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Differences Between Genetic and Physical Centromere Distances in the Case of Two Genes for Male Sterility in Barley

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Summary. Linkage studies with thirty translocations (one of the two chromosomes involved being number 4) in relation to *msg24* (chromosome 4) and thirteen translocations (one of the two chromosomes involved being number 6) in relation to *msg6* (chromosome 6) show without exception close linkage for all combinations tested. The results indicate that both genes are located genetically in or close to the centromere regions of their chromosomes.

Cytological analysis of two BTT stocks (balanced tertiary trisomics) ascertained the respective chromosome arms (both *msg24* and *msg6* on the short arms) and revealed marked differences between genetic and physical centromere distances. The reason is obviously the high content of centromeric heterochromatin occupying both the chromosome arms involved.

Key words: Genic male sterility – Chromosome location – Balanced tertiary trisomics – Heterochromatin – *Hordeum vulgare*

Introduction

In the cytogenetic literature on barley occasional reference was made to the "well known" phenomenon that most chiasmata take place in the distal portion of the chromosome arms. From such indications the far-reaching speculation was deduced that large blocks of genes exist in the central part of the chromosomes where recombination very rarely occurs, and this situation has been considered as a possible handicap in cross-breeding projects (Hagberg 1966, 1967; Hagberg and Hagberg 1969).

However, the view that chiasmata are largely missing in the middle of the barley chromosomes seems to be mainly based on conclusions derived indirectly, e.g., from cytological studies of heterozygous inversions. The low frequency of bridge and fragment formation observed at meiosis I was taken to support the view mentioned above (Holm 1960; Nilan et al. 1968; Kreft 1969). Another indication in the same direction came from results of gene-translocation linkage studies (Persson 1969a, c).

There seems to be only one clear-cut case showing distinct differences between genetic and physical gene-centromere distances in barley. It is exemplified by studies of the locus *ert-d* belonging to linkage group 1 on chromosome 1 and the translocation T 1–5 a which is nearly totally linked with *ert-d* (Tjio and Hagberg 1951; Hagberg and Hagberg 1969). When studying root tip chromosomes in plants of T 1–5 a, the exchanged chromosome segments were found to be unequal enough in size to localize cytologically the break in chromosome 1 at a position rather distal in the long arm (Tjio and Hagberg 1951; Hagberg 1958). As the genetic centromere distance for *ert-d* has been estimated to exceed not more than 15 per cent recombination (Persson 1969b), a situation resulted where the proximal part of the long arm of chromosome 1 with more than three fourths of the cytological length corresponded with only about one third of the genetic map length. However, these results have lost some of their conclusiveness since it became clear from more recent findings that linkage group 1 (with *ert-d*) belongs to a chromosome with a centromere of nearly median position (Tuleen 1973; Künzel 1976; Linde-Laursen 1978; Noda and Kasha 1978; Singh and Tsuchiya 1981) and not to the longest submetacentric chromosome of the complement as originally thought by Tjio and Hagberg.

This paper presents evidence for differences between genetic and cytological centromere distances in the case of two genes for male sterility belonging to barley chromosomes 4 and 6. The proof was obtained by gene-translocation linkage studies in connection with cytological chromosome analysis of two translocations, each showing close linkage to one of the genes mentioned above. Determination of the physical gene positions was made possible by cytological analysis of balanced tertiary trisomics derived from both the translocations studied cytologically.

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Materials and Methods

Two genes for male sterility, *msg6* (chromosome 6) and *msg24* (chromosome 4), both backcrossed from 'Heines Hanna' (*msg6cf*) and 'Betzes' (*msg24v*) respectively into the two-rowed spring barley varieties 'Elgina' or 'Trumpf', and 37 different translocations (with one of the chromosomes involved in the exchange being 4 or 6) were used for F_3 linkage studies. The translocations had been induced by X-rays, and were isolated and identified with regard to the chromosomes involved following the particulars described by Künzel (1976). The experimental treatment and the calculation of recombination percentages from F_3 data were described in detail by Künzel and Scholz (1982).

Fixation, staining and preparation of root tips for measurements of somatic chromosomes and the determination of translocation break points followed the route outlined by Künzel (1976) and Künzel and Nicoloff (1979).

Results

Thirty translocations with one of the two chromosomes involved being number 4 were included in linkage studies with *msg24* (chromosome 4). All of the translocations showed close linkage in relation to *msg24*, regardless of the break positions that were differently distributed over the short and long arms of chromosome 4. Recombination percentages ranged from 0.0 to 6.9 with less than 1.0% in 26 cases. Among the translocations tested, T 32 (see below) gave 0.2% recombination in relation to *msg24* with a 95% confidence interval from 0 to 1.7 (Künzel and Scholz 1982).

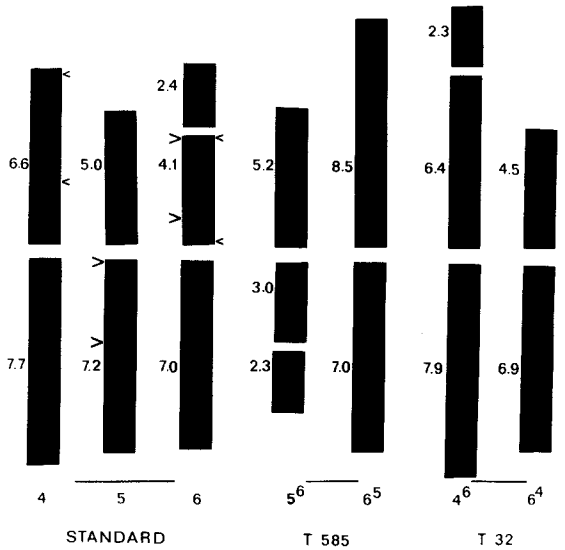

Fig. 1. Schematic representation of standard chromosomes 4, 5, 6 and translocated chromosomes of T 585 (5^6 and 6^5) and T 32 (4^6 and 6^4). Relative arm lengths of standard chromosomes according to Künzel (1976); arm lengths of translocated chromosomes measured from two well-spread metaphases of homozygous translocations. Arrows indicate presumable translocation points: big arrows T 585, small arrows T 32

Similar results were obtained for 13 different translocations with one of the chromosomes involved being number 6 in relation to *msg6* (chromosome 6). Hence, all of the translocations showed close linkage; recombination percentages ranged from 0.0 to 4.1 with less than 1.0% in 11 cases. For the combination *msg6* × T 585 (see below) the recombination value was 0.0% with a 95% confidence interval from 0 to 0.5 (Künzel and Scholz 1982).

In T 32 and T 585, chromosomes 4–6 and 5–6 are involved in the interchanges, respectively. The analysis of chromosome morphology in mitosis revealed that, due to unequal size of the interchanged segments and to the involvement of the satellite bearing arm of chromosome 6, the translocated chromosomes can be recognized. The satellite bearing short arm of chromosome 6 is interchanged with part of the short arm of chromosome 4 in T 32 and with a segment of the long arm of chromosome 5 in T 585. The mean relative arm lengths represented in Fig. 1 are based upon measurements of well-spread metaphases from lines homozygous for T 32 and T 585. Comparing these measurements with the arm lengths of standard chromosomes 4, 5, and 6, it was possible to calculate the chromosome regions in which the translocation breaks occurred (Fig. 1).

With respect to the search for balanced tertiary trisomics (BTT's) the following aspects have been taken into consideration. The self progeny of an individual heterozygous for a translocation contains an enhanced frequency of trisomics due to occasional 3 : 1 disjunction of the four chromosomes of the meiotic pairing complex. In the absence of crossing-over in the interstitial segments (regions between the centromeres and the translocation point) 8 different trisomics are expected. Two of the 8 different types represent tertiary trisomics. Tertiary trisomics contain a normal diploid complement of chromosomes plus an extra translocated chromosome. A tertiary trisomic is called balanced (BTT) if the break-point of the extra translocated chromosome is tightly linked with a marker gene in such a way that the dominant allele of the marker locus is carried on the translocated extra chromosome and recessive alleles are carried on the two normal chromosomes that constitute the diploid complement. Therefore, in the self progeny of a heterozygous translocation showing close linkage with a marker gene, one among the eight different trisomics represents a BTT (Ramage 1964).

Since an extra chromosome is normally not transmitted through male gametes in barley, the self progeny of an BTT-plant consists mostly of recessive diploids and again BTT's. Once identified, such a system remains balanced with respect to its cytogenetic

Table 1. Segregation ratios in plant progenies of two BTT stocks

Stock and number of plant	Progeny		Total	Disomics
	BTT's (fertile)	Disomics (male-sterile)		(%)
msg6/T585				
1	13	28	41	68.3
2	18	27	45	60.0
3	17	45	62	72.6
4	13	21	34	61.8
5	15	26	41	63.4
6	17	28	45	62.2
7	15	34	49	69.4
8	16	27	43	62.8
9	17	24	41	58.5
10	10	22	32	68.8
1 – 10	151[a]	282	433	65.1
msg24/T32				
1	9	19	28	67.9
2	6	25	31	80.6
3	4	25	29	86.2
4	4	24	28	85.7
5	10	24	34	70.6
6	9	21	30	70.0
7	4	23	27	85.2
8	4	21	25	84.0
9	6	24	30	80.0
10	7	24	31	77.4
1 – 10	63[b]	230	293	78.5

[a] Trisomics are rather viable; their morphological appearance differs only slightly from that of normal diploids

[b] Trisomics show reduced tillering and are shorter than diploids; their spikes are slender and the basal rachis internode shows twist-like deformations

constitution and breeding behaviour provided linkage is close enough and pollen transmission of the extra chromosome does not occur (Ramage 1965).

We succeeded in selecting BTT's for both translocation T 32 linked with *msg24* and T 585 linked with *msg6*. Seed of semi-sterile (translocation heterozygous) plants derived from progenies (F_2 or F_3) of the crosses *msg24*×T 32 and *msg6*×T 585 were sieve fractionated and the fractions consisting of small kernels were screened for trisomics by chromosome counts in root tips. This screening resulted in 44 and 41 trisomics out of 535 and 251 analysed seeds descending from T 32 and T 585, respectively. Progeny tests were possible with part of the trisomic plants resulting finally in one BTT stock each for *msg24*/T 32 and *msg6*/T 585. Table 1 represents segregation ratios (fertile BTT's to male-sterile diploids) in plant progenies.

Karyotype analyses were made using root tip meristem cells of seeds harvested from BTT-plants. In this way it was possible to identify the extra translocated chromosomes 6^4 of T 32 and 5^6 of T 585. The chromosome complements of both BTT stocks are illustrated by examples shown in Figs. 2 and 3.

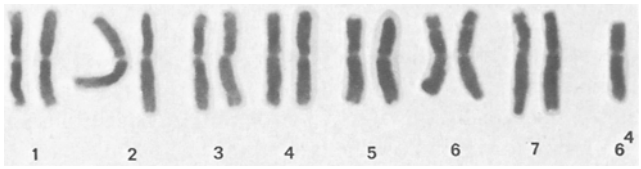

Fig. 2. Mitotic chromosome set of the balanced tertiary trisomic *msg24*/T 32

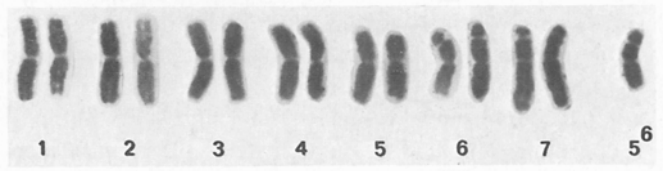

Fig. 3. Mitotic chromosome set of the balanced tertiary trisomic *msg6*/T 585

Discussion

In heterozygous translocations, genetic recombination in interstitial segments (the regions between the centromeres and the translocation point) can be strongly modified (Burnham 1966). When crossing-over in an interstitial segment is followed by alternate chromosome disjunction from the meiotic pairing complex, the crossover chromatids reach gametes which abort due to deficient and duplicated chromosome segments. Therefore, in barley, which exhibits high excess of alternate disjunction, recovered crossovers are reduced considerably in interstitial chromosome regions (Hanson 1952). Taking theoretical aspects into consideration, Kramer and Blander (1961) estimated that the crossover frequencies, determinable in interstitial segments of barely, probably never exceed 10%. Values ascertained experimentally (e.g. Ramage 1964; Persson 1969 a, b) were always even lower so that 3 to 5% were considered as an upper limit.

The reduction of recombination values in interstitial segments of translocation heterozygotes of barley is commonly regarded for the interpretation of gene-translocation linkages as follows: If the gene under study is located close to the centromere, all regions between the gene in question and any translocation point in this chromosome behave as interstitial segments, i.e., all break-points in this chromosome show less than 5% recombination with the gene regardless of the break positions. If the gene is located at a distance from the centromere, three different situations may arise: (1) Translocation points distal to the gene show less than 5% recombination with the gene; (2) Translocation points between the gene and the centromere give a recombination value that is a measure of unmasked recombination between the gene and the breakpoint; (3) Translocation points in the other arm of the chromosome give a recombination value that is a measure of unmasked recombination between the gene and the centromere

plus a small amount of recombination (up to 5%) depending on the location of the break-point (Ramage 1964).

Considering these interpretations the results of linkage studies with 30 different translocations in relation to *msg24* and with 13 different translocations in relation to *msg6* allow the following conclusions:

(1) Both *msg24* and *msg6* are genetically located in or very close to the centromere regions of their chromosomes. The conclusion is deduced from the finding that all of the translocations, regardless of their break positions, which are divided differently among the short and long arms of chromosomes 4 and 6, resp., show the low recombination percentages characteristic for interstitial chromosome segments. The highest recombination value obtained amounts to 6.9% (95% confidence interval 1.1 to 13.0) as to *msg24* and 4.1% (0 to 9.2) as to *msg6*. However, the vast majority of translocations showed recombination percentages lower than 1.0. This conclusion is in conformity with results reported by other authors who studied the same two genes (Eslick 1971; Lehmann and Hagberg 1978; Falk et al. 1980).

(2) The results provide further evidence for the view that the amount of recoverable recombination is consistently low in interstitial chromosome regions in barley. If, as in case of *msg24* and *msg6*, the genetic distance between the locus and the centromere is small, recombination values with any translocation involving the respective chromosome scarcely surpass 5.0% (Ramage 1964; Persson 1969 b).

Balanced tertiary trisomics offer a clue to locate genes physically on their chromosomes provided the extra translocated chromosomes are recognizable in mitosis. The reason is that the respective gene, tightly linked to the translocation break-point, must be located on the extra translocated chromosome of the BTT, either on the translocated or the centromere segment.

The association of *msg24* with linkage group 4 (chromosome 4) is well founded (Eslick 1971). However, it is, according to the most updated linkage maps, not known whether the gene is located on the short or the long arm (Tsuchiya 1981). The identification of the extra translocated chromosome of the BTT stock *msg24*/T 32 as 6^4 shows that *msg24* is located on the translocated segment of the extra chromosome. According to the results of cytological studies of translocation line T 32, chromosome 6^4 has the break-point in the short arm of chromosome 6 which is replaced by part of the short arm of chromosome 4 (Fig. 1). These findings allow the following conclusions:

(1) The gene *msg24* is located on the short arm of chromosome 4, and the break-point of T 32 is proximal to the gene.

(2) The gene is physically located quite far apart from the centromere. The distance amounts to at least about one third of the mitotic arm length and may even be greater.

The position of *msg6* in relation to several marker genes near the centromere of chromosome 6 (linkage group 6) is relatively well known (Lehmann and Hagberg 1978; Falk et al. 1980). However, its location is not known with respect to the arms of the chromosome (Tsuchiya 1981). As the extra chromosome of the BTT stock *msg6*/T 585 has been identified as a translocated chromosome 5^6, *msg6* must be located on the translocated segment. In translocation line T 585 the long arm of chromosome 5 has been found interchanged with part of the short arm of chromosome 6 (Fig. 1). It may thus be concluded:

(1) The gene *msg6* is located on the short arm of chromosome 6, and the break-point of T 585 is proximal to the gene.

(2) The locus shows a physical centromere distance of at least one sixth of the mitotic arm length.

The results obtained demonstrate significant differences between genetic and physical gene-centromere distances, especially for *msg24* located on the short arm of chromosome 4. The reason is presumably the rather high content of constitutive heterochromatin in both the short arms of chromosomes 4 and 6, as revealed by Giemsa C-banding (Linde-Laursen 1975; Linde-Laursen et al. 1982; Noda and Kasha 1978). Chromosome 4 is the most extensively banded barley chromosome, with two larger bands surrounding a smaller one in the short arm near the centromere. The short arm of chromosome 6 is also characterized by prominent centromeric C-bands (Linde-Laursen 1978; Linde-Laursen et al. 1982).

In animals (e.g. Baker 1958; Gropp et al. 1969; Natarajan and Gropp 1971; Zenzes and Wolf 1971) as well as in plants (e.g. Hyde 1953; Linnert 1955; Snoad 1963; Dyer 1963), it has been demonstrated repeatedly that within chromosome regions composed of heterochromatin meiotic crossing-over is largely missing. The same seems to hold true at least for the short arms of barley chromosomes 4 and 6. If chromosome regions without meiotic crossing-over are in fact identical with heterochromatic segments, each of the individual chromosome arms of a species' chromosome complement may show its own characteristics as to possible differences between genetic and physical measures.

In barley, a large fraction of constitutive heterochromatin occupies the chromosome regions near the centromeres, as indicated by Giemsa C-banding. Since the DNA of constitutive heterochromatin does not, as a rule, code for proteins (for review, see Lewin 1980), the general postulate that "large" blocks of genes exist in the central part of barley chromosomes where crossing-over very rarely occurs (Hagberg 1966) seems to be not convincing. On the other hand, this may not exclude that certain genes, some times physically quite far apart from each other, appear to be closely linked around the

centromere regions as has been demonstrated also in other species than barley, e.g., maize (Kasha 1979) and *Drosophila* (Becker 1974).

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