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# C-Banding Analyses of *Bromus inermis* Genomes

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# C-Banding Analyses of *Bromus inermis* Genomes

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## ABSTRACT

**Smooth bromegrass (*Bromus inermis* Leyss.) has both tetraploid (2*n* = 28) and octaploid (2*n* = 56) ploidy levels that have been difficult to characterize cytogenetically because of similar chromosome morphology. Objectives of this study were to identify individual chromosomes of tetraploid and octaploid *B. inermis* with C-banding procedures along with chromosome length and arm length ratios, develop more detailed karyotypes than those previously available, and use the karyotypes to examine the genomic relationship of tetraploid and octaploid *B. inermis*. Root tips of the plants from four tetraploid and three octaploid accessions were used to produce chromosome squash preparations for cytogenetic analysis. The tetraploid *B. inermis* genome consisted of 12 chromosomes with a telomeric band on each arm and sixteen chromosomes with only one telomeric band on one arm. All of the chromosomes of the tetraploid form, except for four chromosomes, were identified by C-banding patterns, chromosome length, and arm length ratio. The octaploid *B. inermis* genome consisted of four chromosomes with no C-bands, ≈14 chromosomes with two telomeric bands, and ≈38 chromosomes with only one telomeric band on either the short or long arm. The combined use of C-banding, chromosome size, and arm length ratio only enabled groups of 2, 4, 6, or 8 similar chromosomes to be identified because of similarities in chromosome morphology of the octaploids. Results indicate that tetraploid *B. inermis* is an allotetraploid since all chromosomes except four could be separated into identifiable pairs. Because of differences between expected and actual numbers of satellite chromosomes and chromosomes with specific C-banding patterns, octaploid *B. inermis* is probably not a doubled form of the tetraploid *B. inermis*.**

SMOOTH BROMEGRASS is a polyploid with reported tetraploid (2*n* = 28) (Carnahan and Hill, 1960; Tan and Dunn, 1977; Armstrong, 1987), hexaploid (2*n* = 42) (Stahlin, 1929; Knobloch, 1943; Tan and Dunn, 1977), and octaploid (2*n* = 56) (Avdulov, 1931; Knobloch, 1943; Hill and Myers, 1948; Tan and Dunn, 1977; Armstrong, 1987) ploidy levels. However, Tuna et al. (2001b) reported mostly octaploid, few tetraploid, and no hexaploid plants in a recent survey of >255 *B. inermis* accessions in the USDA National Plant Germplasm System. In North America, only the octaploid is used in agriculture. To date, only two genomes, A and B, which are believed to be closely related, have been recognized in bromegrasses (Armstrong, 1991).

Octaploid *B. inermis* behaves as an autoallooctaploid, forming predominantly quadrivalents and bivalents at meiosis (Elliott and Love, 1948; Armstrong 1973, 1980). Tetraploid *B. inermis* behaves as an allotetraploid since it forms predominantly bivalents at meiosis (Elliott and Wilsie, 1948; Armstrong, 1980). Pairing behavior in the hybrids of tetraploid and octaploid cytotypes indicated homology between the genomes and suggested genomic formulas AABB and AAAABBBB for the tetraploid and octaploid, respectively (Hill and Carnahan, 1957; Armstrong, 1980). However, karyotypic evidence in *B. inermis* does not support the results of these genetic and chromosome pairing studies since octaploid *B. inermis* has been reported to contain two pairs or sets of homologous chromosomes with large satellites and only one pair with small satellites (Ghosh and Knowles, 1964; Wilton, 1965; Armstrong, 1973). Armstrong (1980) later reported that tetraploid *B. inermis* had one pair of chromosomes with a large satellite and one pair with small satellites. Armstrong (1980) observed two pairs of chromosomes with large satellites and two pairs of chromosomes with small satellites in colchicine-induced octaploid *B. inermis* produced from tetraploid *B. inermis*. Two pairs of small satellites might be expected in natural octaploid *B. inermis*, but since they were not found it has been speculated that one of the genomes differentiated and *B. inermis* should be AAAABBCC (Ghosh and Knowles, 1964; Armstrong, 1977).

Rychlewski (1970) did not find small satellites in octaploid *B. inermis* from Poland, which suggests that the species may be polymorphic for these satellites (Armstrong, 1981). On the basis of chromosome size which ranged between 3.5 and 7.5 μm, Rychlewski (1970) grouped the chromosomes of the octaploid *B. inermis* into putative pairs. Armstrong (1977) reported karyotypes for *B. inermis* using the karyotype of 4*x* cytotype of *B. erectus* Hudson as a model for the A genome. Karyotypes were constructed from *B. inermis* and interspecific hybrids from *B. erectus* (2*n* = 28) × *B. inermis* (2*n* = 56) and *B. arvensis* L. (2*n* = 14) × *B. inermis* (2*n* = 56). The A genome consisted of one chromosome with a large satellite, one submetacentric and five metacentric chromosomes. The B genome was characterized by two submetacentric chromosomes; one of which had a small satellite and five median chromosomes. Armstrong (1977) stated that gross chromosome morphology in *B. inermis*, particularly in the interspecific hybrids, provides no evidence for allooctaploidy. Rychlewski (1970), however, concluded that the karyotype of *B. inermis* had features consistent with an allooctoploid condition.

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**Abbreviations:** GISH, genomic in situ hybridization; NOR, nucleolus organizer region.

Both Rychlewski (1970) and Armstrong (1977) reported difficulties in karyotyping octaploid *B. inermis* using the feulgen staining method due to the large number of chromosomes, small morphological differences between the chromosomes and variability from cell to cell for chromosome length and arm length ratio.

Giemsa C-banding technique, which stains constitutive heterochromatin, is a powerful technique that can be used to identify individual chromosomes and has been successfully used to establish genomic relationships among several species including *Allium*, *Medicago*, *Cicer*, and Triticeae species (Vosa, 1975; Cai and Chinnappa, 1987; Gill and Sears, 1988; Tayyar et al., 1994; Falistocco et al., 1995; Bauchan and Hossain, 1999). In a previous report, we described the utility of C-banding in identifying chromosomes of diploid *B. riparius* Rehm ($2n = 14$) (Tuna et al., 2001a).

Objectives of this study were to identify individual chromosomes of tetraploid and octaploid *B. inermis* with C-banding procedures and chromosome length and arm length ratio; to develop more detailed karyotypes than those previously available; and to use the karyotypes to examine the genomic relationship of tetraploid and octaploid *B. inermis*.

## MATERIALS AND METHODS

Plants from four tetraploid (PI 440202, PI 440203, PI 440204, and PI 499401) and two octaploid *B. inermis* (PI 574512 and 574514) accessions, and the widely cultivated octaploid cultivar 'Lincoln' were used in this study. Seeds of Lincoln bromegrass were obtained from the USDA Forage Research Laboratory at Lincoln, NE, while seeds of tetraploid and octaploid accessions were obtained from the USDA Regional Plant Introduction Station, Pullman, WA.

Techniques for chromosome squash preparations and C-banding are described by Tuna et al. (2001a) and are summarized as follows. Actively growing root tips were collected from potted plants 3 to 4 wk after plants were clipped or from seedling roots from seeds which were germinated in germination boxes containing germination paper saturated with distilled water. For the latter, imbibed seeds were kept at room temperature for 1 d before they were transferred to a refrigerator at 0 to 4°C for one to a few days (until majority of seeds appeared to be germinating). Boxes were then placed in the dark at room temperature and fast growing root tips were collected when they reached 1 to 1.5 cm in length. Harvested root tips from potted plants or germinated seeds were placed in vials containing 0.05% colchicine (w/v). Colchicine was drained from the vials after 1 to 1.5 h and replaced in ethanol and glacial acetic acid (3:1, v/v) for two weeks to a few months. The C-banding method used was that described by Giraldez et al. (1979) and slightly modified as follows. Root tips were stained with 1% acetocarmine (1 g carmine per 100 mL of 45% acetic acid) for about 30 min before making preparations by the squash technique. Slides were examined under microscope and those which exhibited a relatively high mitotic index were quickly frozen by placing them in a −80°C freezer for at least 2 h. Cover slips were then removed and slides were placed in 95% ethanol overnight at room temperature. After dehydration, slides were air dried at room temperature for 3 to 4 h. Subsequently, the dried slides were incubated for 3 min in 0.2 N HCl at 60°C in a water bath and washed briefly in distilled water. Slides were placed in saturated $Ba(OH)_2$ solution at room temperature for 8 min. Slides were washed carefully in distilled water until all the barium crystals were removed. Slides were then placed in 2 × SSC solution (0.3 *M* NaCl plus 0.03 *M* citric acid) at 60°C for 1 h before transferring them to the staining solution containing 4% solution of Giemsa stain (v/v) in phosphate buffer for 12 min. Phosphate buffer was comprised of 62% 0.07 *M* $Na_2HPO_4$ and 38% 0.07 *M* $KH_2PO_4$ solutions. After staining, slides were quickly rinsed in distilled water and dried for several hours. For observations, slides were mounted in Permount.

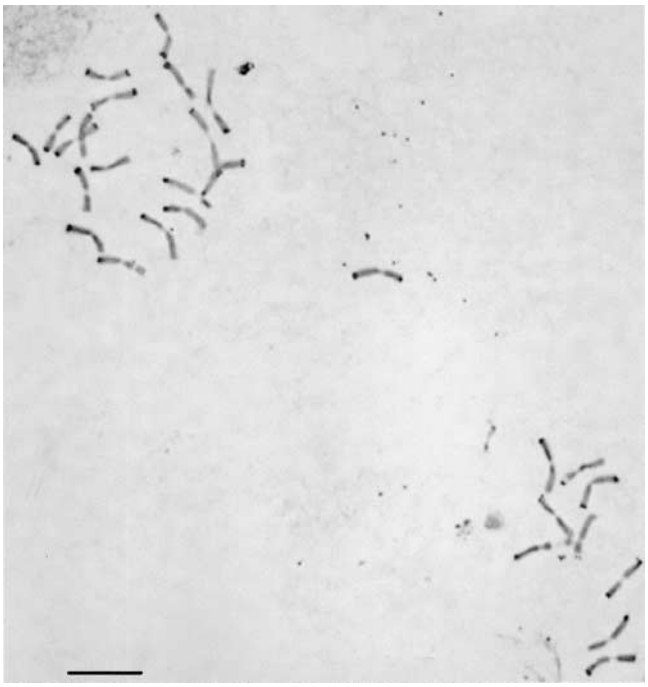

**Fig. 1. C-banded mitotic metaphase chromosomes of the tetraploid *Bromus inermis* ($2n = 28$). Scale Bar is 10 μm in length.**

Cells with well-spread chromosomes were identified and an image of the each cell was captured by a Spot I digital camera (Diagnostic Instruments Inc., Sterling Heights, MI). Printed enlarged pictures (3000 ×) of 10 cells from six different plants of the four tetraploid accessions were used for analysis and to construct the tetraploid karyotype. Printed enlarged pictures (3000 ×) of six cells from four different plants of Lincoln bromegrass and the two octaploid accessions plus additional cells from these which were studied under the microscope were used for analysis and construction of an octaploid karyotype.

Chromosome measurements were made on the enlarged prints and converted to microns by relating measurements from enlarged prints with measurements made in a microscope with a micrometer. The chromosomes were identified on the basis of their total length, arm length ratio, C-banding patterns, and presence or absence of satellites (secondary constrictions).

## RESULTS

### Karyotype and C-Banding Patterns in Tetraploid *Bromus inermis*

C-banding patterns were found on specific chromosomes of both the tetraploid and octaploid forms of *B. inermis*. The genome of tetraploid *B. inermis* consisted of 18 metacentric (Groups I, II, III, IV, V, VI, VII, VIII, XI), six submetacentric (Groups IX, X), and four satellite (Groups XII, XIII) chromosomes (Fig. 1, 2).

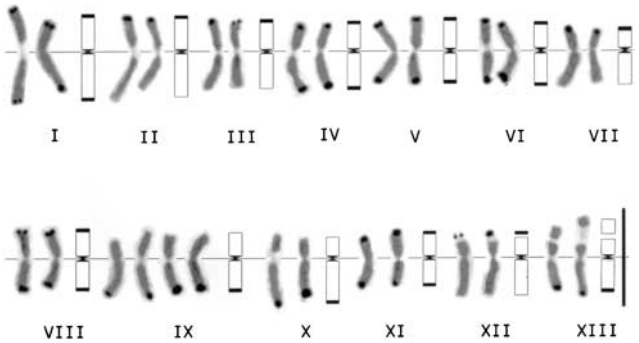


**Fig. 2. Karyotype of tetraploid *Bromus inermis* (PI 440204) based on C-banding and chromosome morphology. Scale Bar is 10 μm in length.**

The arm length ratio varied from 1.73 to 1.06 (Table 1). The total haploid genome length of tetraploid *B. inermis* was determined as 85.81 μm and contained 5.87 pg per 1 C DNA (Tuna et al., 2001b), which is ≈6000 Mb.

Constitutive heterochromatin was located only at telomeric regions in tetraploid *B. inermis*, and all the chromosomes had major C-bands (Fig. 1, 2). Six pairs of chromosomes had telomeric bands on both arms and eight pairs had a telomeric band on only one arm. Occasionally, chromosomes with a large satellite had a C-band at the nucleolus organizer region (NOR) site of one or both chromosomes depending on the cell analyzed. Except for four of the chromosomes, it was possible to identify all homologous chromosomes by chromosome lengths, arm length ratios, and C-banding patterns. Chromosomes of the tetraploid *B. inermis* were divided into two main classes based on their C-banding patterns, one with telomeric bands on both arms and the other with telomeric bands on one arm.

One class consisted of six groups of chromosomes (I, IV, V, VI, VIII, XI) with telomeric bands on both arms (Fig. 1, 2). It was possible to separate chromosomes of this class into groups based on differences in chromosome length and arm length ratios (Table 1). Chromosomes of Group I were the longest within the karyotype (Fig. 2, Table 1) and arm length ratio of the group differed from that of Group IV. Chromosomes of Group IV had the highest arm length ratio in this class, allowing the chromosomes of the group to be differentiated from chromosomes of the other groups with two telomeric bands. Arm length ratios of chromosomes of Group V were similar to those of the chromosomes of Group XI, but chromosomes of Group XI were shorter in length. Chromosomes of Group XI can be differentiated from chromosomes of Groups VI and VIII by arm length ratio and chromosome length (Table 1). Chromosomes of Groups VI and VIII had the same arm length ratio but chromosomes of Group VI were 0.3 μm longer. Chromosomes of Group XI were unique because of their short length and their arm length ratio.

The second class of tetraploid chromosomes had the single telomeric band on either the short or long arm of the chromosomes. Chromosomes of Groups IX, X, and XIII had a telomeric band only on the long arm. Chromosomes of Group X, which had the highest arm length ratio within the karyotype, can be distinguished from chromosomes of Group IX by size and arm length ratio (Fig. 2, Table 1). Group IX was the only chromosome group within the karyotype that consists of four similar chromosomes in terms of banding patterns, size, and arm length ratio. Chromosomes of Group XIII could be identified from Group IX and X chromosomes by the large satellite on the short arm.

Chromosome Groups II, III, VII, and XII had a telomeric band on their short arm (Fig. 2, Table 1). Chromosomes of Group XII were easily identifiable since they had a small satellite on their short arm and a higher arm length ratio than the other three groups. Group III had the lowest arm length ratio in comparison with Groups II and VII. Chromosomes of Group II are the longest while those of Group VII are the shortest among groups with a telomeric band on their short arm (Fig. 2, Table 1).

## Karyotype and C-Banding Patterns in Octaploid *Bromus inermis*

Chromosomes of the octaploid *B. inermis* could be grouped into 14 different chromosome groups based on C-banding, chromosome length, and arm length ratio

**Table 1. The chromosomes of tetraploid *Bromus inermis* Leyss.**

| Group | Long arm mean ± SD | Short arm mean ± SD | Total length mean ± SD | Satellite size mean ± SD | Arm ratio† mean ± SD | Chromosome type | C-banding arm location |
|---|---|---|---|---|---|---|---|
| | μm | | | | | | |
| I | 3.66 ± 0.77 | 3.31 ± 0.68 | 6.97 ± 1.45 | | 1.09 ± 0.05 | median‡ | both |
| II | 3.70 ± 0.74 | 3.12 ± 0.71 | 6.82 ± 1.45 | | 1.18 ± 0.06 | median | short |
| III | 3.37 ± 0.73 | 3.08 ± 0.76 | 6.46 ± 1.48 | | 1.09 ± 0.07 | median | short |
| IV | 3.34 ± 0.79 | 2.72 ± 0.59 | 6.37 ± 1.31 | | 1.35 ± 0.13 | median | both |
| V | 3.26 ± 0.62 | 3.03 ± 0.63 | 6.30 ± 1.25 | | 1.07 ± 0.05 | median | both |
| VI | 3.31 ± 0.59 | 2.78 ± 0.53 | 6.10 ± 1.11 | | 1.19 ± 0.08 | median | both |
| VII | 3.43 ± 0.83 | 2.63 ± 0.66 | 6.07 ± 1.55 | | 1.34 ± 0.27 | median | short |
| VIII | 3.03 ± 0.59 | 2.75 ± 0.47 | 5.79 ± 1.05 | | 1.19 ± 0.12 | median | both |
| IX | 3.49 ± 0.87 | 2.27 ± 0.46 | 5.70 ± 1.11 | | 1.54 ± 0.25 | submedian§ | long |
| X | 3.59 ± 0.84 | 1.84 ± 0.27 | 5.43 ± 1.09 | | 1.73 ± 0.30 | submedian | long |
| XI | 2.69 ± 0.37 | 2.55 ± 0.36 | 5.24 ± 0.73 | | 1.06 ± 0.06 | median | both |
| XII | 3.73 ± 0.88 | 2.41 ± 0.69 | 6.20 ± 1.49 | 0.54 ± 0.10 | 1.57 ± 0.29 | satellite | short |
| XIII | 3.37 ± 0.74 | 1.81 ± 0.46 | 6.65 ± 1.39 | 1.47 ± 0.43 | 1.10 ± 0.08 | satellite | long |

† Arm ratio = length of the long arm/length of the short arm.
‡ Median = arm ratio is <1.50.
§ Submedian = arm ratio is >1.50.

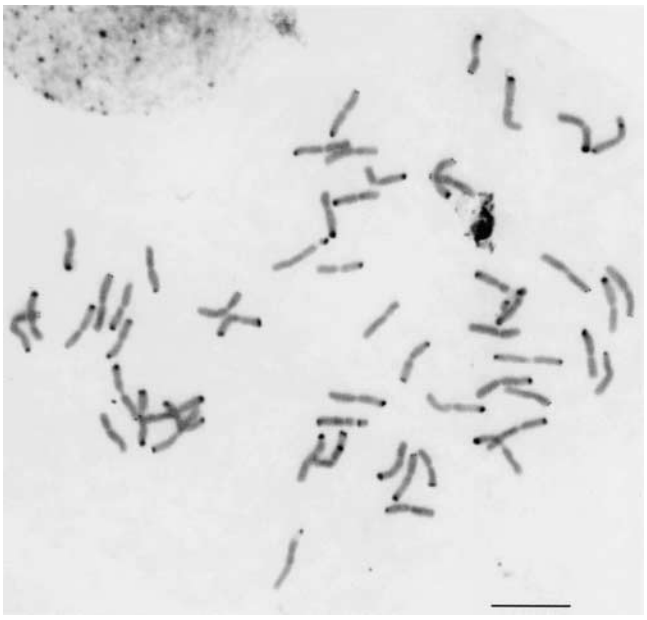

**Fig. 3. C-banded mitotic metaphase chromosomes of octaploid *Bromus inermis* L. (2*n* = 56, Lincoln bromegrass). Scale Bar is 10 μm in length.**

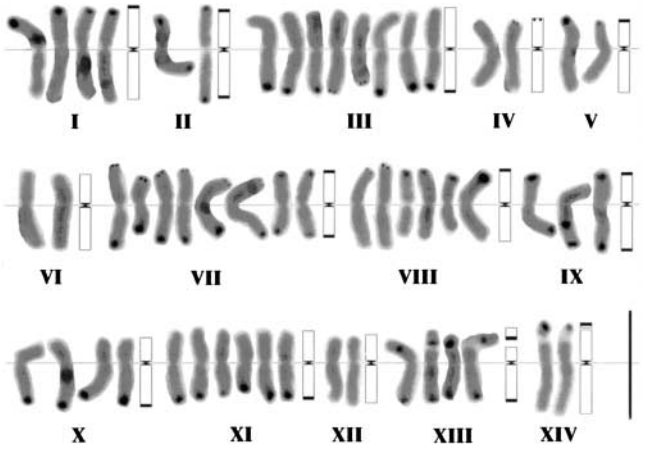


**Fig. 4. Karyotype of octaploid *Bromus inermis* L. (Lincoln bromegrass) based on C-banding and chromosome morphology. Scale Bar is 10 μm in length.**

(Fig. 3, 4; Table 2). The genome of the octaploid *B. inermis* consisted of 42 metacentric chromosomes (Groups I, II, III, V, VI, VII, VIII, IX, XI), eight submetacentric chromosomes (Groups IV, X, XII) and six chromosomes with satellites including two pair with large satellites and one pair with small satellites. A number of metacentric chromosomes could be classified as submetacentric since their arm length ratio was very close to the minimum arm length ratio of 1.5 for submetacentric chromosomes. Although satellite chromosomes could be identified, we were able to clearly observe all of the satellite chromosomes together only in a limited number of cells. Because of disagreement in the literature on the number of satellite chromosomes in octaploid *B. inermis* and their importance in determining genomic relationships, one of these cells was chosen to illustrate the karyotype in this study even though one chromosome (IX) is missing (Fig. 3, 4). Chromosomes varied in length from 6.78 to 5.28 μm while the arm length ratio varied from 1.90 to 1.11 (Table 2). The total haploid genome length of octaploid *B. inermis* was determined as 164.84 μm and contains 11.15 pg per 1C DNA (Tuna et al., 2001b) which is approximately 11 000 Mb.

Constitutive heterochromatin was located only at telomeric regions in octaploid *B. inermis*. Most of the chromosomes had major telomeric C-bands while others had faint telomeric bands and a few of the chromosomes had no bands at all (Fig. 3, 4). Approximately 38 chromosomes had a telomeric band on only one arm. Approximately 14 chromosomes had telomeric bands on both arms while four chromosomes had no bands. One of the chromosomes in Group IX (Fig. 4, Group IX, center chromosome) had an unusual interstitial band which was observed in only one plant of the several hundred evaluated. Meiotic studies will be needed to confirm if it is due to a chromosomal rearrangement. In the octaploids, chromosomes with large satellites usually had a C-band at the NOR site of one or both chromosomes depending on the cell analyzed.

In octaploid *B. inermis*, chromosomes could be separated into three main classes based on their C-banding patterns. The classes consisted of chromosomes with

**Table 2. The chromosomes of octaploid *Bromus inermis* Leyss.**

| Group | Long arm mean ± SD | Short arm mean ± SD | Total length mean ± SD | Satellite size mean ± SD | Arm ratio† mean ± SD | Chromosome type | C-banding arm location |
|---|---|---|---|---|---|---|---|
| | μm | | | | | | |
| I | 3.62 ± 0.25 | 3.14 ± 0.33 | 6.78 ± 5.19 | | 1.16 ± 0.10 | median‡ | short |
| II | 3.46 ± 0.47 | 2.89 ± 0.39 | 6.38 ± 0.78 | | 1.19 ± 0.12 | median | both |
| III | 3.36 ± 0.31 | 2.91 ± 0.30 | 6.29 ± 0.54 | | 1.15 ± 0.10 | median | long |
| IV | 3.80 ± 0.30 | 2.00 ± 0.19 | 5.81 ± 0.43 | | 1.90 ± 0.16 | submedian§ | short |
| V | 3.47 ± 0.63 | 2.32 ± 0.35 | 5.80 ± 0.95 | | 1.48 ± 0.15 | median | short |
| VI | 3.41 ± 0.26 | 2.36 ± 0.23 | 5.77 ± 0.46 | | 1.44 ± 0.09 | median | none |
| VII | 3.01 ± 0.59 | 2.69 ± 0.52 | 5.70 ± 1.07 | | 1.11 ± 0.09 | median | both |
| VIII | 2.99 ± 0.31 | 2.65 ± 0.24 | 5.65 ± 0.47 | | 1.13 ± 0.09 | median | short |
| IX | 3.33 ± 0.45 | 2.26 ± 0.31 | 5.60 ± 0.66 | | 1.48 ± 0.21 | median | both |
| X | 3.49 ± 0.35 | 2.01 ± 0.18 | 5.52 ± 0.49 | | 1.73 ± 0.13 | submedian | long |
| XI | 2.90 ± 0.35 | 2.44 ± 0.19 | 5.36 ± 0.52 | | 1.18 ± 0.12 | median | long |
| XII | 3.32 ± 0.24 | 1.94 ± 0.24 | 5.28 ± 0.48 | | 1.71 ± 0.15 | submedian | none |
| XIII | 3.22 ± 0.40 | 1.47 ± 0.19 | 6.11 ± 0.82 | 1.40 ± 0.26 | 1.12 ± 0.07 | satellite | long, NOR region |
| XIV | 3.55 ± 0.65 | 2.33 ± 0.51 | 6.17 ± 1.19 | 0.80 ± 0.18 | 1.36 ± 0.19 | satellite | short |

† Arm ratio = length of the long arm/length of the short arm.
‡ Median = arm ratio is <1.50.
§ Submedian = arm ratio is >1.50.

telomeric bands on both arms, chromosomes with telomeric bands on one arm, and chromosomes without any C-bands (Fig. 3, 4; Table 2).

Chromosome Groups II, VII, and IX had telomeric bands on both arms (Fig. 3, 4). Chromosomes of Group IX had the largest arm length ratio in comparison with chromosomes of Groups II and VII (Table 2). Chromosomes of Group II were longer than those of Groups VII and IX.

Four groups of chromosomes (III, X, XI, XIII) had a telomeric band on their long arm (Fig. 3, 4). Of these four groups, chromosomes of Group X had the second highest arm length ratio (1.73) within the karyotype (Table 2). Chromosomes of Group XIII had a large satellite on the short arm. Chromosomes of Group III and XI were distinguishable since chromosomes in Group III were longer in length than the chromosomes in Group XI (Table 2).

Five groups of chromosomes (Groups I, IV, V, VIII, XIV) had telomeric bands on their short arm (Fig. 3, 4). Chromosomes of Group IV were the most easily identifiable since they had the highest arm length ratio in the karyotype while chromosomes of Group XIV had a small satellite on their short arm (Table 2, Fig. 4). Chromosomes of Group V were shorter in length and had a larger arm length ratio than chromosomes of Group XIV (Table 2). Chromosomes of Group V and XIV may not be distinguished if the small satellite is not visible on the short arm of chromosomes in Group XIV. Chromosomes of Group V could be distinguished from Group VIII by arm length ratio (Table 2). Chromosomes of Group V could be distinguished from chromosomes of Group I due to shorter chromosome length and a higher arm length ratio (Table 2). Group I had the longest chromosomes in the karyotype and could be differentiated from others by size.

The third class consisted of two groups of chromosomes (IV and VI) with no C-bands (Fig. 3, 4). These chromosome groups were easily differentiated from each other on the basis of chromosome length and arm length ratios (Table 2).

## DISCUSSION

Armstrong (1980) reported a karyotype based on Feulgen staining for tetraploid *B. inermis*. He separated the chromosomes of the tetraploid *B. inermis* into 10 pairs of metacentric chromosomes, two pairs of submetacentric chromosomes and two pairs of satellite chromosomes (one pair large and one pair small satellite). The karyotype that is reported in this study is generally in agreement with Armstrong's karyotype but is more detailed because of C-banding which enabled individual chromosomes of tetraploid *B. inermis* to be identified. Unfortunately, it was not possible to make an exact comparison between the two karyotypes since the karyotype in the previous report was presented without any scale. Relative comparison between previous and present results were made by making measurements on the chromosomes shown in the Armstrong's (1980) karyotype. The arm length ratio estimated from Armstrong's karyotype does not appear to exceed 1.62 in any chromosome and in the second smallest chromosome pair it seems to be the highest. The chromosome with the highest arm length ratio (1.73) was the second smallest chromosome in the present karyotype, which is in agreement with Armstrong's results. In both studies, the smallest and largest chromosome pairs had approximately the lowest arm length ratio. The average arm length ratio of the Armstrong's karyotype was 1.25 compared with 1.28 in this study. These results indicate that these two karyotypes are largely in agreement for observations other than C-bands.

Although we observed two pairs of satellite chromosomes, one pair with a large satellite and the other with a small satellite, it was difficult to consistently identify both of the chromosomes with small satellites. They were usually observed as submetacentric chromosomes with a telomeric C-band on the short arm. Sometimes the telomeric band was extremely faint in one chromosome or both. Similar difficulties were encountered by Armstrong (1980) in documenting the small satellites in karyotypes of *B. inermis*.

The present study demonstrated that the C-banding technique is effective in identifying individual chromosomes and pairing homologs in tetraploid *B. inermis*. Furthermore, the results of this study support the previous research that indicates tetraploid *B. inermis* is an allotetraploid since all chromosomes but four chromosomes were arranged into groups of two. If tetraploid *B. inermis* was an autopolyploid, more groups containing four chromosomes would be expected.

The karyotype of octaploid *B. inermis* was difficult to analyze. Small morphological differences among chromosome pairs, individual variability of arm length and chromosome size, and banding pattern similarity made an exact designation and arrangement of all homologous pairs impossible. Chromosomes were more similar in cells with highly contracted chromosomes. Therefore, only cells with less contracted chromosomes were used in karyotype analysis. Some chromosome groups in the karyotype contain more than four, six, or eight chromosomes since it was not possible to separate them due to their morphological similarity. However, by adding C-banding patterns, chromosome differences were better defined.

Previous karyotypes of octaploid *B. inermis* based on Feulgen staining (Rychlewski, 1970; Armstrong, 1977) and our karyotype are generally in agreement. Most of the chromosomes of octaploid *B. inermis* are metacentric and only a few of the chromosomes are clearly submetacentric. Our karyotype supports the previous karyotype reported by Armstrong (1977) on the number of satellite chromosomes. Both karyotypes have four large and two small satellite chromosomes. In contrast, Rychlewski (1970) found only four large and no small satellites in octaploid *B. inermis* plants from Poland and Romania. The presence of two small satellites has been reported in other studies of *B. inermis* (Schulz-Schaeffer, 1960; Ghosh and Knowles, 1964; Wilton, 1965; Armstrong, 1973). Armstrong (1981) suggested that *B. inermis* could be polymorphic for small satellites.

Armstrong (1977) did not report the actual length and arm length ratios of chromosomes while Rychlewski (1970) reported the size and arm length ratio of the octaploid *B. inermis* chromosomes as 7.50 to 3.25 μm and 3 to 1, respectively. The length and arm length ratio varied between 6.78 and 5.28 μm and 1.90 to 1.11, respectively, in this study. Differences in length and arm ratio between the two studies could be due to different slide preparation and chromosome measurement methods.

The karyotype of octaploid *B. inermis* presented in this study suggests that at least one of the genomes is disomic since there are groups with only two chromosomes including a group with small satellites. Groups of four, including one with four chromosomes with a large satellite, may indicate the similarity between at least two genomes of the octaploid *B. inermis*.

The octaploid *B. inermis* karyotype has only two small satellite chromosomes, while the expected number of chromosomes with a small satellite would be four if the octaploid was a result of the doubling of the tetraploid genomes since tetraploid *B. inermis* had two small satellites. This evidence suggests diffentiation within the B genomes as suggested by Armstrong (1977). The C-banded karyotype of tetraploid *B. inermis* contains 12 chromosomes with two telomeric bands and all chromosomes possessing major telomeric bands (Fig. 2). However, the C-banded karyotype of octaploid *B. inermis* contained only 14 chromosomes with two telomeric bands while the expected number of chromosomes with two telomeric band would be twice that of the tetraploid form (Fig. 4). Four chromosomes of the octaploid *B. inermis* did not have major C-bands and some chromosomes had only faint telomeric bands (Fig 4). Different C-banding patterns of tetraploid and octaploid *B. inermis* support the idea of differentiation at least in one of the genomes of octaploid *B. inermis*. Our results do not support the AAAABBBB genomic structure of octaploid *B. inermis* as suggested by Hill and Carnahan (1957) and Armstrong (1980). Additional information is needed on C-banded karyotypes of other related *Bromus* species and GISH (genomic in situ hybridization) before more definite conclusions can be reached. The GISH hybridization between diploid and polyploidy *Bromus* species may be a useful method of discerning parental genomes.

Previous hybridization and chromosome pairing studies suggest that diploid *B. variagatus* and tetraploid *B. erectus* are the progenitor of the A genome while the origin of the B genome is still unknown (Walton, 1980; Armstrong, 1991). Armstrong (1987) suggested that diploid *B. riparius* (PI 440215) collected from Chimkent in Kazakhstan could be a progenitor of the *B. inermis* complex since its morphology resembles that of the tetraploid *B. inermis* collected from the same region. On the basis of only C-banded karyotypes of the diploid *B. riparius* (Tuna et al., 2001a) and polyploid *B. inermis* (Fig 2 and 4), it is unlikely that diploid *B. riparius* is a progenitor of polyploid *B. inermis*, although chromosome morphology is quite similar. One important difference between the karyotypes is the lack of chromosomes with an interstitial band in the karyotypes of polyploid *B. inermis* while the diploid *B. riparius* karyotype contains one pair of chromosomes with a large interstitial band. The other difference is the location of the telomeric band on the satellite chromosomes, although both had large satellite chromosomes with similar morphology. The satellite chromosome of the diploid *B. riparius* had a telomeric band on the satellite arm while the satellite chromosome of the polyploid *B. inermis* had a telomeric band in the other arm of the chromosome. The GISH analysis and chromosome pairing studies in hybrids between diploid *B. riparius* and polyploid *B. inermis* are needed to make a final conclusion.

It was not possible to identify all the chromosomes of polyploid bromegrasses, especially the ones with higher ploidy levels (octaploid) and separate the chromosomes into genomes based on C-banding patterns and chromosome morphology. However, it may be possible to separate chromosomes of polyploid *B. inermis* into genomes by comparing the present karyotype with the C-banded karyotypes of A genome progenitors (*B. variagatus*, $2n = 14$ or *B. erectus*, $2n = 28$). Additional measures have to be taken, including FISH (fluorescence in situ hybridization) and GISH to identify chromosomes accurately and separate them into genomes. However, combining C-banding and chromosome morphology made it possible to develop karyotypes that are more informative than previous karyotypes of *B. inermis*. Therefore, the C-banding technique should be useful for studying species relationships in the genus *Bromus* when C-banded karyotypes are prepared for other species, including diploids. C-banding analysis of other related species, including another widely cultivated decaploid species *B. riparius*, are in progress.

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