Chromosome identification of seven primary trisomics in inbred rye (*Secale cereale* L.) by acetocarmine Giemsa C-banding¹

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ABSTRACT

Seven primary trisomics were isolated in the progeny of autotriploids of an inbred rye line (*Secale cereale* L.). Each triplicated chromosome was cytologically identified by the improved acetocarmine Giemsa C-banding method. This method elucidates qualitative and quantitative traits of the individual chromosomes in the complement.

Chromosomes 1 and 2 were non-satellited, while the others (chromosomes 3, 4, 5, 6, and 7) had secondary constrictions in the short arms with different sizes of satellites. Chromosomes 4 and 5 had clear tertiary constrictions in their long arms. After C-banding, prominent bands were observed on both terminal ends of chromosomes 1 and 3. Chromosome 2 had somewhat weak bands on both terminal ends. The satellites of chromosomes 4, 5, 6, and 7 were heavily stained, but the long arm of chromosome 4 had three weakly stained bands, whereas chromosome 5 had two weak and two still weaker bands. Two weakly stained bands were observed in the long arm of chromosome 6. A prominent band on the end of the long arm and a weak one close to the terminal band were recognized in chromosome 7. A diamond shaped centromere was detected in some cells. These characteristics were cytologically confirmed in all seven types of primary trisomics.

Homoeologous relationships to the wheat chromosomes were discussed. Rye chromosomes 1 to 7 in this study are homoeologous with wheat chromosomes and designated as 2R, 7R, 3R, 4R, 6R, 5R, and 1R, respectively.

1. INTRODUCTION

The usefulness of aneuploids for genetic and cytogenetic studies is well known. Trisomics are produced and used most frequently in diploid organisms. Blakeslee et al. (1920) first obtained trisomic plants in *Datura stramonium*, and since then many reports concerning trisomic plants have been accumulated (Khush, 1973).

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Kamanoi and Jenkins (1962) first developed a complete set of seven primary trisomics in a winter rye, *Secale cereale* L. cv. Petkus. Balkandschiewa and Mettin (1974) and Pilch (1978) reported seven types of primary trisomics from triploid hybrids between tetraploids and diploids of different rye cultivars. Zeller et al. (1977) obtained six of the seven types of primary trisomics in rye cultivar Heines Hellkorn. Sturm and his associates developed primary trisomics and used them for genetic analysis (Sturm and Engel, 1980; Sturm et al., 1981). Unfortunately, all of these sets of trisomic lines have been partly or completely lost (M. Kamanoi, R. Schlegel, A. Winkel, and F. J. Zeller, personal communication). In any case some of these sets are not practically useful because of their heterogeneous background, due to hybrid origin between different cultivars. This is a special hinderance to the study of the effects of the extra chromosome on specific character expression in the trisomics. It is, therefore, necessary to develop a complete set of primary trisomics from a homogeneous strain of rye.

We have been involved in producing a complete series of primary trisomics in an inbred rye line (Fujigaki and Tsuchiya, 1988). In this paper the results of chromosome identification of these trisomics by an improved acetocarmine-Giemsa C-banding method, will be presented as well as a discussion of homoeologous relationships with barley and wheat chromosomes.

2. MATERIALS AND METHODS

Diploid plants of an inbred rye line, *Secale cereale* L., and its autotetraploid have been maintained by selfing or sibcrossing in the Laboratory of Cytogenetics, Department of Agronomy, Colorado State University. Trisomic plants were recovered in the progeny of autotriploid plants derived from crossing the autotetraploid and the diploid. The trisomics were isolated cytologically and classified into seven groups by morphological characteristics (Fujigaki and Tsuchiya, 1986, 1988).

Accurate identification of extra chromosomes in the trisomic plants was carried out by the cytological method of Fujigaki and Tsuchiya (1985), which is briefly described as follows:

1. Pretreatment of newly developed root tips in ice water for 16 hours.
2. Fix in Farmer's solution.
3. Stain the fixed materials in 0.6% acetocarmine for 1 to 3 hours.
4. Make squash preparations in 45% acetic acid.
5. Use phase contrast for photomicrographs.
6. Remove cover glass after dry ice freezing.
7. Air dry and store in a desiccator.
8. 5% Barium hydroxide treatment at 60°C for 5 minutes.
9. Rinse the slides in distilled water.
10. Incubate preparations in 2×SSC (0.3 M sodium chloride containing 0.03 M

tri-sodium citrate, adjusted to pH 7) at 60°C for 1 hour.

11. Rinse slides in distilled water.
12. Stain in 5% Giemsa solution diluted with phosphate buffer (pH 6.8).
13. Rinse slides in distilled water and air dry.
14. Mount by Permount or Eukitt.
15. Take photomicrographs of the same cells taken in step 5.

Identification and designation of rye chromosomes were the same as those reported in haploid rye by Fujigaki and Tsuchiya (1985).

3. RESULTS

All seven types of the primary trisomics of the inbred rye line were identified cytologically as shown in Figures 1 and 2. The basic banding pattern and the overall karyotype were the same as those of the haploid plants previously reported (Fujigaki and Tsuchiya, 1985), though polymorphism of the banding pattern was detected for the first time.

According to Fujigaki and Tsuchiya (1985), main points for identification of the individual chromosomes of the inbred rye are as follows: Two pairs of metacentric chromosomes (chromosome 1 and 2) are non-satellited and the remaining five pairs (chromosome 3, 4, 5, 6, and 7) are SAT-chromosomes with different sizes of satellites. Moreover, two (chromosomes 4 and 5) of these five pairs have a tertiary constriction consistently in their long arm. In addition to these characteristics detected in the acetocarmine preparations, specific banding patterns were observed after the Giemsa C-banding procedure was applied. Diamond shaped centromeres reported by Singh and Tsuchiya (1982) in barley were also observed in some cells (Fig. 2b).

Chromosome 1 has two prominent bands; one for each terminal end.

Chromosome 2 is the smallest non-satellited chromosome and the terminal bands at both ends are somewhat weak. There are four faint bands; two are in the long arm at the middle and distal portion close to the terminal band; the other two are in the short arm near the centromere and at the middle portion.

Chromosome 3 has two prominent bands; one at the satellite and the other at the terminal end of the long arm. Sometimes small satellites of this chromosome are not clearly observed in acetocarmine-stained preparations. A faint dot is observed close to the centromere on the short arm in some cells.

Chromosome 4 has a prominent band at the satellite and three weakly stained bands in the long arm, one being on the tertiary constriction, another at the terminal end, and the third between the two but closer to the distal end.

Chromosome 5 has a prominent band at the satellite. There is a weak but clear band on the long arm one third of the distance from the terminal end. Another weak band is on the tertiary constriction. There are two faint dots on the long arm, one at the terminal end and the other between the centromere and the

Fig. 1. Karyotypes of three primary trisomies in an inbred rye line (Triplo 1, 2, 3). a, A somatic metaphase cell of Triplo 1 ($2n=15$) stained with acetocarmine, showing three chromosome 1 (arrows). b, The same cell as a, stained with Giemsa-C banding technique after destaining the acetocarmine. c-e, Karyotype arranged in the numerical order of chromosomes 1 through 7 from left to right. The upper rows are chromosomes from acetocarmine stained cells, and the lower rows are Giemsa C-banded chromosomes from the same cell as the upper row. c, Triplo 1; d, Triplo 2; e, Triplo 3. Bar indicates $10\ \mu\text{m}$.

Fig. 2. Karyotypes of four primary trisomies in an inbred rye line (Triplo 4, Triplo 5, Triplo 6, Triplo 7). Karyotypes arranged in the numerical order in the same way as Fig. 1 c-e. a, Triplo 4; b, Triplo 5; c, Triplo 6; d, Triplo 7. Bar indicates 10 μ m.

tertiary constriction.

Chromosome 6 has the second largest satellite, which is heavily stained by Giemsa. There are two weakly stained bands on the long arm.

Chromosome 7 has the largest satellite. There are two prominent heterochromatic bands; one on the satellite and the other at the terminal end of the long arm. There are three weakly stained bands, one located close to the terminal band in the long arm, another in the middle of the long arm, and the other at the distal end of the short arm.

The results shown confirm that the seven morphologically identified primary trisomic lines are independent and carry each of the seven chromosomes of the rye genome.

4. DISCUSSION

The seven rye chromosomes were unquestionably identified and designated in an inbred rye line by a combined acetocarmine-Giemsa banding method in haploid plants (Fujigaki and Tsuchiya, 1985). The triplicated chromosomes in seven morphologically identified primary trisomics in this inbred rye line have been identified and designated based on the previously established chromosomal designation in the haploids (Fujigaki and Tsuchiya, 1985). Therefore, the identification of seven primary trisomic lines has now been morphologically and cytologically confirmed, and a complete set of primary trisomics has been unequivocally established in an inbred line of rye.

The usefulness of this primary trisomic set already has been demonstrated by the morphological study of Fujigaki and Tsuchiya (1988) and the comparison of plant morphology of primary trisomics for corresponding homoeologous chromosomes between rye and barley (Table 1).

However, there are some problems in the identification and designation of rye chromosomes. Rye chromosomes have been investigated and designated by many scientists (Tjio and Levan, 1950; Jain, 1960; Bhattacharyya and Jenkins, 1960; Heneen, 1962; Sybenga and Wolters, 1972; Sarma and Natarajan, 1973; Vosa, 1974; Gill and Kimber, 1974; Hadlaczky and Koczka, 1974; Weimarck, 1975; Darvey and Gustafson, 1975; Singh and Robbelen, 1975; Gustafson et al., 1976; Zeller et al., 1977; de Vries and Sybenga, 1976; Giraldez et al., 1979). Their unique ways of chromosome designation complicated the identification of each chromosome in the complement (Schlegel and Mettin, 1982). There are several reasons for this:

1. There is no numbering system associated with marker genes of linkage groups as in maize, tomato, and barley.
2. Rye chromosomes do not possess as many intercalary C-bands on their arms compared to barley; C-bands of rye are mainly on the terminal ends.
3. Rye species are naturally outcrossing. Most workers, however, have paid

Table 1. Homoeologous relationship inferred from morphological characteristics of primary trisomics of barley and rye

Homoeologous group (wheat)	Barley trisomics	Rye trisomics	Common characteristics in both trisomics
1	Pseudonormal (Triplo 5)	Triplo 7 (SAT)	Nearly normal appearance but slightly short culms, revolute leaves, short and dense spikes
2	Slender (Triplo 2)	Triplo 1	Bushy habit, narrow and slightly yellowish green leaves, thin culms and spikes
3	Pale (Triplo 3)	Triplo 3	Pale green, long drooping leaves, reversely twisted leaf, dense spikes with short awn
4	Robust (Triplo 4)	Triplo 4	Vigorous, long, wide and dark green leaves, slightly lax spikes with large glumes, emaciated seeds
5	Semi-erect (Triplo 7) (SAT)	Triplo 6	Semi-erect habit, short and erect leaves, lax spikes with long glumes and large seeds
6	Purple (Triplo 6) (SAT)	Triplo 5	Thick, wide, long, coarse and dark green leaves, purple color at base of leaf sheath, short spikes with coarse, longer awns
7	Bush (Triplo 1)	Triplo 2	Bushy habit, fused, dark green and short leaves at seedling stage, long glumes

little attention to the heterozygosity of their materials, including chromosome heteromorphism.

In order to standardize rye chromosome nomenclature an international workshop was held at Wageningen, The Netherlands, and adopted 'Imperial' rye additions to 'Chinese Spring' wheat as the standard rye chromosome set (Sybenga, 1983). Once the standard karyotype was accepted, all researchers should comply with the system. However, even after standardization there still seems to be some confusion because the workshop report completely ignored previous results of karyotype analyses based on the conventional method. The karyogram in that report (Sybenga, 1983) showed only the 1R chromosome as a SAT-chromosome. However, five pairs of the rye chromosomes have secondary constrictions with different sizes of the satellites (Levan, 1942; Heneen, 1962; Heneen and Caspersson, 1973) and these were confirmed in the haploid complement (Fujigaki and Tsuchiya, 1985). In addition to the secondary constriction, a tertiary constriction on the long arm (chromosomes 4 and 5 in the present study) is also a useful landmark on these two chromosomes (Bhattacharyya and Jenkins, 1960; Heneen, 1962; Koller and Zeller, 1976; Nakata et al., 1977; Fujigaki and Tsuchiya, 1985). Although there are many claims for reliability of chromosome analysis by conventional methods, these traits of chromosome morphology combined with quantitative measurements might yield reliable results for chromosome identification.

As described above, Giemsa C-bands on rye chromosomes are located mostly on the terminal ends and only rarely on the intercalary segments or even centromere

regions. Rye is originally outcrossing so that most materials are heterozygous and the C-banding pattern is often not uniform on homologous chromosomes between varieties nor within a variety (Verma and Rees, 1974; Gill and Kimber, 1974; Merker, 1973; Singh and Robbelen, 1975; Bennett et al., 1977; Giraldez et al., 1979). Moreover, even in inbred rye lines the Giemsa C-banding polymorphism was detected (Weimarck, 1975; Lelley et al., 1978; and present study). These facts make it difficult to identify individual chromosomes, especially 2R, 3R, and 7R, when one depends solely upon Giemsa C-bands, since these three are almost the same size and have the similar C-banding pattern in the karyogram.

Nakata et al. (1977) developed a sequential staining procedure using acetocarmine and Giemsa C-banding in the same cells. Fujigaki and Tsuchiya (1985) improved this method and accurately identified individual chromosomes in the haploid complement of an inbred rye line. The advantage of this combined staining method is that it compensates for deficiencies of the individual stainings and gives quantitative and qualitative information on the chromosomes from the same cells. This is clearly shown in Figs. 1 and 2. The diamond-shaped structure of the centromere seen after Giemsa C-banding was first observed in rye. This should help identify C-banding chromosomes of rye because C-banding procedures often cause the centromere position to be obscure, hence arm ratios cannot be calculated. Even faint bands detected by the N-banding (Schlegel and Gill, 1984) were also observed in this combined staining method. Therefore, improvement of the present method will enable scientists to detect more clearly not only prominent terminal bands but also minor or faint bands and centromeres.

For accurate chromosome identification in rye, Sybenga and Wolters (1972) developed a translocation tester set and it was chosen as the standard translocation tester set (Sybenga, 1983). de Vries and Sybenga (1976) classified their chromosomes by Giemsa banding; chromosomes VII, II, I, V, VI, IV, and III in the translocation lines being 1R to 7R in order. Zeller et al. (1977) examined 'Imperial' rye addition lines, Sybenga's translocation lines and six types of Heines Hellkorn trisomics, and concluded the relationships as follows: Rye chromosomes 1R to 7R in the addition lines corresponded to VII, I, II, IV, VI, V and III in the Sybenga's translocations, and 1/23, ?, 4/11, 4/9, 1/19, 1/21 and 3/23 of the Heines Hellkorn trisomics, respectively. Schlegel and Mettin (1982), in their comprehensive review, proposed a uniform symbolization system of rye chromosomes. In their review the chromosomes in the translocation lines, VII, II, I, IV, VI, V, and III, were named as 1R, 2R, 3R, 4R, 5R, 6R, and 7R, respectively.

Even though these three papers dealt with the same chromosomes in the translocation lines, they gave rather different chromosome designations. More recently Sybenga et al. (1985) intercrossed the translocation lines to the 'Imperial' rye addition lines and finally concluded based on the pairing data that chromosomes VII, III, II, IV, VI, V, I in the translocation lines being 1R to 7R in order. Melz et al. (1988) identified the extra chromosomes of 'Esto' trisomics cytologically

with the translocation lines and confirmed the result of Sybenga et al. (1985).

Though the chromosome relationship between the translocation testers and the primary trisomics in the present report is under investigation by Fujigaki (unpublished), the rye chromosomes I, II, III, IV, V, VI and VII in the microphotograph of Fig. 2b of de Vries and Sybenga (1976) are in good agreement with our chromosome identification (Fujigaki and Tsuchiya, 1985), 2, 3, 1, 4, 5, 6 and 7, respectively. This means that the extra chromosomes 1, 2, 3, 4, 5, 6, and 7 in the present trisomics should be 2R, 7R, 3R, 4R, 6R, 5R, and 1R, respectively.

Homoeologous relationships to wheat chromosomes were inferred by similarity of the morphological characteristics which were expressed by the primary trisomic plants of barley and rye during their growth (Fujigaki and Tsuchiya, 1986, 1988). The plant morphology corresponded most closely to that of tetrasomics of hexaploid wheat (Sears, 1954) and rye addition lines of wheat (Miller, 1984; Miller and Reader, 1987). The common characteristics in trisomic sets of barley and rye are briefly described in Table 1.

Based on the similarities in the results of chromosome analyses and that of plant morphology mentioned above, we can safely conclude that the homoeologous designation of the extra chromosomes in the present trisomics being as follows: triplo 1=2R, triplo 2=7R, triplo 3=3R, triplo 4=4R, triplo 5=6R, triplo 6=5R, and triplo 7=1R, respectively.

If this is accepted, 7R in the present study is the shortest in the complement and is metacentric with slightly weak bands on both terminal ends. On the other hand, 7R in the standard karyogram (Sybenga, 1983) is the longest with heavy bands on both ends, whereas 3R is the shortest metacentric chromosome. The conflict should be resolved by further investigations. It should not be ruled out the possibility that some segmental differences exist between barley and rye. As noted by Tsuchiya (unpublished) and Tsuchiya and Wang (1989) there is more morphological effect of long-arm telotrisomics than short-arm telotrisomics, so that some segmental changes would not cause obvious phenotypic changes.

The establishment of cytogenetic linkage maps in rye with the use of these trisomics would be another approach to solve the problems in identification and designation of rye chromosomes.

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