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F. Marthe · G. Künzel

Localization of translocation breakpoints in somatic metaphase chromosomes of barley

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Abstract Karyotype analyses based on staining by acetocarmine followed by Giemsa N-banding of somatic metaphase chromosomes of *Hordeum vulgare* L. were carried out on 61 reciprocal translocations induced by X-irradiation. By means of computer-based karyotype analyses all of the 122 breakpoints could be localized to defined sites or segments distributed over the seven barley chromosomes. The pre-definition of translocations with respect to their rearranged chromosome arms from other studies rendered it possible to define the break positions even in translocations having exchanged segments equal in size and the breakpoints located distally to any Giemsa band or other cytological marker. The breakpoints were found to be non-randomly spaced along the chromosomes and their arms. All breaks but one occurred in interband regions of the chromosomes, and none of the breaks was located directly within a centromere. However, short and long chromosome arms recombined at random. An improved tester set of translocations depicting the known break positions of most distal location is presented.

Key words *Hordeum vulgare* · Translocation chromosomes · Breakpoint mapping · Giemsa N-banding · Computer-based karyotype analysis

Introduction

In barley about 1000 lines exist with single translocations (Ramage 1985). At Gatersleben 415 lines with single translocations and 261 with multiple translocations (up to nine interchanges per genome) have been produced (Künzel 1993), and selected lines of this material have been used successfully in studies contributing to basic knowledge in cytogenetics (e.g., Künzel 1976, 1982; Künzel and Nicoloff 1979; Nicoloff et al. 1979, 1981; Schubert et al. 1980; Schubert and Künzel 1990) and also to particular aspects of breeding research (e.g., Scholz and Künzel 1981a,b, 1987).

However, the efficiency of translocation techniques as analytical tools is often handicapped by an insufficient number of localized chromosomal break positions, the reason for this being that barley chromosomes are not amenable to pachytene analysis. Up to now, several other methods have been used, which in combination may overcome this handicap to certain degree (for review see Hagberg 1986).

Except for linkage studies using translocations and marker gene stocks, all of the known conventional methods have been used to localize the breakpoints of translocations from the Gatersleben stocks (Künzel 1989), i.e., (1) analyses of homogeneously stained somatic karyotypes in homo- and heterozygous translocations (cf. Tjio and Hagberg 1951; Hagberg 1986; Hagberg et al. 1975; Hagberg et al. 1978); (2) studies on chromosomal configurations at meiotic metaphase I in F_1 hybrids with translocation-tester sets (cf. Burnham 1962; Hagberg 1986); and (3) analyses of chromosomal configurations at meiotic metaphase I, improved by the Giemsa N-banding of meiotic chromosomes (Künzel 1987; Künzel and Marthe 1991), and combined with fertility studies (cf. Hagberg 1958, 1986; Kasha and Burnham 1965b) in F_1 hybrids of lines having breakpoints in homologous chromosomes. The summarizing result of these studies, especially those of Giemsa N-banding in meiosis, was that 792 of the 830 breakpoints in 415 single translocations could be assigned to individual chromosome arms (Künzel 1993).

The Giemsa banding patterns of somatic metaphase chromosomes can be used to localize the breakpoints of barley translocations more precisely than previously used methods (Linde-Laursen 1988; Kakeda and Yamagata 1991). Nevertheless, this method also has limitations since most of the Giemsa bands are situated close to centromeres (Linde-Laursen 1981). Conse-

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F. Marthe · G. Künzel (✉)
Institute of Plant Genetics and Crop Plant Research, D-06466 Gatersleben, FRG

quently, only few cases describe well-defined break positions in segments distal to bands, which is especially desirable for a more efficient application of translocation techniques. Therefore, large numbers of translocations should be analyzed in order to identify a sufficient diversity of well-defined chromosomal reconstructs.

The research presented in this paper presents further progress in this field by localizing 122 breakpoints to specified regions of somatic chromosomes. Using Giemsa N-banding and a computer-facilitated karyotype analysis, we cytologically mapped breakpoints to defined segments or sites. Such basic "breakpoint mapping" will be useful in constructing an integrated map for the barley genome that relates recombinative distances from linkage studies with cytomorphological distances measured in metaphase chromosomes using translocation breakpoints as cytological markers in addition to Giemsa bands. On the basis of our own work an improved tester set of translocations is presented.

Materials and methods

Translocations and terminology

Sixty-one translocations of two-rowed spring-type barley (*Hordeum vulgare* L.), induced by X-rays in varieties 'Frigga', 'Elgina', 'Trumpf', and St. 13559 (breeding stock from Hadmersleben, phenotypically like 'Elgina'), were used as specified in Tables 1–7. The translocations were chosen to cover all of the seven chromosomes with a preference for chromosome 5. All of the 61 translocations were pre-defined as to their rearranged chromosomes and chromosome arms by methods previously described (Künzel 1987, 1989; Künzel and Marthe 1991). The designation of the translocations corresponds to the nomenclature used for their description in the World Collection of translocations (Künzel 1993); i.e., capital T followed by two numbers that indicate the chromosomes involved and one or more lowercase letters that specify the translocation: e.g., T1–3ak. Designations of the translocated chromosomes are according to Ramage (1985); e.g., the two chromosomes involved in T1–3 ak are designated T13 ak and T31 ak, with the first number referring to the chromosome with the centromere-bearing segment and the second to the chromosome providing the translocated segment.

Cytological technique and analytical procedure

Seeds of homozygous translocation lines were germinated, and the root tips with colchicine-arrested metaphases were fixed in ethanol-acetic acid (3:1), stained for 30 min in 2% acetocarmine, and squashed in 45% acetic acid.

Using a microscope 'Jenaval' (Carl Zeiss, Jena) combined with a CCD-camera 'Sony AVC-D7CE', a frame grabber-equipped personal computer and an improved version of the image analyzing software package AMBA/R-CHRO (Ahne et al. 1989; Houben et al. 1990), we were able to digitally store phase-contrast images of well-spread metaphases with complete sets of 14 chromosomes. After storage of the pictures, the slides were subjected to the following N-banding procedure. After removal of the coverslip by freezing (liquid nitrogen), the slides were placed in 96% ethanol for 1 h, air-dried, kept in a desiccator over silica gel for at least 1 week, destained in 45% acetic acid (60 °C, 15 min), air-dried, incubated in 1 *M* $NaH_2PO_4 \cdot H_2O$ (92 °C, 2 min), shortly rinsed in Sörensen's phosphate buffer (1/15 *M*, pH 6.81), stained for 3–4 h in 2.8% Giemsa Merck No. 9204 (in Sörensen's phosphate buffer, 1/15 *M*, pH 6.81), rinsed in distilled water, air-dried, and finally mounted in Euparal.

N-banded metaphases of the same cells were used as reference. Each chromosome arm of the stored images was individualized, and the two pairs of translocated chromosomes were identified. The images of complete metaphases were interactively improved by longi-

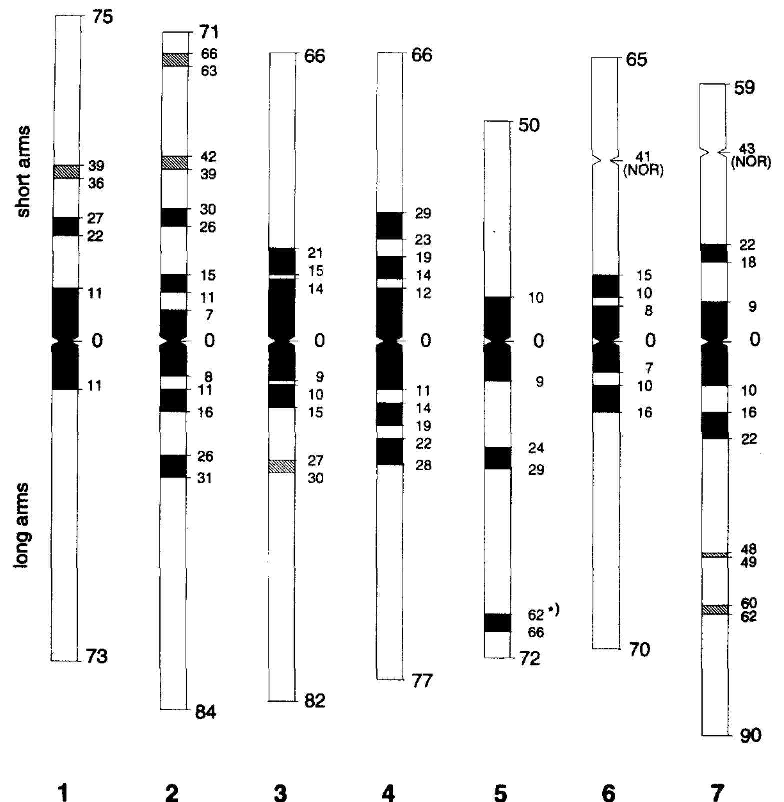

Fig. 1 Idiogram of the barley chromosomes with the combined Giemsa N-banding patterns observed in the parental lines of the studied translocations. Positions and sizes of the constrictions, bands, and chromosome ends are given in 'milliGeNomes' as defined in the Materials and methods. *Black segments* represent distinct N-bands consistently observed; *hatched segments*. faint, unstably expressed N-bands; *) band observed only in translocation lines from 'St. 13559'

tudinal straightening of the chromosomes, separation of attached chromosomes, or by a more precise setting of the measuring points for chromosome ends, centromere, satellite, or band positions before length measurements were automatically computed. The software provided length data for each of the 28 chromosome arms and four satellites, the short/long arm length ratios for the seven chromosomes and, on the basis of relative units (% of the total mitotic genome length), the length differences for each measured arm relative to the standard chromosome arm (Fig. 1). The data compiled in Tables 1–7 represent the means of the measurements from 2 to 5 complete metaphases per translocation.

Construction of the idiogram

The standard idiogram (Fig. 1) was based on chromosome measurements of the parental lines of the translocations studied according to Künzel (1976). It has been constructed following in part the proposals by Jensen and Linde-Laursen (1992): (1) total mitotic genome length is defined as "one GeNome" (GN); (2) distances on the idiogram are given in milliGeNomes (mGN); (3) centromeres are given the position 0 (zero); and (4) positions of the bands, translocation breakpoints, secondary constrictions, and chromosome ends are defined by their distances in mGN from the zero positions. However, the designation of chromosome arms followed the proposal by Singh and Tsuchiya (1982), i.e., with respect to the nearly metacentric chromosome 1, the interstitially Giemsa-banded arm is designated as the short arm despite being physically somewhat longer than the arm without an interstital band (Linde-Laursen 1985; Fukui and Kakeda 1990).

The sizes and positions of the bands were measured in each of 10 selected chromosomes having particularly clear and distinct bands. The idiogram combines all of the bands observed in 'Frigga', 'Elgina', 'Trumpf', and St. 13559, i.e., the genotypic background of the studied translocations (see legend Fig. 1).

Results

With the idiogram of the Giemsa N-banded chromosomes (Fig. 1) as a reference, Tables 1–7 define the 122 breakpoints involved in the 61 translocations. Figure 2 depicts the distribution of the breakpoints on the seven barley chromosomes. Additionally, previously unnoticed pericentric inversions were identified in 2 translocation lines: one involving chromosome 4 with breakpoints in the 43–66/28–51 mGN short/long arm segments (translocation line T1–5 am, Table 1); the other involving chromosome 7 with breakpoints in the 22–43/53–74 mGN segments (line T5–6ap, Table 5).

Breakpoints in relation to Giemsa bands or NORs

Rearranged banding patterns were observed in 56 of the 122 translocated chromosomes. Except for chromo-

Table 1 Location of 10 translocation breakpoints in barley chromosome 1

Translocation chromosome	Genotypic background	Characterization of the break positions Arm/length[a]	Segment[b]	Bands[c]
T13ak	Frigga	L/−6	L:22 to 67	L:−
T13am	Frigga	L/−4	L:17 to 69	L:−
T13as	St. 13559	L/−13	L:11 to 60	L:−
T14af	St. 13559	S/−16	S:27 to 59	S:−[e]
T14ah	St. 13559	L/+4	L:11 to 14	L:−
T14al	Trumpf	S/−14	S:27 to 61	S:−[e]
T15ak	St. 13559	S/−28	S:27 to 47	S:−[e]
T15am	St. 13559[d]	S/−21	S:27 to 36	S:+
T15an	Elgina	S/−22	S:27 to 36	S:+
T17an	Elgina	L/−33	L:24 to 40	L:−

[a] Arm or region affected (*S/L* short/long arm, *Cen* centromere, *Sat* satellite, *NOR* nucleous organizing region) and length difference of the translocated arm when compared to the standard arm (−/+, shorter/longer by the given mGN units (for reference see Fig. 1))
[b] Segment within which the breakpoint is located, defined by the difference between the two mGN values
[c] Remarks concerning Giemsa bands on the chromosome arm that is involved in the translocation: −, all bands remained at their standard positions; +, one or more bands transferred by translocation
[d] Additional pericentric inversion in chromosome 4 with break positions in the short arm segment between 43 and 66 mGN and in the long arm segment between 28 and 51 mGN
[e] Faint distal band in 1 S not observed

Table 2 Location of 10 translocation breakpoints in barley chromosome 2

Translocation chromosome	Genotypic background	Characterization of the break positions Arm/length[a]	Segment[b]	Bands[c]
T23aj	St.13559	S/−19	S:42 to 52	S:−[d]
T23am	Elgina	S/+30	S:49 to 63	S:+
T24ak	Elgina	L/−17	L:31 to 67	L:−
T24al	Elgina	S/−3	S:10 to 11	S:+
T24aw	Trumpf	S/−55	S:7 to 11	S:+
T25ad	Frigga	L/−23	L:31 to 61	L:−
T25af	St. 13559	S/+19	S:30 to 39	S:+
T25ah	St. 13559	L/0	L:78 to 84	L:−
T25as	Trumpf	L/−27	L:31 to 57	L:−
T27ad	Frigga	L/+22	L:38 to 84	L:−

[a] to [c] see Table 1
[d] Faint distal band in 2 S not observed

Table 3 Location of 13 translocation breakpoints in barley chromosome 3

Translocation chromosome	Genotypic background	Characterization of the break positions Arm/length[a]	Segment[b]	Bands[c]
T31ak	Frigga	S/+6	S:21 to 66	S:−
T31am	Frigga	L/+4	L:30 to 82	L:−
T31as	St. 13559	L/+13	L:33 to 82	L:−
T32aj	St. 13559	S/+19	S:56 to 66	S:−
T32am	Elgina	L/−30	L:30 to 44	L:−
T34ae	Frigga	L/−55	L:15 to 27	L:+
T34ag	Frigga	L/+12	L:45 to 82	L:−
T34ap	St. 13559	S/−11	S:21 to 55	S:−
T35ad	St. 13559	L/−22	L:20 to 27	L:+
T35ae	St. 13559	L/+13	L:55 to 82	L:−
T35af	Mut. 4841[d]	S/+37	S:40 to 55	S:−
T36aa	St. 13559	L/−28	L:30	L:−
T37ao	Elgina	L/−9	L:45 to 73	L:−

[a] to [c] see Table 1
[d] Induced mutant of cv 'Saale'; dense ear (*erectoides*), short culm

Table 4 Location of 22 translocation breakpoints in barley chromosome 4

Translocation chromosome	Genotypic background	Characterization of the break positions Arm/length[a]	Segment[b]	Bands[c]
T41af	St. 13559	L/+16	L:45 to 77	L:−
T41ah	St. 13559	L/−4	L:11 to 14	L:+
T41al	Trumpf	L/+14	L:43 to 77	L:−
T42ak	Elgina	L/+17	L:41 to 77	L:−
T42al	Elgina	L/+3	L:19 to 20	L:+
T42aw	Trumpf	S/+55	S:57 to 61	S:−
T43ae	Frigga	S/+55	S:54 to 66	S:−
T43ag	Frigga	L/−12	L:28 to 65	L:−
T43ap	St. 13559	S/+11	S:32 to 66	S:−
T45ac	Frigga	S/+26	S:29 to 44	S:−
T45ad	Frigga	S/+31	S:54 to 66	S:−
T45af	St. 13559	L/+2	L:36 to 69	L:−
T45ag	St. 13559	L/−15	L:28 to 62	L:−
T45ah	St. 13559	L/+12	L:28 to 41	L:−
T45ai	Elgina	L/−37	L:28 to 40	L:−
T45aj	Elgina	L/−24	L:28 to 53	L:−
T45aq	Trumpf	S/−13	S:12 to 13	S:+
T45ar	Trumpf	S/−8	S:19 to 23	S:+
T46z	St. 13559	S/−18	S:0 to 7	S:+(split)
T46ab	St. 13559	S/+8	S:29 to 66	S:−
T47v	St. 13559	L/+11	L:38 to 47	L:−
T47ae	Elgina	L/−16	L:45 to 61	L:−

[a] to [c] see Table 1

some T46z (Table 4), all of the breakpoints were found in interband regions.

In 29 translocations 1 or both breakpoints are located proximally to one or more bands or an NOR. Hence, the size of the segment within which the breakpoints are located corresponds to the respective interband distances in 19 translocations: T1–4 ah, T1–5 am, T1–5 an, T2–4 aw, T2–5 af, T3–4 ae, T3–5af, T4–5ac, T4–5 af, T4–5 ar, T4–7v, T5–6aa, T5–6 aj, T5–7 ac, T5–7ad, T5–7 af, T5–7 aj (band-NOR distance), T5–7 ao, T5–7 ap. Segments smaller than the interband regions could be defined on the basis of the unequal size of the interchanged segments or reciprocally exchanged bands between both of the chromosomes involved, respectively, in the remaining 11 translocations: T2–3 am, T2–4 al, T3–5 ad, T4–5ah, T4–5aq, T5–6x (band-NOR distance), T5–6z, T5–7 ag, T5–7ah, and T5–7 ak.

Two translocations (T3–6aa and T5–7ab) have break positions splitting the NORs. In 29 translocations both breakpoints are located distally to Giemsa bands or NORs. For 3 translocations the size of the defined segments harboring the breakpoints corresponds to the satellite length since part of the satellite is exchanged for a larger part of the respective chromosome arm (T1–7an, T4–7ae, T6–7 au). For 8 translocations the corresponding segments are equal to the distance between the most terminally located band and the chromosome end, 1–3ak, T1–3 am, T2–5 ah, T3–7ao, T4–6ab, T5–6ab, T5–6ak and T5–7an, and for 18 translocations the segments are smaller because of exchanged segments unequal in length: T1–3as, T1–4af, T1–4al, T1–5ak, T2–3aj, T3–4ag, T2–4ak, T2–5ad, T2–5as, T2–7ad, T3–4ap, T3–5ae, T4–5ad, T4–5ag, T4–5ai, T4–5aj, T5–6ao, T5–6ap.

Table 5 Location of 38 translocation breakpoints in barley chromosome 5

Translocation chromosome	Genotypic background	Characterization of the break position Arm/length[a]	Segment[b]	Bands[c]
T51ak	St. 13559	S/+28	S:30 to 50	S:–
T51am	St. 13559[d]	S/+21	S:23 to 32	S:–
T51an	Elgina	S/+22	S:24 to 33	S:–
T52ad	Frigga	L/+23	L:42 to 72	L:–[g]
T52af	St. 13559	L/–19	L:12 to 21	L:+
T52ah	St. 13559	L/0	L:66 to 72	L:–
T52as	Trumpf	L/+27	L:46 to 72	L:–[g]
T53ad	St. 13559	S/+22	S:10 to 17	S:–
T53ae	St. 13559	S/–13	S:10 to 37	S:–
T53af	Mut. 4841[e]	L/–37	L:9 to 24	L:+
T54ac	Frigga	L/–26	L:9 to 24	L:+
T54ad	Frigga	L/–31	L:29 to 41	L:–[g]
T54af	St. 13559	L/–2	L:29 to 62	L:+
T54ag	St. 13559	S/+15	S:16 to 50	S:–
T54ah	St. 13559	L/–12	L:11 to 24	L:+
T54ai	Elgina	S/+37	S:38 to 50	S:–
T54aj	Elgina	S/+24	S:25 to 50	S:–
T54aq	Trumpf	S/+13	S:10 to 11	S:–
T54ar	Trumpf	S/+8	S:11 to 15	S:–
T56x	Frigga	S/+6	S:10 to 32	S:–
T56z	St. 13559	L/–13	L:29 to 59	L:+
T56aa	St. 13559	L/–13	L:9 to 24	L:+
T56ab	St. 13559	S/+8	S:10 to 50	S:–
T56aj	Trumpf	S/+17	S:10 to 12	S:–
T56ak	Trumpf	S/+4	S:10 to 50	S:–
T56ao	Trumpf	S/–2	S:10 to 48	S:–
T56ap	Trumpf[f]	S/–4	S:10 to 46	S:–
T57ab	Frigga	S/–9	S:25	S:–
T57ac	Frigga	S/+42	S:24 to 50	S:–
T57ad	Frigga	S/+41	S:11 to 17	S:–
T57af	St. 13559	L/+22	L:29 to 62	L:+
T57ag	St. 13559	L/–23	L:33 to 49	L:+
T57ah	St. 13559	S/+15	S:10 to 35	S:–
T57aj	St. 13559	S/+6	S:19 to 40	S:–
T57ak	Mut. 4841[e]	S/+30	S:12 to 50	S:–
T57an	Elgina	L/+21	L:66 to 72	L:–
T57ao	Elgina	L/+22	L:29 to 62	L:+[g]
T57ap	Trumpf	L/+2	L:9 to 24	L:+[g]

[a] to [c] see Table 1
[d] Additional pericentric inversion in chromosome 4; see Table 1, T15am
[e] Induced mutant of cv 'Saale'; dense ear (*erectoides*), short culm
[f] Additional pericentric inversion in chromosome 7 with break positions in the short arm segment between 20 and 43 mGN and in the long arm segment between 53 and 74 mGN
[g] Distal band in 5 L not present

Table 6 Location of 12 translocation breakpoints in barley chromosome 6

Translocation chromosome	Genotypic background	Characterization of the break positions Arm/length[a]	Segment[b]	Bands[c]
T63aa	St. 13559	NOR/+28	S:41	S:–
T64z	St. 13559	L/+18	L:22 to 29	L:–
T64ab	St. 13559	L/–8	L:25 to 62	L:–
T65x	Frigga	S/–6	S:19 to 41	S:–
T65z	St. 13559	L/+13	L:40 to 70	L:–
T65aa	St. 13559	S/+13	S:15 to 30	S:–
T65ab	St. 13559	L/–8	L:22 to 62	L:–
T65aj	Trumpf	S/–17	S:8 to 10	S:+
T65ak	Trumpf	L/–4	L:26 to 66	L:–
T65ao	Trumpf	L/+2	L:32 to 70	L:–
T65ap	Trumpf[d]	L/+4	L:34 to 70	L:–
T67au	St. 13559	Sat/+40	S:41 to 65	S:–

[a] to [c] see Table 1
[d] Additional pericentric inversion in chromosome 7, see Table 5, T56ap

Breakpoints in relation to centromeres

None of the 122 breakpoints have been localized directly within a centromere. In T4–6z, the N-band in chromosome T46z, positioned between 0 and 12 mGN of the short arm, is obviously split close to the centromere (Table 4 and Fig. 1). The break occurred probably in a small region adjacent to the centromere which, in contrast to N-banding, is not preferentially stainable by Giemsa C-banding (Islam 1980; Linde-Laursen 1981). Breakpoints in pericentromeric regions of 19 chromosomes were assigned to interband regions next to the

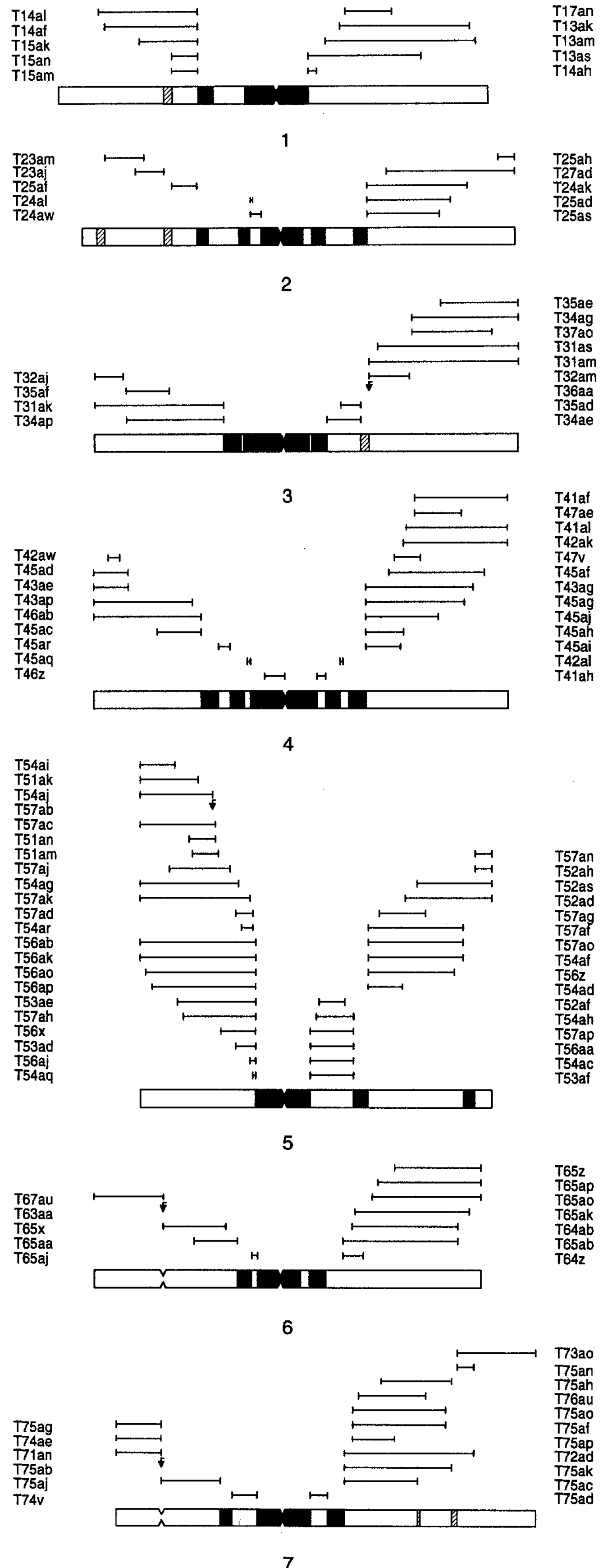

Fig. 2 Distribution of 122 translocation breakpoints on the seven barley chromosomes. For idiogram see legend of Fig. 1. Breakpoint positions marked by *lines* or *arrows*

Table 7 Location of 17 translocation breakpoints in barley chromosome 7

Translocation chromosome	Genotypic background	Characterization of the break positions Arm/length[a]	Segment[b]	Bands[c]
T71an	Elgina	Sat/+33	S:43 to 59	S:–
T72ad	Frigga	L/–22	L:22 to 68	L:–[e]
T73ao	Elgina	L/+9	L:62 to 90	L:–
T74v	St. 13559	S/–11	S:9 to 18	S:+
T74ae	Elgina	Sat/+16	S:43 to 59	S:–
T75ab	Frigga	NOR/+9	S:43	S:–
T75ac	Frigga	L/–42	L:22 to 48	L:+
T75ad	Frigga	L/–41	L:10 to 16	L:+
T75af	St. 13559	L/–22	L:25 to 58	L:+[f]
T75ag	St. 13559	Sat/+23	S:43 to 59	S:–
T75ah	St. 13559	L/–15	L:35 to 60	L:+[f]
T75aj	St. 13559	S/–6	S:22 to 43	S:–
T75ak	Mut. 4841[d]	L/–30	L:22 to 60	L:+[f]
T75an	Elgina	L/–21	L:62 to 68	L:–
T75ao	Elgina	L/–22	L:25 to 58	L:+[f]
T75ap	Trumpf	L/–2	L:25 to 40	L:+[f]
T76au	St. 13559	L/–40	L:26 to 50	L:–[e]

[a] to [c] see Table 1
[d] Induced mutant of cv 'Saale', dense ear (*erectoides*), short culm
[e] Both faint interstitial and distal bands in 7 L not observed
[f] Faint interstitial band in 7L not observed

centromeres: T14ah, T24al, T24aw, T34ae, T41ah, T42al, T45aq, T53ad, T53af, T54ac, T54aq, T54ar, T56aa, T56aj, T57ad, T57ap, T65aj, T74av, T75ad.

Distribution of breakpoints

For 59 translocations the breakpoints are assigned to segments of sizes ranging from 1 mGN in T2–4al and T4–5aq (Tables 2, 4, and 5) up to 52 mGN length in T1–3am (Tables 1 and 3). About 50% of the breakpoints could be assigned to segments of less than 20 mGN in length. In 2 translocations, T3–6aa and 5–7ab, the 4 breakpoints (Tables 3, 5–7) could be precisely localized since the nucleolus organizing regions of chromosomes 6 and 7 were split and parts of the secondary constriction, together with the satellite, were translocated to the long arm of chromosome 3 and the short arm of chromosome 5, respectively.

Discussion

Measurements of acetocarmine-stained chromosomes with clearly visible constrictions and ends followed by Giemsa N-banding for reliable individualization of each chromosome of the same metaphase ensured that precise quantitative and qualitative information could be obtained for all chromosomes of the same cell. Com-

puter-based karyotype analyses proved to be helpful in managing the quantity of numerical data. Manual corrections at each step of the automatically performed analysis turned out to be the deciding prerequisite for obtaining reliable results.

This procedure revealed the regions of break positions in 43 of the 61 translocations studied on the basis of changed banding patterns and/or interchanged segments of unequal size or breakpoints located within or close to the NORs. The results obtained confirmed and refined the break positions of translocations with respect to the chromosome arms involved as determined from preceding studies, especially by Giemsa banding in meiosis (Künzel 1993; Künzel and Marthe 1991), with two exceptions. (1) In T2–4al (former T 2028), Künzel and Marthe (1991) assumed the breakpoints to involve centromeres; the revised break positions involve pericentromeric regions of the short arm of chromosome 2 and the long arm of chromosome 4 (Tables 2 and 4). (2) With respect to T5–7ag with break positions in the long arm of chromosome 5 and the satellite of chromosome 7 (Tables 5 and 7), the former 5S–7L positions (Künzel 1993) were a mistake in writing.

In Giemsa-banded karyotypes of 70 (Linde-Laursen 1988) and 58 (Kakeda and Yamagata 1991) barley translocations, the interchanged chromosomes of 18 and 10 translocations, respectively, could not be identified owing to unchanged banding patterns and exchanged segments of nearly equal size. The present study revealed the break positions even in these cases since the translocations were reliably pre-defined as to their rearranged chromosome arms.

None of the 122 breakpoints were located within a centromere. This is in agreement with the findings of Kakeda and Yamagata (1991). Several of the breakpoints localized to centromeres of chromosomes 6 and 7 by previous studies on non-banded chromosomes (e.g., Hagberg et al. 1978) might in fact be located at the transition to the chromosome arms rather than directly within the centromere, as has been suggested by Linde-Laursen (1988) and supported by the translocation chromosome T46z of the present study.

Including the present study, an altogether fairly high number of Giemsa-banded translocation chromosomes have been studied in barley (Finch and Bennett 1982; Georgiev et al. 1985; Konishi and Linde-Laursen 1988; Linde-Laursen 1988; Gecheff 1989; Kakeda and Yamagata 1991; Xu and Kasha 1992). The results of these studies have shown that break positions within Giemsa bands are exceptions, i.e., the breakpoints are non-randomly spaced along the chromosomes and their arms. This was also observed for X-ray-induced aberrations that occur preferentially in the interband regions of *Vicia faba* chromosomes (Rieger et al. 1977; Döbel et al. 1978).

Nevertheless, the large numbers of barley translocations studied by Hagberg (1986) and the 792 breakpoints of the 396 translocations identified in both the present and former studies (Künzel 1993) have revealed a length-proportional distribution over the chromosome arms. With respect to the S-S, S-L, and L-L chromosome arm combinations of the 396 translocations studied, a 1:2:1 ratio might be expected under the assumption that short and long arms are randomly recombined after irradiation. However, the ratio actually observed is significantly different from 1:2:1; there is a deficit of S-S and a surplus of L-L combinations (66:203:127; $\chi^2 = 19.05$, $P < 0.001$). If arm length differences are compensated for by comparing S-S plus L-L versus S-L combinations, then there is conformity with the expected 1:1 ratio (193:203; $\chi^2 = 0.25$, $P > 0.6$). The deviation from the 1:2:1 ratio, therefore, can be attributed exclusively to the arm length-dependent frequency of breakpoints.

Barley translocations have been widely used as testers in genetic linkage studies (e.g., Ramage et al. 1961; Kasha and Burnham 1965a, b; Persson 1969a,b; Jensen 1971; Tuleen 1971; Hagberg et al. 1978; Künzel and Scholz 1982). Tester sets for this study were established that represent all of the 14 chromosome arms with translocation breakpoints (Hagberg and Hagberg 1968). They have been updated several times (Persson 1969a; Linde-Laursen 1988). In barley, the efficiency of translocation breakpoints in linkage studies is determined mainly by their physical location, which should be as distal as possible (Ramage 1964). This aspect becomes particularly important since there is accumulating knowledge that, at least in cereals with large genomes, the hitherto mapped genes tend to be physically located in the terminal chromosome regions and that recombination seems to be largely restricted to the distal chromosome regions. This has been indicated in barley (Hagberg and Hagberg 1969; Linde-Laursen 1982; Künzel 1982), rye (Gustafson et al. 1990; Heslop-Harrison 1991), and wheat (Dvorak and Chen 1984; Snape et al. 1985; Tsujimoto and Noda 1990; Curtis and Lukaszewski 1991; Werner et al. 1992). Linde-Laursen (1988) proposed a set of barley tranlocations with breakpoints cytologically "localized as distally as possible and to as short chromosome segments as possible". If some of his proposed translocations are substituted by others from the present study, with break positions on comparatively short and distally located segments, the tester set can be improved for 8 of the 14 chromosome arms, as shown in Fig. 3.

At present, efforts are being made to isolate individual chromosomes in plants (e.g., Arumuganathan et al. 1991; Fukui et al. 1992; Lucretti et al. 1993), which would enable the construction of chromosome-specific DNA libraries. When complete libraries are available for individual barley chromosomes, further progress in localizing translocation breakpoints can be expected from in situ hybridization techniques, e.g., via 'chromosome painting'. Painting procedures of plant chromosomes have so far been shown to be highly efficient in identifying and defining alien chromosome segments translocated to wheat chromosomes (e.g., Le et al. 1989; Heslop-Harrison

Fig. 3 Proposed translocation tester set of barley depicting the most distal location of breakpoints. *Hatched regions* or *arrowheads* breakpositions according to the proposal by Linde-Laursen (1988); *black regions* break positions identified by the present study as being more distally located or at similar positions as previously proposed;
*) distal break position in terms of meiotic recombination as indicated by the predominant occurrence of chain quadrivalents in interchange heterozygotes

et al. 1990; Anamthawat-Jonsson et al. 1990; Schwarzacher et al. 1992; Mukai et al. 1993).

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