arm with a 32% distal euchromatic deficiency (Figs. 1 and 5).

Chromosome 5

This chromosome has an arm ratio (0.71) similar to chromosome 3 and is the shortest chromosome among

Table 2. Relative chromosome arm length (%), mean arm ratios of *H. vulgare'* Shin Ebisu (SE 16)

Cell No.	Chromosome no. and relative arm length (%)															
	1		2		3		4		5		6		7		6 Sat	7 Sat
	L	S	L	S	L	S	L	S	L	S	L	S	L	S		
1	7.69	7.69	9.23	6.92	9.23	6.15	7.69	6.92	7.69	5.38	7.69	4.61	8.46	4.61	2.30	1.53
2	7.69	8.09	8.90	7.29	9.72	6.47	8.90	5.67	7.29	4.85	7.29	4.04	9.72	4.04	2.42	1.62
3	6.98	6.98	9.56	8.08	8.46	6.61	8.08	6.99	7.35	5.15	6.61	4.42	9.56	5.14	2.20	1.47
4	8.43	7.23	8.43	7.23	9.04	6.62	7.83	6.63	7.23	5.42	7.23	4.21	9.64	4.82	2.41	1.80
5	8.41	7.96	8.85	7.52	8.85	6.19	7.08	6.19	7.08	5.31	7.08	4.42	10.62	4.42	1.77	1.77
6	7.14	7.93	9.52	7.14	9.52	6.74	7.93	5.95	6.74	4.76	7.14	4.37	9.92	5.16	2.38	1.58
7	7.41	7.41	9.05	6.58	9.47	6.58	7.41	6.58	7.41	4.94	8.23	4.94	9.05	4.94	2.47	1.65
8	6.36	7.07	9.19	7.06	9.89	7.06	7.77	7.36	7.77	5.65	7.42	4.24	9.54	4.59	2.82	2.12
9	7.64	7.64	8.92	7.32	9.55	7.01	7.64	6.37	7.64	5.09	7.01	4.45	8.92	4.77	2.22	1.27
10	8.62	7.66	8.62	6.71	8.62	5.75	7.66	6.70	7.66	5.75	6.70	3.83	10.92	4.78	1.91	1.91
Mean	7.64	7.57	9.03	7.19	9.24	6.52	7.80	6.44	7.39	5.23	7.24	4.36	9.64	4.73	2.29	1.67
95% confidence limit	±0.51	±0.27	±0.26	±0.29	±0.34	±0.28	±0.34	±0.29	±0.23	±0.23	±0.34	±0.21	±0.53	±0.24	±0.21	±0.17
Relative length[b]	65.89	65.28	77.88	62.06	79.68	56.24	67.29	55.53	63.72	45.14	62.46	37.57	83.12	40.78	19.75	14.42
95% confidence limit	±4.39	±2.34	±2.23	±2.56	±2.95	±2.48	±2.93	±2.56	±1.97	±2.04	±2.90	±1.85	±4.58	±2.07	±1.81	±1.48
Arm ratio (S/L)	0.99		0.79		0.71		0.83		0.71		0.60[a]		0.49[a]			

L = Long arm, S = short arm

[a] Arm ratios do not include satellite

[b] Based on 100 units for both arms of chromosome 6

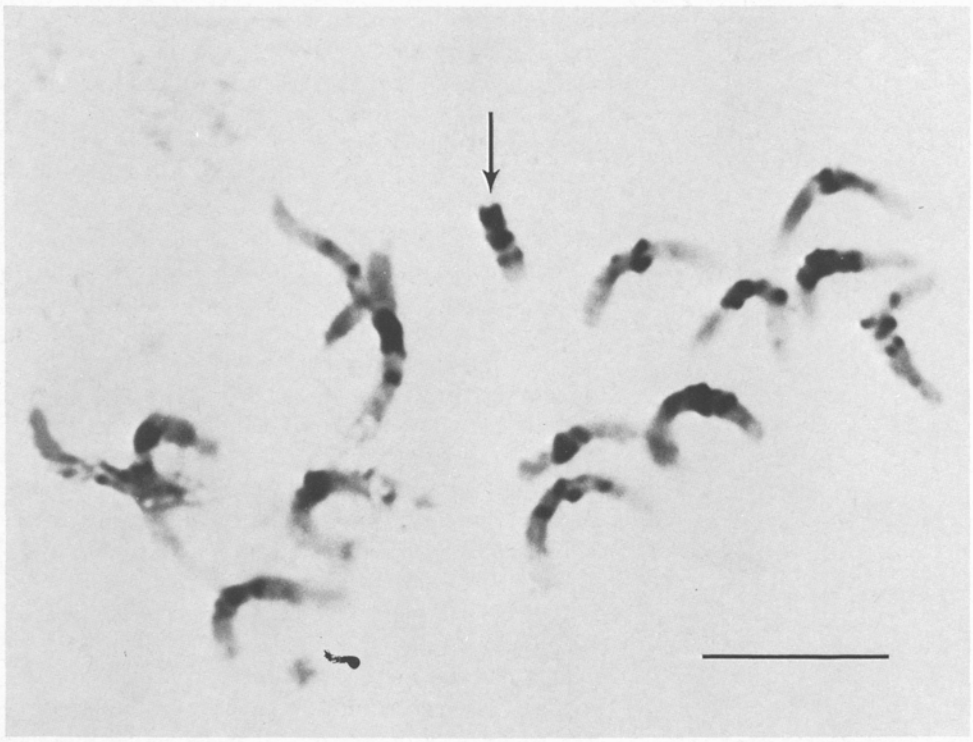

Fig. 5. Giemsa N-banded prophase chromosome of Triplo 4. Arrow indicates the telocentric chromosome. Bar represents 10 μm

the five nonsatellited chromosomes (Table 2). Similar results were also reported earlier by other researchers (Table 3). It has a centromeric band and an intercalary band on the long arm and a band on the short arm that was darker than those of the long arm (Figs. 1 and 2). The karyotype analysis and the banding pattern of the telocentric chromosome indicated that it is the long arm of chromosome 5 (Fig. 1). This confirms the results of previous work by Tsuchiya (1972 b).

Chromosome 6

This chromosome has a larger satellite than chromosome 7 and has an arm ratio of 0.60 (without the satellite). A similar observation was also recorded by other workers (Table 3). Chromosome 6 showed a dark centromeric band in both arms (Figs. 1 and 2). However, a faint intercalary band on the long arm and a faint dot on each chromatid on the telomere of the satellite were observed at prometaphase (Fig. 2). Telo 6S was readily identified (Fig. 1) which confirms the previous report of Seip (1980).

Chromosome 7

This chromosome has the longest long arm in the barley karyotype (Tables 2 and 3) and showed an equally dense centromeric band on each arm. A dark band proximal to the centromere and a narrow band at the distal portion of the long arm was observed (Fig. 2). A faint intercalary band was also observed on the short arm.

Discussion

The chromosome arms of barley were cytologically designated by several authors (Table 4). The original designation of the telocentric chromosomes was mainly based upon the morphological characteristics of telotrisomic plants and genetic/linkage analysis (Fedak et al. 1971, 1972; Singh and Tsuchiya, 1977; Tsuchiya 1971, 1972 c; Tsuchiya and Singh 1982). Some telotrisomic types such as Triplo 3S, 4S and 5S showed conflicting results between morphological characteristics and the genetic/linkage data. Previously identified Triplo 5S was later proved to be Triplo 5L by karyotype analysis (Tsuchiya 1972 b) which was in agreement with the results of morphological studies. The application of the improved Giemsa N-banding technique and a conventional staining method applied to the same cell (Nakata et al. 1977) provided precise identification of the telocentric chromosomes of barley (Figs. 1 and 2).

Linde-Laursen (1978 b) revised the previous identification of telocentric chromosome 1L and 1S (Linde-

Table 3. Comparison of relative chromosome arm length and arm ratios in barley observed by several authors

Authors	1		Arm ratio	2		Arm ratio	3		Arm ratio	4		Arm ratio	5		Arm ratio	6		Arm ratio	7		Arm ratio
	L	S	S/L	L	S	S/L	L	S	S/L	L	S	S/L	L	S	S/L	L	S	S/L	L	S	S/L
Tjio & Hagberg (1951)	78.4 63.7	58.5 58.6	0.746 0.919	71.7	61.1	0.859	63.7 78.4	58.6 58.5	0.919 0.746	67.2	51.8	0.771	60.7	44.3	0.729	62.1	37.9	0.610	78.5	32.2	0.410
Tuleen (1973)	64.5	61.3	0.950	75.0	60.5	0.806	74.5	56.8	0.762												
Künzel (1976)	66.5	64.2	0.965	73.9	63.0	0.852	72.4	58.5	0.808	68.2	58.0	0.850	63.6	44.4	0.698	62.1	36.7	0.590	79.8	37.7	0.472
Noda & Kasha (1978)	69.6	62.2	0.893	79.9	66.0	0.826	80.2	52.1	0.649	68.2	60.6	0.888	60.9	42.0	0.689	64.1	35.9	0.560	88.5	40.4	0.456
Present study from Table 1	65.9	65.3	0.990	77.9	62.1	0.797	79.7	56.2	0.705	67.3	55.5	0.824	63.7	45.1	0.708	62.5	37.6	0.601	83.1	40.8	0.490

Laursen 1975; Singh and Tsuchiya 1977, 1981b) to 1S and 1L based on his results from the Giemsa C-banding technique. However, as shown in Figs. 2 and 4 and Table 2, it is obvious that chromosome 1 is a metacentric and the arm length varies from cell to cell because of artifacts. Ambiguity of the centromere position in simple Giemsa banded chromosomes by Linde-Laursen (1975, 1978a, b) made it more difficult for accurate measurement. According to Linde-Laursen (1975) the short arm of chromosome 1 (1S) has a centromeric band and an intercalary band while 1L has only a centromeric band. However, in a later paper, Linde-Laursen (1978a) switched these arms and showed that the short arm had only a centromeric band. These conflicting results by the same author clearly indicate the difficulty in measurement of arm length of simple Giemsa banded chromosomes, especially of those with a median centromere such as chromosome 1. The median centromere of chromosome 1 has also been observed by Tuleen (1973), Künzel (1976) and Noda and Kasha (1978). It should be pointed out that the quantitative measurement of arm length in the present study was made in the acetocarmine stained chromosomes after individual chromosomes in the same cell were qualitatively identified by the Giemsa N-banding technique (Singh and Tsuchiya 1982).

Another aspect to be considered in the identification of chromosome arms, especially a difficult case such as chromosome 1 in barley, is the effect of the extra telocentric chromosome on the morphology of the telotrisomic plants. It has been shown that the long arm has more effect on plant morphology than the short arm, as demonstrated in tomato telotrisomics (Khush and Rick 1968) and other cases of barley telotrisomics (Singh and Tsuchiya 1977). The telotrisomic for 1L (Triplo 1L) showed almost the same morphology as the primary trisomic for complete chromosome 1 (Triplo 1), while Triplo 1S expressed no effect of the extra chromosome 1S, showing the same morphology and fertility as the normal diploid sibling.

Based on the present results and previous work (Singh and Tsuchiya 1977), and information obtained from other research (Khush and Rick 1968 in tomato), it is concluded that the previous designation for Telo 1L and 1S by the present authors (Singh and Tsuchiya 1977, 1981b) was correct.

Chromosome 2 is submetacentric (Figs. 1, 2, 4, Table 2). Similar results have been reported by Tjio and Hagberg (1951), Tuleen (1973), Künzel (1976), and Noda and Kasha (1978). Telo 2L and Telo 2S have been correctly identified and designated. Morphologically Triplo 2L was similar to its primary trisomic, Slender, while Triplo 2S, having some morphological effects, was neither similar to Slender nor to its diploid

sibs (Singh and Tsuchiya 1977). In addition, most of the genes located on the long and short arm of the linkage map of chromosome 2 showed association with their respective arms (Singh 1974; Tsuchiya and Singh 1982). The Giemsa N-banding technique in the present study confirmed the identification of Telo 2L and Telo 2S, as banding patterns were similar to the long and short arm of chromosome 2. This conclusion is in agreement with Linde-Laursen (1975, 1978 b).

The identification of chromosome 3 and designation of its telocentric chromosomes needs special consideration. Chromosome 3 was observed as a submedian chromosome with an arm ratio of 0.71 (Table 2). Similar results were reported by Tuleen (1973), Künzel (1976), and Noda and Kasha (1978). However, Tjio and Hagberg (1951) identified chromosome 3 as a median (arm ratio = 0.919) chromosome. If chromosome 1 and 3 of Tjio and Hagberg (1951) are switched, their results will agree with the recent observations (Table 3).

The Giemsa N-banding pattern showed that the long arm of chromosome 3 had a centromeric band (more faint than that in the short arm) and an intercalary band, while the short arm had a centromeric band only (Figs. 1 and 2). The banding pattern and karyotype analysis of the previously identified Telo 3S (Singh and Tsuchiya 1977, 1981 b; Tsuchiya and Singh 1982) was identical to the long arm of chromosome 3 (Linde-Laursen 1975, 1978 b). Morphology of the previously designated Triplo 3S plants was the same as that of the primary trisomic for chromosome 3, Pale (Singh and Tsuchiya 1977). Furthermore, the recently isolated Telo 3S (Singh and Tsuchiya 1981 c) which corresponded to the short arm of chromosome 3 (Fig. 1) confirmed that Telo 3L was erroneously identified as Telo 3S, although most of the genes located on the short arm (3S) of the linkage map of chromosome 3 (Robertson 1971) showed association with the present Telo 3L. This demonstrates that the linkage map prepared by Robertson (1971) for chromosome 3 (Fig. 6 a) should be reversed, long arm to short arm and vice versa (Fig. 6 b). The morphology of newly obtained true Triplo 3S is similar to the normal diploid in most of the morphological traits with a few exceptions (Singh and Tsuchiya 1981 c; Tsuchiya et al. 1982).

Similarly the N-banding technique demonstrated that the previously identified Telo 4S (Linde-Laursen 1978 b; Singh 1974; Singh and Tsuchiya 1977, 1981 b; Tsuchiya and Singh 1982) was actually the long arm of chromosome 4 (4L). The new designation of Triplo 4L corresponds with the morphology of this telotrisomic (Triplo 4L) which showed almost the same characteristics as the primary trisomic (Triplo 4). Based on these results the linkage map of chromosome 4 (Fig. 7 a) prepared by Robertson (1971) should also be reversed (Fig. 7 b). The linkage map of chromosome 5 was reversed based on the results of karyotype and genetic analysis (Tsuchiya 1972 b).

The apparent loss of a distal portion of Telo 4L did not affect its morphology, transmission rate (Singh and Tsuchiya 1977) or meiotic behaviors (Singh and Tsu-

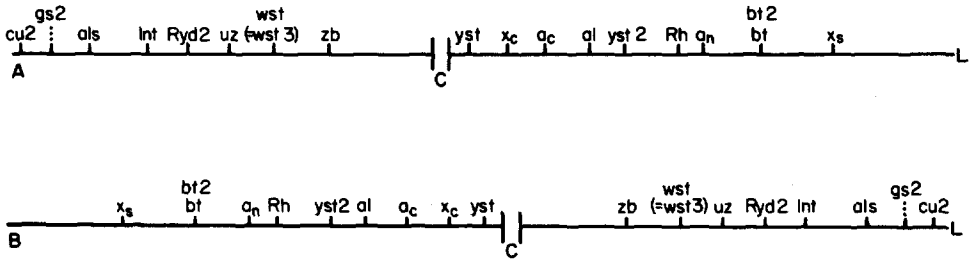

Fig. 6 A and B. The linkage map of chromosome 3. **A** Prepared by Robertson 1971, **B** Revised linkage map

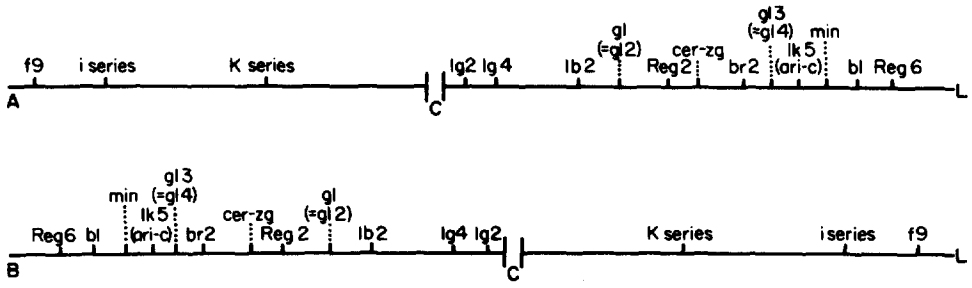

Fig. 7 A and B. The linkage map of chromosome 4. **A** Prepared by Robertson 1971, **B** Revised linkage map

Table 4. Proposed and previous designation of telotrisomic types in barley

Proposed designation	Previous designations				
	Tsuchiya 1971	Fedak et al. 1971	Tsuchiya 1972c	Fedak et al. 1972	Singh and Tsuchiya 1977, 1981b Tsuchiya and Singh 1982
Triplo 1L	Telotri 1L	–	Triplo 1L	–	Triplo 1L
Triplo 1S	Telotri 1S	Telotri 1S	Triplo 1S	Telotri 1S	Triplo 1S
Triplo 2L	Telotri 2L	Telotri 2L	Triplo 2L	–	Triplo 2L
Triplo 2S	Telotri 2S	–	Triplo 2S	–	Triplo 2S
Triplo 3L	Telotri 3A	–	Triplo 3S	–	Triplo 3S
Triplo 3S[a]					
Triplo 4L	Telotri 4A	Telotri 4L	Triplo 4S	–	Triplo 4S
Triplo 5L	Telotri 5A	Telotri 5S	Triplo 5L	Telotri 5L	Triplo 5L
Triplo 6S[b]					

[a] Singh and Tsuchiya 1981c
[b] Triplo 6S was originally identified by Seip (1980)

chiya 1981b). In this case it is likely that the broken end healed and started functioning as a normal telomere (Hang 1981).

Based on all the information available, the present authors propose the designation of chromosome arms and the telocentric chromosomes of barley as shown in Table 4.

It is worthy of mention that the telocentric chromosomes of barley appear to contain half of the centromere (Fig. 1), although it was speculated previously that barley telocentric chromosomes might have a complete centromere because they were highly stable (Singh and Tsuchiya 1981b). The appearance of the diamond-shape centromere in complete chromosomes and a half-diamond in the telocentric chromosomes (Figs. 1 and 4a, b) with the N-banding technique (Fig. 2) demonstrate that the breakage occurred in the middle of the centromere. The stability of barley telocentrics (Singh and Tsuchiya 1981b) and the lack of secondary trisomics in the progeny of telotrisomics (Singh and Tsuchiya 1977) indicate that stability of barley telocentric chromosomes does not depend upon completeness of the centromere. It would be interesting to study the structure of the centromere of some telocentric chromosomes with different stability in wheat (Steinitz-Sears 1966) by Giemsa N-banding technique (Singh and Tsuchiya 1982).

It is not expected that major heterochromatin polymorphism will occur in barley because the cultivars studied here and in other experiments were highly self-pollinated. This may be the reason that all the barley cultivars show a similar basic banding pattern (Islam 1980; Linde-Laursen 1975, 1978a, b; Noda and Kasha 1978; Singh and Tsuchiya 1981a). Although minor variation in the banding patterns has been observed, it may be ascribed to the different techniques used and different stages of somatic chromosomes studied. However, it was observed with the same techniques that prometaphase chromosomes expressed maximum banding patterns (Singh and Tsuchiya 1982). Heterochromatin polymorphism has been reported in cross-pollinated species such as *Anemone* (Marks and Schweizer 1974), *Tulipa* (Filion 1974) and *Secale cereale* (Singh and Röbbelen 1975; Weimarck 1975).

During the present research it was found that two telocentric chromosomes, Telo 2S and 4L, each had a deficiency. Telo 2S showed a 50% distal deletion, while Telo 4L had a 32% deficiency. If telotrisomic plants with this type of extra telocentric chromosome having a deficiency were used in genetic/linkage analysis, a wrong conclusion could be drawn, because genes located in the deficient segment would show a disomic ratio. A strange result obtained in telotrisomic analysis with Triplo 2S – yst3 (Tsuchiya and Hang 1979) may be ascribed to a deficiency in Telo 2S. These results indicate the importance of detailed study of the karyotype of telotrisomic plants.

Telotrisomic plants have proved to be very useful in various cytogenetic studies, especially in linkage mapping in barley (Tsuchiya 1982a). The centromere position was located in the linkage maps of four chromosomes (1 through 4) and the gene-chromosome arm relationships were established in those four chromosomes (Tsuchiya and Singh 1982). Long and short arm relationships for three chromosomes (3, 4 and 5) were reversed (this paper and Tsuchiya 1972b). However, in view of the findings of a deficiency in two of nine telocentric chromosomes, it is important to realize the problems associated with these materials (Tsuchiya 1982a).

Conclusion

With the use of an improved Giemsa N-banding technique (Singh and Tsuchiya 1982), all seven chromosomes and fourteen chromosome arms were definitely identified and designated. Nine telocentric chromosomes have each shown the same banding pattern as the corresponding arm of the chromosomes, thus identified and designated as follows: 1L, 1S, 2L, 2S, 3L, 3S, 4L, 5L, and 6S (Table 4).

In the previous paper (Singh and Tsuchiya 1977, 1981b; Tsuchiya and Singh 1982), the present authors stated that there were some problems in the identification and designation of telocentric chromosomes in some telotrisomic lines, especially those for chromosome 3 and 4. However, based on the results from telotrisomic analysis with marker genes located in the previously established linkage maps (Robertson 1971; Tsuchiya 1980), the telocentric chromosomes for chromosome 3 and 4 were designated as 3S and 4S, respectively. The present experiment has shown that these telocentric chromosomes are the long arm of each chromosome, 3L and 4L, respectively. Accordingly, the previous designation of 3S and 4S should be reversed as 3L and 4L, respectively. The linkage maps for chromosomes 3 and 4 developed by Tsuchiya and Singh (1982) based on the results from telotrisomic analysis and previously established genetic linkage maps (Tsuchiya 1980) should also be reversed as shown in Figs. 6 and 7, respectively.

Deficiency was detected in the telocentric chromosome 2S in one of the Triplo 2S plants and many of the telocentric chromosome 4L plants. These telotrisomic plants with a deficiency in the telocentric chromosome may lead to an erroneous conclusion in genetic analysis if the gene(s) tested were located in the deficient segment. This is one of the problems in the use of telotrisomics in genetic analysis.

The definite cytological identification of all barley chromosome arms and the designation of available nine telocentric chromosomes, was useful in developing recent cytogenetical linkage maps of barley (Tsuchiya 1982).

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