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Production and identification of new structural chromosome mutations in barley (*Hordeum vulgare* L.)

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Abstract A total of 52 reciprocal translocations and 9 pericentric inversions were induced and identified in both standard and cytologically marked barley karyotypes using gamma-rays as the clastogenic agent. An analysis based upon Giemsa N-banding patterns and arm length measurements of the reconstructed chromosomes enabled a rather precise cytological localization of intra- and interchange breakpoints. This analysis was significantly facilitated and improved, especially for the identification of pericentric inversions, when the reconstructed karyotype T-1586 was used as starting material. The majority, if not all, of the aberration breakpoints proved to be localized in interband regions or in medial and terminal parts of the chromosomes, i.e., in regions which are deficient in constitutive heterochromatin. A great number of the structural mutations produced in this study contain specific cytological markers covering nearly all of the chromosomes of barley karyotype. This material might be of considerable interest in solving various problems of barley cytogenetics and chromosome engineering and especially in constructing a physical map of barley genome.

Key words Reciprocal translocations · Pericentric inversions · Giemsa N-banding · *Hordeum vulgare*

Introduction

Chromosomal rearrangements are one of the most frequently produced class of mutations (gene or point mutations are the other important class of genetic changes) that result from the action of both physical and chemical mutagenic agents. The different aspects of chromosome reconstruction have been thoroughly discussed in the literature (Hagberg 1986; Schubert et al. 1991; Gupta and Gupta 1991).

The karyotype of cultivated barley (*Hordeum vulgare* L.) has been subjected to extensive experimental reconstruction. Similar to other diploid organisms (Schubert and Rieger 1990), barley does not tolerate the loss of large cytologically visible chromosome segments. Thus, reciprocal translocations and paracentric inversions have proved to be the most common of the surviving induced chromosomal rearrangements in this species (Hagberg 1986; Künzel et al. 1984; Nilan et al. 1968; Ekberg 1974). As a result of extensive studies along these lines a large number of chromosomal structural mutations (mainly reciprocal translocations) have been identified. However, only a small portion of the rearrangements produced so far have been precisely analysed with respect to the location of the intra- or interchange breakpoints along the chromosomes (Linde-Laursen and Olsen 1976; Finch and Bennett 1982; Georgiev et al. 1985; Konishi and Linde-Laursen 1987; Linde-Laursen 1988; Gecheff 1989). This hampers the efficient use of most of the structural rearrangements in barley cytogenetics and chromosome engineering.

For a long time our work has been aimed at the induction and selection of new structural chromosome mutations in barley that contain specific cytological markers for different chromosomes and chromosome arms. This paper details data concerning the cytological localization of the breakpoints of a portion of the chromosomal rearrangements produced so far.

Materials and methods

Dry seeds of both standard two-rowed spring barley variety 'Freya' and translocation line T-1586 (originating from the same variety), which have cytologically easily interdistinguishable chromosomes (Gecheff 1978), were irradiated with gamma-rays (doses between 100 and 200 Gy) in order to produce chromosome structural mutations.

Since the main interest of our work were the rearrangements that resulted in a marked alteration of the morphology of the chromosomes, the first very important step in the isolation of the structural

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mutants was the metaphase analysis of the Feulgen-stained root-tip metaphase chromosomes of M1 plants that showed well-expressed partial sterility. For this purpose, the examination of Feulgen squashes of the root tips of about 4–5 seeds of such M1 plants proved to be quite adequate. About 30 seeds of each M1 plant showing karyotype reconstructions of interest were grown in a glasshouse or in the field and the plant allowed to self-fertilize; homozygous structural mutations were then isolated among the fertile M2 plants.

The location of intra- and interchange breakpoints along the chromosomes was established using both arm ratio measurements of Feulgen-stained reconstructed chromosomes and their Giemsa banding patterns (up to ten samples of each chromosome type, the number being dependent on the position of the breakpoints). Further, the newly produced structural mutants were test-crossed onto the respective original lines ('Freya' or T-1586) or, when necessary, onto the suitable tester set of translocations, and the meiotic metaphase I of F_1 plants was examined to specify the reconstruction of the karyotype as a whole.

All data concerning both conventional and Giemsa banding cytological techniques have been given in previous paper (Gecheff 1978, 1989).

Results

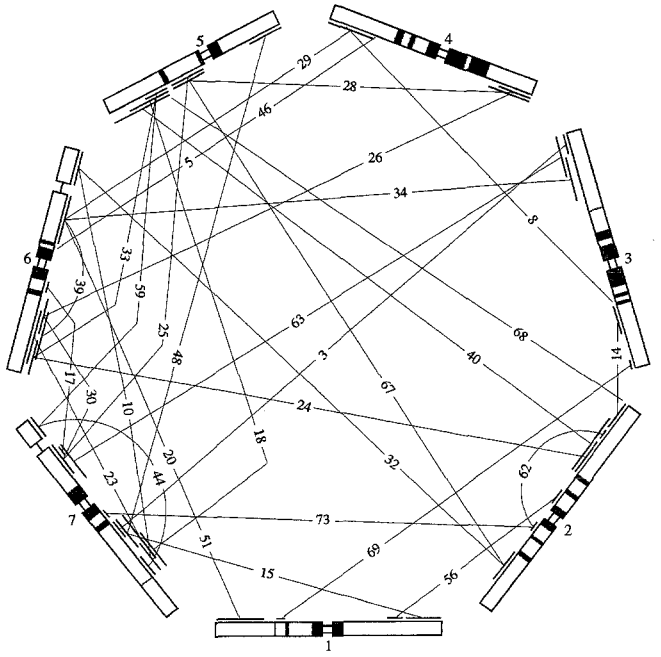
Figure 1 shows a schematic representation of the chromosomal localization of the breakpoints of gamma-induced pericentric inversions and reciprocal translocations in standard variety 'Freya'. It was observed that only about 11% of the partially sterile M1 plants of this variety showed some alterations in the morphology of their root-tip metaphase chromosomes. Obviously, partial sterility observed in the first generation may be due to reasons other than the induction of heterozygous chromosome mutations (cf. Künzel et al. 1984). The low selective capacity of the karyogramme analysis applied in this case might also be due to the fact that a great portion of the induced translocations have resulted in the exchange of approximately equal chromosome segments. This type of interchange can be easily identified using a traditional approach (Hagberg 1960). However, since this study was aimed at the production of structural mutants containing specific cytological markers in different chromosomes, the technique used here proved to be the most suitable for the purposes of the investigation.

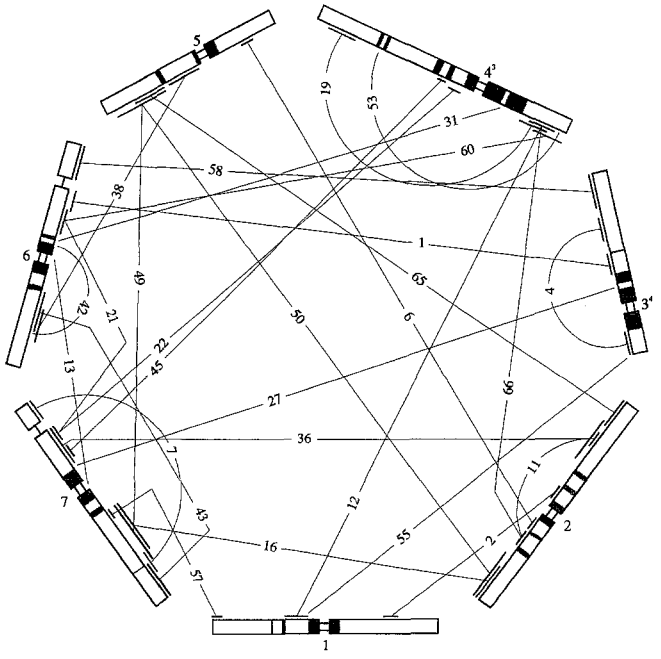
The identification of induced chromosomal rearrangements was significantly facilitated when the cytologically marked karyotype T-1586 (Fig. 2), which contains a single reciprocal translocation between chromosomes 3 and 4 (marked as T-8 in Fig. 1), was used as starting material. As a result of this translocation all chromosome pairs of the karyotype became easily interdistinguishable, and the resolution power of the karyogramme analysis was improved. This was an important step in the development of efficient techniques for detection of chromosome rearrangements, especially pericentric inversions. More than 26% of the total number of partially sterile M1 plants contained some kind of karyotype reconstruction. What is interesting in this investigation is that at least one-fifth of the induced chromosomal rearrangements in T-1586 were found to be pericentric inversions.

As expected, the reciprocal translocations and pericentric inversions proved to be the main types of

Fig. 1 Schematic representation of the breakpoint distribution along the chromosomes of 30 reciprocal translocations (the *straight lines* connecting the arms of different chromosomes) and three pericentric inversions (the *parabolic lines* connecting the two arms of one and the same chromosome) in standard variety 'Freya'. The *hatched areas* of the chromosomes show the size and the position of heterochromatic regions, as revealed by Giemsa N-banding

Fig. 2 Schematic representation of chromosomal localization of the breakpoints of 22 reciprocal translocations and six pericentric inversions in reconstructed karyotype T-1586. More detailed explanations are given in the legend of Fig. 1





gamma-induced structural mutations in both 'Freya' and T-1586. The localization of the breakpoints of these rearrangements along the chromosomes (Fig. 1 and 2) was done using analysis based upon Giemsa N-banding patterns in combination with arm length measurements of the reconstructed chromosomes. An indispensable prerequisite for this analysis is, however, a good knowledge of the Giemsa N-banding patterns of the chromosomes of standard variety 'Freya'. N-banded chromosomes of this variety and an idiogram of the karyotype showing the relative size and position of the bands are given in Fig. 3. All of the bands presented in the idiogram, with the exception of the faint bands located distally in the short arm of chromosome 1 and the long arm of chromosome 7, showed a very high appearance frequency (more than 80%). The appearance of NOR-associated heterochromatic segments in chromosomes 6 and 7 was rather variable however (see Georgiev et al. 1985), and these are not given in the idiogram.

The comparative analysis of good metaphase spreads stained with acetocarmin and subsequently N-banded (Fig. 4) allowed precise localization of the position of the centromeres and direct identification of the chromosomes that were involved in definite rearrangements. Further detailed analysis of enlarged microphotographs of Giemsa-banded reconstructed chromosomes enabled the precise localization of translocation and inversion breakpoints. This analysis was especially efficient when chromosome reconstruction resulted in the transfer of particular bands (Fig. 5, A–E, G, I). Since the heterochromatic segments in barley chromosomes are mainly concentrated proximal to the centromere (Fig. 3), the sites of the breakpoints in this case were found to be confined within a very small interband region. However, a precise identification of the position of rearrangement breakpoints was possible even if no transfer of bands took place, provided the reconstruction markedly affected the morphology of the chromosomes (Fig. 5, F, H, J). The putative site of breakpoints in this case could not exceed the size of the chromosome segment standing terminally to the most distal Giemsa band in the shortened arm.

As the structural mutations produced in this study were mostly of an unequal (or asymmetric, as termed by Linde-Laursen 1988) type, i.e., chromosome segments of different sizes were exchanged, they could be very well characterized with respect to their localization along the chromosomes. The most remarkable result concerning the chromosome distribution of these mutations, which can be seen in Figs. 1 and 2, was that the majority, if not all, of the breakpoints of both reciprocal translocations and pericentric inversions were localized in interband regions. T-8 (Fig. 1) seems to be the only indication in this study that the breakpoints might also be located within heterochromatic regions, since an additional faint band was very often observed in the short arm of reconstructed chromosome 3^4 (Fig. 5, B).

All of the F_1 hybrids between the newly produced translocations and original lines ('Freya' or T-1586, respectively) showed in metaphase I of meiosis 1 a quadrivalent and five bivalents, and in analogous crosses of the pericentric inversions a normal meiotic configuration was observed. These data indicate that the newly produced structural mutants contained only rearrangements that have been identified by root-tip analysis. The conclusions concerning chromosome involvement in translocations drawn by Giemsa banding were also confirmed by translocation tester set analysis.

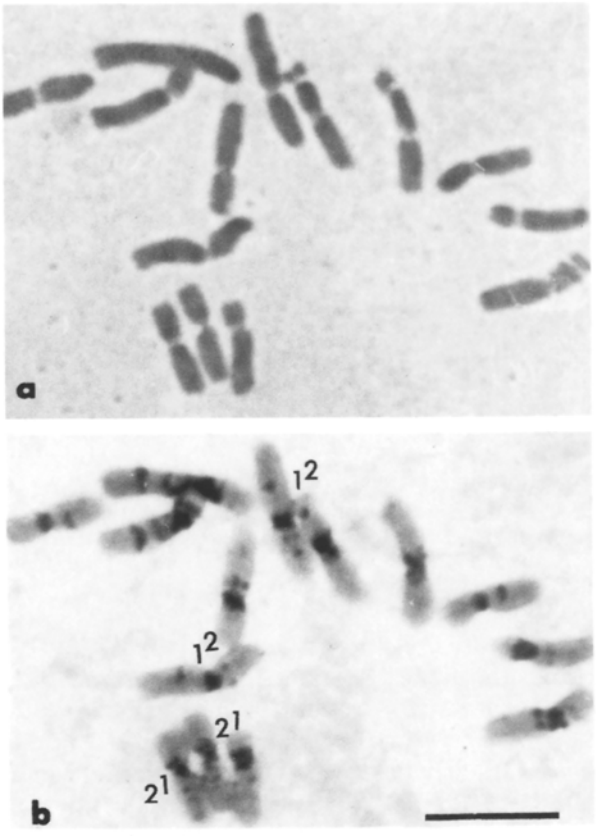


Fig. 4a, b Somatic metaphase chromosomes of T-2 stained first with acetocarmin (**a**) and subsequently by Giemsa banding (**b**). *Bar*: 10 μm

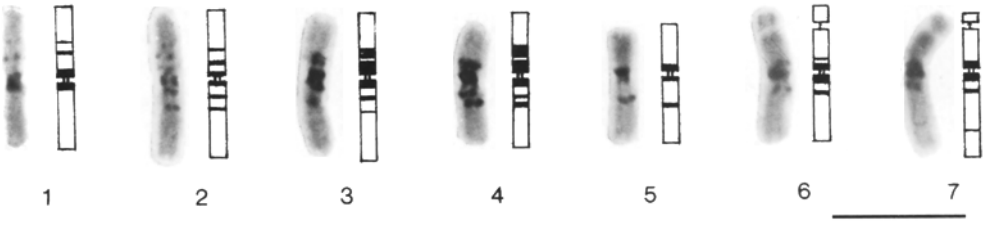


Fig. 3 Idiogram of Giemsa N-banded chromosomes of standard variety 'Freya'. *Bar*: 10 μm

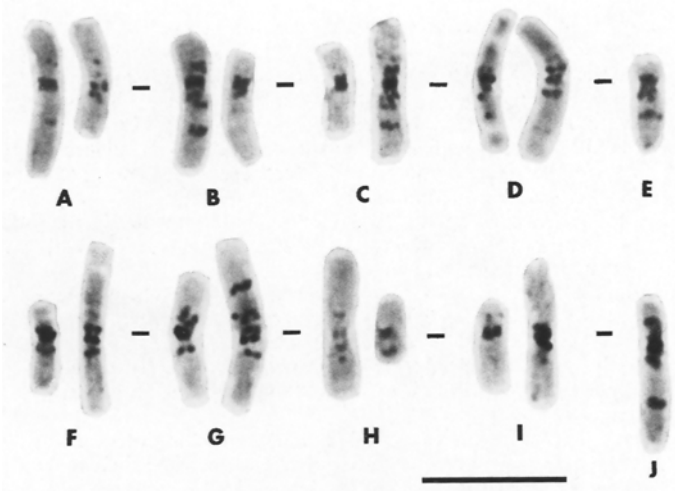


Fig. 5 Distribution patterns of Giemsa bands in reconstructed chromosomes of: *A* T-2, *B* T-8, *C* T-12, *D* T-13, *E* I-42, *F* T-21, *G* T-45, *H* T-68, *I* T-69 and *J* I-19. Compare the schematic representations of *B*, *H*, *I* and Fig. 1, and *A*, *C*, *D*, *E* and Fig. 2. *Bar*: 10 μm

Discussion

The main result of this study is that a number of gamma-induced homozygous reciprocal translocations and pericentric inversions could be isolated and analyzed with respect to their chromosome location.

The Giemsa banding technique in combination with karyogramme analysis offers essential gain in resolution power and time-saving over other procedures used so far in the identification of chromosomes involved in rearrangements and location of the breakpoints of structural mutations (Burnham 1962; Kasha and Burnham 1965; Hagberg and Hagberg 1968; Hagberg 1986) although not all genetic changes of this type are amenable to detection by this approach. This method, which has been used by other authors (Finch and Bennett 1982; Georgiev et al. 1985; Linde-Laursen 1988), allowed a rather precise chromosome mapping of the induced structural mutations. An essential step in improving the resolution power of this analysis was the use of cytologically marked karyotype T-1586 as starting material for mutation induction, and this was especially in identifying pericentric inversions. Since the procedures usually used for the isolation of reciprocal translocations are completely inadequate for the detection of inversions and since barley proves to be unsuitable for pachytene analysis, the development of effective techniques for selection and identification of this type of structural mutations seems to be very important. All of the rearrangements induced in translocation stock T-1586 resulted in the production of double structural mutants, but each of them, except for those involving chromosomes 3 and 4, could be easily recovered as single one in the M2-segregating progeny of crosses between newly produced mutants and standard variety 'Freya'.

As far as the problem of distribution of the heterochromatic segments (as revealed by Giemsa banding) along barley chromosomes is concerned, it should be pointed out that although the banding pattern observed in this study has much in common with those established by other authors (Singh and Tsuchiya 1982; Fukui and Kakeda 1990), there are also some differences. As is well known, there are large differences among various barley lines with respect to the size of particular bands and the number and position of bands on the same chromosome (intervarietal polymorphism) (Kakeda et al. 1991). Moreover, the pattern of Giemsa banding seems to depend on the technical procedure used (Singh and Tsuchyia 1982).

Although the amount of data obtained in this study is not enough to draw a general conclusion about the distribution pattern of gamma-induced structural mutations, surprisingly, a number of breakpoints proved to be localized in medial and terminal parts of the chromosomes, i.e., in regions which are deficient in constitutive heterochromatin. Besides, as mentioned, nearly all of the breakpoints proved to be localized in interband regions of the chromosomes, just as was established earlier by Linde-Laursen (1988) for a number of barley translocations. The method used in this study do not have a power of resolution sufficient for identification of exchanges between heterochromatic regions themselves, but the data show that the subdivision of Giemsa bands by gamma-irradiation is probably a very rare event. Thus, the results seem to be in a good agreement with the "winding up-concept" of Kaina (1979) in accounting for the transformation of primary DNA lesions into rearrangements at the chromosome level. According to this hypothesis, aberrations are expected to be located exclusively in actively transcribing euchromatic chromosome regions (corresponding to the interband regions in Figs. 1 and 2), while aberrations in constitutive heterochromatin (Giemsa bands) should be rare or absent. The data concerning preferential involvement in structural rearrangements of heterochromatin-containing chromo some segments (Rieger et al. 1977) do not contradict the hypothesis mentioned above since it has been established that the most sensitive sites of aberration induction appear to be the frontiers between hetero- and euchromatin, but not be heterochromatic regions themselves (Seabright 1973; Döbel et al. 1978).

Up to now, the structural chromosome mutations reported in this paper have been extensively used in creating multireconstructed karyotypes with cytologically marked chromosome complements. These karyotypes have enabled a successful investigation of the regional specificity of the mutagens and the effects of segment transposition on the involvement of different chromosome segments in induced structural mutations (Gecheff 1989, 1992).

Among the different possibilities of application of the structural mutations, their use in constructing a physical map of the genome is of great importance. Recently, a new approach has been devised for physical localization

of genetically mapped restriction fragment length polymorphism clones to barley chromosomes using morphologically distinct translocation chromosomes (Sorokin et al. 1994). This work provides a number of stocks containing precisely localized cytological markers for different chromosomes which could be successfully used in the construction of a physical map of the barley genome.

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References

Burnham CR (1962) Discussion in cytogenetics. Burgess Publ, Minneapolis

Döbel P, Schubert I, Rieger R (1978) Distribution of heterochromatin in a reconstructed karyotype of *Vicia faba* as identified by banding and DNA-late replication patterns. Chromosoma 69:193–209

Ekberg I (1974) Cytogenetic studies of three paracentric inversions in barley. Hereditas 30:1–30

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

Fukui K, Kakeda K (1990) Quantitative karyotyping of barley chromosomes by image analysis methods. Genome 33:450–458

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) Multiple reconstruction of barley karyotype resulting in complete cytological marking of the chromosome complement. Theor Appl Genet 78:683–688

Gecheff KI (1991) Further evidence for a high position-specific effect in the action of chemical mutagens on the chromosomes of barley. Theor Appl Genet 83:179–184

Gecheff KI (1992) Chromosome reconstruction and chromosomal transposition effects in the induction of barley structural mutations. Genet Plant Breed 25:235–246

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

Gupta PK, Gupta SN (1991) Cytogenetics of chromosome interchanges in plants. In: Gupta PK, Tsuchiya T (eds) Chromosome engineering in plants: genetics, breeding, evolution, part A. Elsevier, Amsterdam Oxford New York Tokyo, pp 87–112

Hagberg A (1960) Cytogenetic analysis of induced mutations. Genet Agrar 12:319–336

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

Hagberg A, Hagberg G (1968) Induced mutations in barley. In: Mutations in plant breeding II. IAEA, Vienna, pp 129–137

Kaina B (1979) Significance and consequences of differential chromatin condensation in the production of chromosome structural changes. Biol Zentalbl 98:129–152

Kakeda K, Fukui K, Yamagata H (1991) Heterochromatic differentiation in barley chromosomes revealed by C- and N-banding techniques. Theor Appl Genet 81:144–150

Kasha KJ, Burnham CR (1965) The location of interchange breakpoints in barley. II. Chromosome pairing and intercross methods. Can J Genet Cytol 7:620–632

Künzel G, Gramatikova M, Hamann S (1984) Isolation of radiation-induced translocations in spring and winter barley. Biol Zentralbl 103:649–653

Konishi T, Linde-Laursen I (1987) Spontaneous chromosomal rearrangements in cultivated and wild barleys. Theor Appl Genet 75:237–243

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

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

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

Rieger R, Michaelis S, Schubert I, Kaina B (1977) Effects of chromosome repatterning in *Vicia faba* L. II. Aberation clustering after treatment with chemical mutagens and X-rays as affected by segment transposition. Biol Zentralbl 96:161–182

Schubert I, Rieger R (1990) Deletions are not tolerated by the *Vicia faba* genome. Biol Zentralbl 109:207–213

Schubert I, Rieger R, Künzel G (1991) Karyotype reconstruction in plants with special emphasis on *Vicia faba*. In: Gupta PK, Tsuchiya T (eds) Chromosome engineering in plants: genetics, breeding evolution, part A. Elsevier, Amsterdam Oxford New York Tokyo, pp 113–140

Seabright M (1973) High resolution studies on the pattern of induced exchanges in the human karyotype. Chromosoma 40:333–346

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

Sorokin A, Marthe F, Houben A, Pich U, Graner A, Kunzel G (1994) Polymerase chain reaction mediated localization of RFLP clones to microisolated translocation chromosomes of barley. Genome 37:550–555