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Multiple reconstruction of barley karyotype resulting in complete cytological marking of the chromosome complement

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Summary. A new reconstructed barley karyotype, PK-88, which is a quadruple homozygote for three unequal translocations, 1–2, 3–4, 5–7, and one pericentric inversion in chromosome 6, was studied. As a result of these chromosome rearrangements, a complete cytological marking of the complement has been achieved. Due to the specific intra- or interchromosomal transfer of particular bands, Giemsa staining of somatic chromosomes provided clear-cut indications about the localization of translocation and inversion breakpoints. It was established that the long arms of chromosomes 1, 2, 4, 5 and 7 and the short arm of chromosome 3 have been involved in interchanges 1–2, 3–4, and 5–7. The breakpoints of pericentric inversion proved to be located proximally to the short (satellite) arm and distally in the long arm of chromosome 6. PK-88 offers an essential gain in resolution power and extension of the areas of application in cytogenetics over other reconstructed karyotypes produced so far in barley.

Key words: Reciprocal translocation – Pericentric inversion – Giemsa banding – Barley

Introduction

Due to its convenience for cytogenetic studies, barley karyotype has been subjected many times to experimental reconstruction. Both theoretical and applied aspects of the rearrangement of barley chromosomes have been thoroughly discussed (Nilan et al. 1968; Hagberg et al. 1972; Hagberg 1986). Among them, the use of chromosomal rearrangements for improvement of the karyotype with respect to the distinction of the individual chromosome types arouses particular interest.

The first attempts along these lines were done in studies dealing with the regional specificity of mutagenic factors. By means of ionizing radiations, single or double translocation lines have been produced in which all chromosome pairs can be easily distinguished in light microscope studies (Nicoloff and Künzel 1976; Gecheff 1976). Further reconstruction concerned the structure of chromosome 1, which is practically metacentric in the standard karyotype and does not allow the identification of its two arms (Künzel and Nicoloff 1979). An outstanding example of a cytologically marked karyotype is the triple translocation homozygote Tuleen 346 investigated by Finch and Bennett (1982). However, even in this case one of the chromosomes in the karyotype, namely, chromosome 4, does not carry any specific cytological marker, so in hybrid materials it cannot be distinguished from the standard homologue.

For a long time, our work was aimed at creation of synthetic karyotypes in which (1) all chromosome types would be cytologically marked, and (2) the individual chromosome could be easily identified in Feulgen preparations. The problem was successfully solved with karyotype PK-88, which is a quadruple homozygote for three unequal translocations and one pericentric inversion. The present paper provides data about the chromosome morphology and positions of intra- and interchange breakpoints concerning this karyotype.

Materials and methods

Parent karyotypes and crosses

PK-88 is a multi-reconstructed karyotype which was synthesized using both "irradiation" and "intercross" methods. It consists of three reciprocal translocations involving chromosomes 1 and 2 (T-2), 3 and 4 (T-1586), 5 and 7 (T-18), and one pericentric inversion in chromosome 6 (T-42). T-1586 (Gecheff 1976) and

Table 1. Relative mean lengths of chromosomes and chromosome arms from somatic cells of standard barley karyotype

Measurements and estimates	Chromosome no.						
	1	2	3	4	5	6	7
Satellite						2.24	1.59
Short arm	7.24	7.07	6.72	6.10	4.96	4.44	4.12
Long arm	7.35	8.61	8.78	7.63	7.15	7.00	9.00
Total length	14.59	15.68	15.50	13.73	12.11	13.68	14.71
95% confidence limit of relative total length	0.46	0.51	0.56	0.42	0.38	0.52	0.60
Short/long arm ratio	0.98	0.82	0.76	0.79	0.69	0.63	0.45

Table 2. Relative mean lengths of chromosomes and chromosome arms from somatic cells of reconstructed barley karyotype PK-88

Measurements and estimates	Chromosome no.						
	1^2	2^1	3^4	4^3	5^7	6^i	7^5
Satellite						1.88	1.29
Short arm	7.36	5.08	3.00	6.40	5.00	3.30	4.13
Long arm	10.75	7.02	8.50	11.78	8.87	8.31	7.33
Total length	18.11	12.10	11.50	18.18	13.87	13.49	12.75
95% confidence limit of relative total length	0.42	0.39	0.42	0.50	0.36	0.49	0.54
Short/long arm ratio	0.68	0.72	0.35	0.54	0.56	0.39	0.56

T-18 were induced independently by gamma-irradiation of dry seeds (doses of 150 Gy and 180 Gy, respectively) of standard variety Freya. Karyotypes T-2 and T-42 (K.I. Gecheff, unpublished data) are produced by gamma-irradiation of T-1586 (dose of 180 Gy). Thus, these karyotypes are identical to T-1586 with respect to translocation 3–4, but additionally contain an interchange between chromosomes 1 and 2, and a pericentric inversion in chromosome 6. Subsequently, T-2 was crossed with both T-18 and T-42 and triple homozygotes for interchanges 1–2, 3–4, 5–7 (T-164) and interchanges 1–2, 3–4, and pericentric inversion 6 (T-171) were selected from the F_2 of each of the crosses, T-2 × T-18 and T-2 × T-42. Finally, these triple homozygotes were crossed together and plants homozygous for all four chromosomal rearrangements were found in their F_2.

Cytological techniques

For measurements of the somatic metaphase chromosomes, germinated seeds (roots about 1–1.5 cm long) were pre-treated with 0.025% or 0.03% colchicine in a saturated solution of α-bromonaphthalene for 2 h at 24 °C, and whole embryos were detached from the seeds and fixed in freshly prepared ethanol : acetic acid (3 : 1) solution. This material was hydrolyzed for 9 min in 1 *N* HCl and stained with Schiff's reagent for 1 h. After an additional maceration with 4% pectinase for 10–12 min, squash preparations were made. The chromosomes were measured from enlarged photomicrographs of the best metaphase cells. The data in Tables 1 and 2 and Fig. 4 are means based on the measurements of ten metaphases of both standard and reconstructed karyotype PK-88 taken from at least five diploid roots. The length of each arm and chromosome is expressed as a percentage of total length of all chromosomes of the genome.

The improved Giemsa (N)-banding technique of Singh and Tsuchiya (1982), some steps of which were modified, was used for differential staining of root tip metaphase chromosomes. Pre treatment and fixation procedures were the same as in Feulgen preparations. The material was kept in the fixative overnight, followed by a transfer into 0.8% acetocarmine for about 30 min, and were then squashed in 45% acetic acid. The coverslip was removed after freezing in liquid N_2 and the slides were placed in absolute ethanol for 1.5 h and kept in a desiccator over silica gel or copper sulphate for about 1 week. Air-dried slides were incubated in 1 *M* $Na_2PO_4 \cdot H_2O$ (pH 4.15) for 5 min at 94 °C, thoroughly rinsed with distilled water, air-dried at room temperature for 1–2 h, and stained in 5% Giemsa in 1/15 *M* Sorensen's phosphate buffer at pH 6.8. After staining, which may last from a few minutes up to a few hours, the slides were rinsed in distilled water, air-dried overnight, put into xylene for 2 h, air-dried (1–2 h), and mounted in Canada balsam. All solutions were prepared immediately before use. The data in Fig. 4 concerning relative sizes and positions of Giemsa bands are based on studies of the parent lines T-1586 (Gecheff 1989), T-2, T-18, T-42, and triple homozygotes T-164 (Fig. 3a) and T-171 (Fig. 3b). The ideograms were produced by measuring the photomicrographs of at least ten well-stained chromosomes of each type.

Results and discussion

Karyotype analysis of Feulgen-stained chromosomes

The first karyogram of barley was established by Tjio and Hagberg (1951) on the basis of measurements of the

relative lengths and arm ratios of the chromosomes. Thereafter, based upon the karyotype analysis of translocation stocks (Tuleen 1973; Künzel 1976; Gecheff 1978) and differential staining of chromosomes (Linde-Laursen 1975; Noda and Kasha 1978; Singh and Tsuchiya 1982), the original designation of barley chromosomes has been revised. The numbering of chromosomes in this study is in agreement with the observation of Singh and Tsuchiya (1982), according to which chromosomes 1 and 3 of Tjio and Hagberg (1951) are switched.

The relative lengths of standard chromosomes and arm ratios established in the present study (Table 1) do not significantly differ from our earlier observations (Gecheff 1976), and are very similar to the data of some other authors (Künzel 1976; Singh and Tsuchiya 1982). The data in Table 1 show that individual chromosomes of barley differ in their lengths and arm ratios. However, even in good spreads there are serious difficulties in identifying submedian chromosomes of the complement, namely, chromosomes 2, 3, and 4.

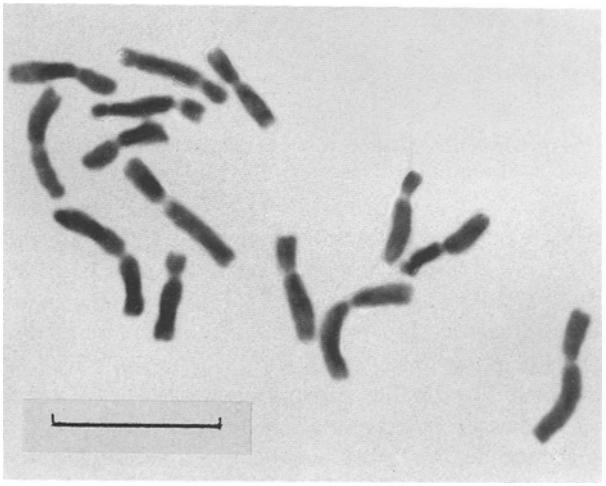

Fig. 1. Somatic metaphase cell of PK-88 barley. *Bar* represents 10 μm

Table 2 gives relative mean lengths and arm ratios of reconstructed karyotype PK-88, and Figs. 1 and 2 show root-tip metaphase and karyogram of this line. Due to the exchange of unequal segments between chromosome arms involved in intra- and interchanges induced, the individual chromosome types of PK-88 significantly differ from each other in their size and shape. Rarely some difficulties may arise in the identification of chromosomes 1^2 and 4^3, which appear to be similar to each other with respect to their total length. However, as can be seen in Figs. 1 and 2, these chromosome types are clearly enough distinguishable because of a significant difference in their arm ratios (Table 2).

In fact, all chromosome rearrangements induced in PK-88 were originally selected and identified by karyotype analysis of partially sterile plants in M_2-segregating families, since they all have resulted in great morphological changes of standard homologues. This analysis was especially efficient in identifying the reciprocal translocation 1–2 (T-2) and pericentric inversion 6 (T-42), which were induced additionally in T-1586, a karyotype whose chromosomes were all interdistinguishable (Gecheff et al. 1988).

The comparative analysis of the data in Tables 1 and 2 regarding relative arm lengths is in very good agreement with the conclusions drawn from other analyses in this study as to which arms are involved in the particular chromosome rearrangements. Thus, summing in pairs the relative lengths of the long arms of standard chromosomes 1 and 2 (Table 1) which are supposed to be involved in interchange 1–2, and the relative lengths of corresponding reconstructed arms in PK-88 (long arm of chromosome 1^2 and short arm of chromosome 2^1 in Table 2), estimates of 15.96 and 15.83 were obtained, respectively. Such a good agreement of the estimates was also observed for interchange 3–4, where the short arm of chromosome 3 and long arm of chromosome 4 appear

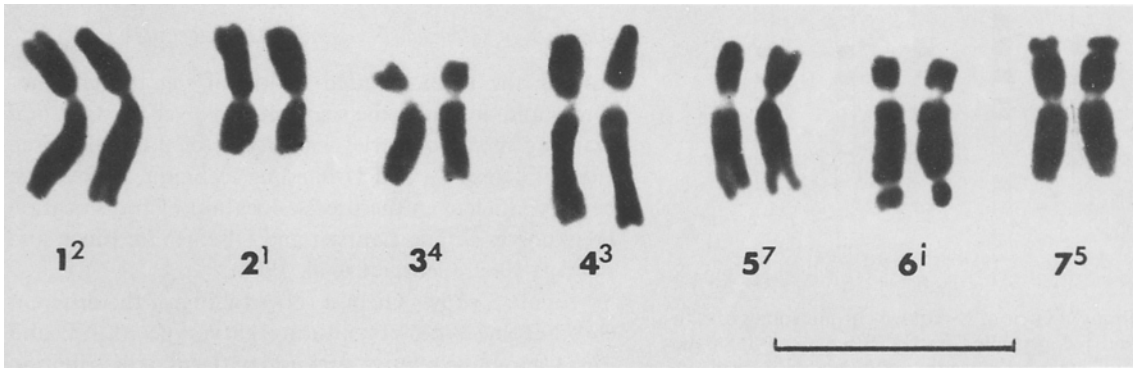

Fig. 2. The karyotype of PK-88 barley. *Bar* represents 10 μm

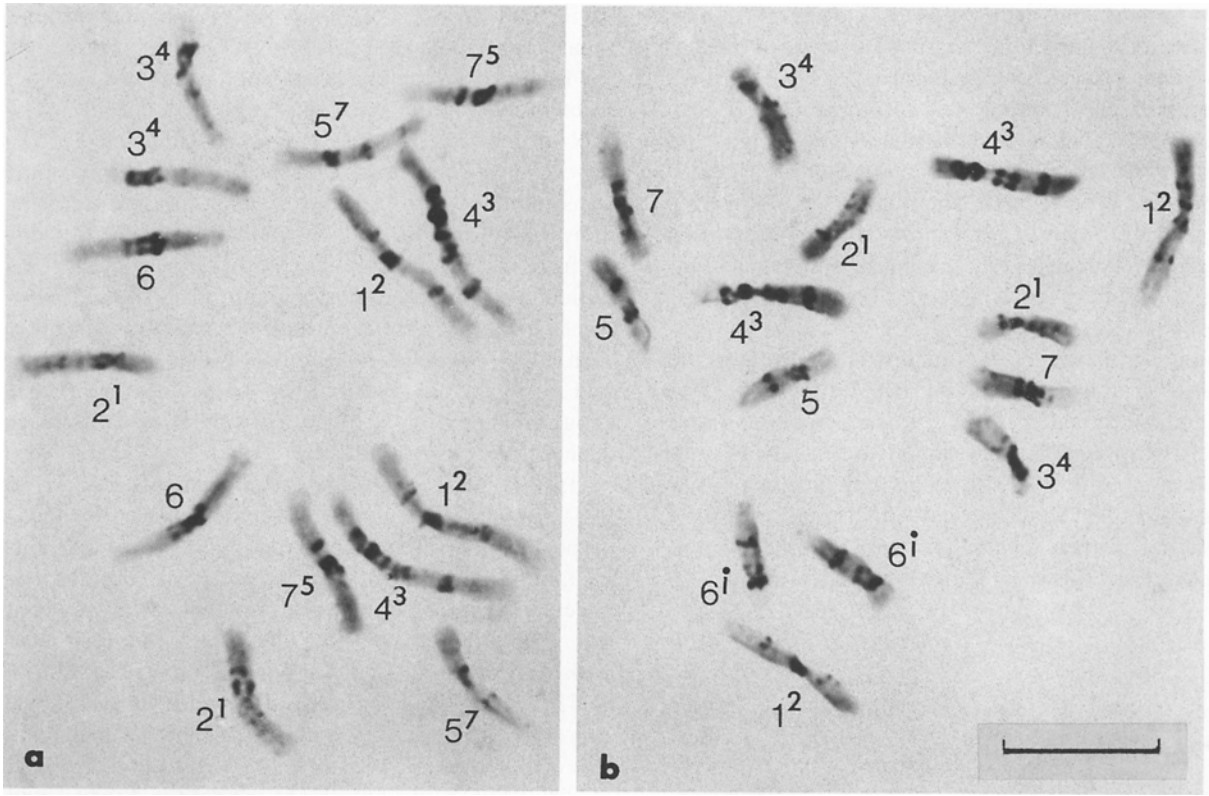

Fig. 3 a and b. Giemsa N-banded chromosome types of PK-88: **a** T-164 – triple homozygote for interchanges 1-2,3-4 and 5-7; **b** T-171 – triple homozygote for interchanges 1-2 and 3-4, and pericentric inversion in chromosome 6. *Bar* represents 10 μm

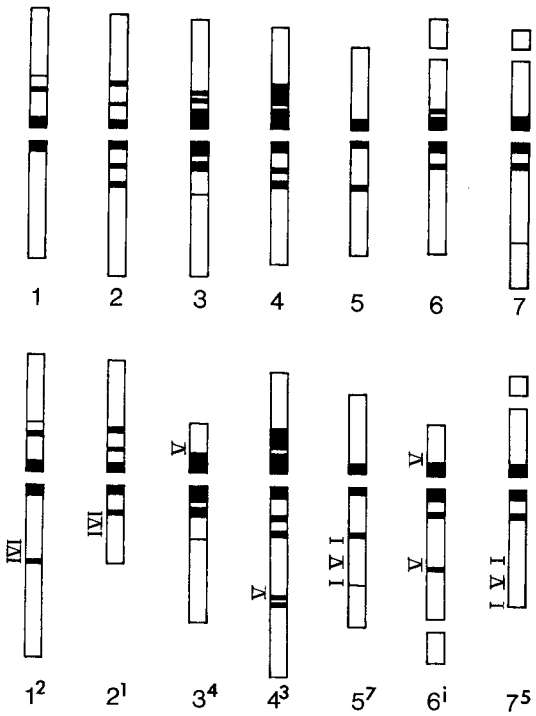

Fig. 4. Ideogramms of Giemsa N-banded chromosomes of standard (above) and reconstructed barley karyotype PK-88 (below). *Arrows* indicate possible sites of intra- or interchange breackpoints

to be involved, and for interchange 5–7 involving the long arms of chromosomes 5 and 7 ($P>0.99$ in all χ^2 tests). The very close values of the relative lengths of standard chromosome 6 (13.68, in Table 1) and reconstructed chromosome 6 of PK-88 (13.49, in Table 2) show that the most probable rearrangement in this case seems to be a pericentric inversion.

Independently, the same conclusions about chromosome involvement in all three interchanges included in karyotype PK-88 were drawn from translocation tester set analysis of parent lines T-1586 (Gecheff 1978), T-2, and T-18 (K.I. Gecheff, unpublished data).

Analysis of Giemsa (N)-banded chromosomes

Among the methods used in identifying the chromosomes and chromosome segments involved in structural rearrangements of barley karyotype (cf. Linde-Laursen 1988), Giemsa C- and N-banding techniques proved to be very efficient in the precise location of translocation breakpoints (Linde-Laursen and Olsen 1976; Finch and Bennett 1982; Georgiev et al. 1985).

Figure 3 shows Giemsa (N)-banding of the different chromosome types constituting karyotype PK-88, and Fig. 4 shows the relative sizes and patterns of distribution of N-bands along the metaphase chromosomes in stan-

dard karyotype and PK-88. These investigations completely confirmed the above-mentioned conclusions drawn from karyotype analysis and, due to the specific intra- or interchromosomal transfer of particular bands, provided clear-cut indications about the localization of the breakpoints of reciprocal translocations 1–2, 3–4, 5–7, and pericentric inversion in chromosome 6, as follows.

Interchange 1–2

The application of Giemsa banding techniques is especially useful in identifying the involvement in rearrangements of chromosome 1. Owing to the median position of the centromere, the karyotype analysis was useless for identifying which of the two arms of this chromosome has been involved in translocation 1–2. As a matter of fact, the identity of the short and long arm of chromosome 1 is still a controversial question (cf. Singh and Tsuchiya 1982). The results of this study show that the two arms of chromosome 1 are practically equal in length (short/long arm ratio = 0.98). Occasionally, the comparative analysis of photomicrographs taken from somatic metaphases which were successively stained with acetocarmine and Giemsa showed conflicting data even for the two homologues of the same cell. The banding pattern of chromosome 1 does not differ from that established by Singh and Tsuchiya (1982). In spite of the absence of clear evidence about arm designation of this chromosome, we have accepted the conception of these authors, according to whom the long arm has only a centromeric band and the short arm has a centromeric and two intercalary bands.

It can be seen in Figs. 3 and 4 that there is very good agreement between the sizes and positions of Giemsa bands in the short arms of standard chromosome 1 and chromosome 1^2 of PK-88, and the same holds true for the short arm of standard chromosome 2 and the long arm of chromosome 2^1. On the other hand, the analysis of banding patterns of the long arm of chromosome 1^2 and the short arm of chromosome 2^1 shows that as a result of reciprocal translocation, the most distal band in the long arm of chromosome 2 is transferred to the long arm of chromosome 1 residing in the median position in the long arm of chromosome 1^2. It was also concluded that the translocation breakpoints are located proximally to the median band in the long arm of chromosome 1^2 and distally to the intercalary band in the short arm of chromosome 2^1 within the segment whose length is equal to the distance confined by the two intercalary bands of the long arm of chromosome 2 (Fig. 4).

Interchange 3–4

The data concerning chromosome location of the breakpoints of interchange 3–4 confirmed the conclusions drawn in our previous Giemsa banding experiments (Georgiev et al. 1985; Gecheff 1989). The transfer of a double proximal band in the short arm of chromosome 3 to the long arm of chromosome 4 in this case allows a precise identification of the interchange breakpoints which are indicated by arrows in Fig. 4.

Interchange 5–7

The distribution of Giemsa bands over chromosomes 5^7 and 7^5 of karyotype PK-88 shows (Fig. 4) that the short arm of chromosome 5^7 and the short satellite arm of chromosome 7^5 reveal unchanged morphology and distribution pattern of Giemsa bands, when compared to the respective chromosome arms of standard karyotype. Long arms of the same chromosomes, however, clearly differ in their morphology and banding pattern. There are clear indications that the faint band located distally in the long arm of chromosome 7 is transferred to the long arm of chromosome 5, the translocation breakpoints being anywhere in segments 2.7 units long shown on the long arms of chromosomes 5^7 and 7^5 in Fig. 4.

Intrachange 6

The first convincing evidence that chromosome 6 of PK-88 contains a pericentric inversion was obtained by karyotype analysis of parent line T-42. It was established that the apparent alteration in the morphology of this chromosome was not accompanied by any sort of morphological changes in the rest of the chromosomes of the karyotype, which were easily interdistinguishable in this case. This assumption was unequivocally confirmed in our Giemsa banding experiments. Due to the transfer of the proximal band of the short (satellite) arm to the long arm of this chromosome (the band in question can be seen on the median part of the long arm of chromosome 6^i in Fig. 3 b), it became possible to identify exactly the inversion breakpoints which are indicated by arrows in Fig. 4.

PK-88 and the cytogenetical research

The ability to identify individual chromosomes and chromosome segments is of great importance in many genetic studies and breeding programs. Most of the aspects of this problem have already been discussed (Michaelis and Rieger 1971; Finch and Bennett 1982). Among the natural species, only a few are known to have chromosomes which are all morphologically distinguishable. Cytologically marked karyotypes produced so far in barley have been used mainly to study the specific distribution along the chromosomes of structural changes induced by mutagenic factors (Nicoloff and Künzel 1976; Gecheff 1976), and to elucidate the position and behaviour of chromosomes in barley and its hybrids (cf. Finch and Bennett

1982). However, it can be expected that such karyotypes would be very useful in all kinds of genetic and breeding studies manipulating at the chromosome level.

Due to the complete cytological marking of the complement and clear morphological distinction of its chromosomes from those of normal barley and its relative species, PK-88 offers an essential gain in resolution power and extension of the areas of application in cytogenetics over other reconstructed karyotypes produced so far in barley.

As far as the phenotype is concerned, PK-88 is a stable line which is normally vigorous and fertile without any apparent qualitative alterations of its habit. At present, this line is used to study the effects of chromosomal transposition on the involvement in induced structural mutations of the different chromosome segments.

References

Finch RA, Bennett MD (1982) The karyotype of Tuleen 346 barley. Theor Appl Genet 62:53–58

Gecheff KI (1976) A new translocation in barley resulting in a cytologically marked karyotype. Mutat Res 36:241–244

Gecheff KI (1978) Cytogenetic study on a barley translocation line and some conclusions concerning chromosome karyogramme designation. Genet Plant Breed 11:17–22

Gecheff KI (1989) Position-specific effects in the mutagenic action of mitomycin C on the chromosomes of *Hordeum vulgare* L. Theor Appl Genet 77:705–710

Gecheff K, Slavov D, Uzounova V (1988) Chromosome reconstruction in barley. I. On the methods of cytogenetic analysis of structural mutations. Genet Plant Breed 21:469–477

Georgiev S, Gecheff K, Nicoloff H, Künzel G, Rieger R (1985) Giemsa banding as a means of identification of reconstructed chromosomes in barley. Biol Zentralbl 104:29–34

Hagberg A (1986) Induced structural rearrangements. In: Jensen CJ, Odenbach W, Schieder O (eds) Genetic manipulation in plant breeding. de Gruyter, Berlin, pp 17–36

Hagberg A, Persson G, Hagberg G (1972) Utilization of induced chromosomal aberrations. Translocations, duplications and trisomics in barley. In: Induced mutations and plant improvement. IAEA, Vienna pp 173–182

Künzel G (1976) Indications for a necessary revision of the barley karyogramme by use of translocations. In: Gaul H (ed) Barley genetics. Proc 3rd Int Barley Genet Symp. Thiemig, München, pp 275–281

Künzel G, Nicoloff H (1979) Further results on karyotype reconstruction in barley. Biol Zentralbl 98:587–592

Linde-Laursen I (1975 Giemsa C-banding of the chromosomes of "Emir" barley. Hereditas 81:285–289

Linde-Laursen I (1988) Giemsa C-banding of barley chromosomes. V. Localization of breakpoints in 70 reciprocal translocations. Hereditas 108:65–76

Linde-Laursen I, Olsen OA (1976) Identification of an interchange between chromosome 2 and 5 in Riso mutant 56-a high lysin line. Barley Genet Newsl 6:43–44

Michaelis A, Rieger R (1971) New karyotype of *Vicia faba* L. Chromosoma 35:1–8

Nicoloff H, Künzel G (1976) Reconstructed barley karyotypes as a tool in the analysis of intrachromosomal distribution of chromatid aberrations. In: Gaul H (ed) Barley genetics Proc 3rd Int Barley Genet Symp. Thiemig, München, pp 236–241

Nilan RA, Powell JB, Conger BV, Muir CE (1968) Induction and utilization of inversions and mutations in barley. In: Mutations in plant breeding II. IAEA, Vienna, pp 193–203

Noda K, Kasha KJ (1978) A proposed barley karyotype revision based on C-band chromosome identification. Crop Sci 18:925–930

Singh RJ, Tsuchiya T (1982) Identification and designation of telocentric chromosomes in barley by means of Giemsa N-banding technique. Theor Appl Genet 64:13–24

Tjio JH, Hagberg A (1951) Cytological studies of some X-ray mutants of barley. An Estac Exp Aula Dei 2:149–167

Tuleen NA (1973) Karyotype analysis of multiple translocation stocks of barley. Can J Genet Gytol 15:267–273