

plants that were aneuploid ($2n = 17$). C-banding analysis of root-tip cells showed that the extra chromosomes were highly heterochromatic B chromosomes. The Giemsa banding pattern of the A-chromosome complement in the aneuploid cells was not altered from the banding patterns of normal cells.

The occurrence of B chromosomes has been reported in many plants and animals (Jones and Rees 1982). They are different from normal or A chromosomes and are also termed supernumerary or accessory chromosomes. B chromosomes typically have the following characteristics: they are usually smaller than A chromosomes and are generally heterochromatic; they normally do not influence the viability and phenotype of the organism; they vary between different cells, tissues, individuals, and populations; they do not pair with A chromosomes; and they affect mitotic behavior by lagging and elimination, polyploidy, or preferential distribution (Rieger et al. 1991).

During a routine cytogenetic investigation of diploid *M. sativa* ssp. *falcata* (L.) Arcangeli accessions from the U.S. National Plant Germplasm System, we discovered two accessions in which three plants each were aneuploid with $2n = 17$. Masoud et al. (1991) showed that it was pos-

sible to detect heterochromatin of alfalfa using the C-banding technique. The purpose of this study was to determine the nature of the extra chromosome using C-banding.

Materials and Methods

Seeds of 13 accessions of diploid *M. sativa* ssp. *falcata* (Table 1) were obtained from the U.S. National Plant Germplasm System, Pullman, Washington. One accession, UAG 1806, was obtained from the Karl Lesins collection (E. Small, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada). Two accessions, PI 115365 and PI 486207, contained aneuploid plants ($2n = 17$). Both accessions originated in Russia and were originally obtained from the Vavilov Institute, St. Petersburg, Russia.

Root tips were obtained 3 days after germination was initiated, pretreated in an ice bath for 20 h, and fixed in Farmer's fixative (3:1, v/v, 95% ethanol : glacial acetic acid) for at least 30 min. The C-banding procedure of Bauchan and Hossain (1997) for alfalfa was followed. Thirty-five seedlings per accession were studied and a minimum of 50 cells were observed from each seedling. Photomicrographs were taken using a Zeiss Axiophot Microscope using Kodak Technical Pan 2415 film. The photomicrograph was obtained through

Identification of B Chromosomes Using Giemsa Banding in *Medicago*

M. A. Hossain and G. R. Bauchan

This is the first verified report of the existence of B chromosomes in the genus *Medicago*. During a routine cytogenetic investigation of diploid *M. sativa* ssp. *falcata* (L.) Arcangeli accessions obtained from the U.S. National Plant Germplasm System, we discovered two accessions, PI 115365 and PI 486207, each with three

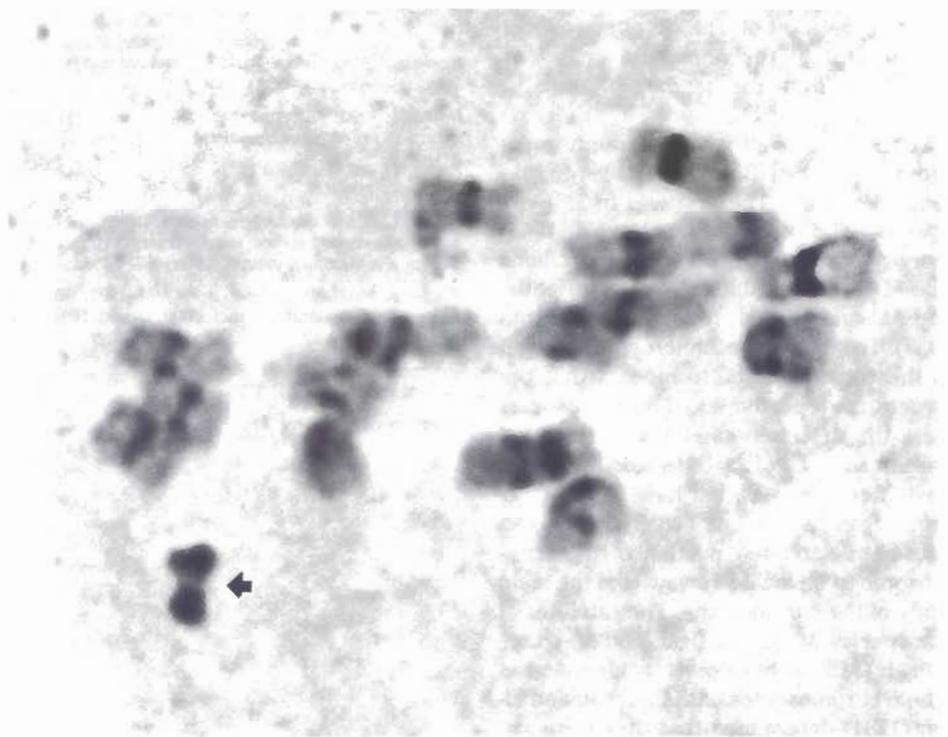


Figure 1. The normally C-banded A chromosomes from PI 486207 plus the highly heterochromatic B chromosome (arrow).

Table 1. List of accessions observed and the country where the accession was collected

Accession number	Country of origin
PI 115365 ^a	Russia
PI 262532	Israel
PI 263154	Russia
PI 307398	Sweden
PI 405064	USA
PI 440527	Russia
PI 464727	Turkey
PI 464728	Turkey
PI 467970	USA
PI 486207	Russia
PI 494662	Romania
PI 577557	Bulgaria
UAG 1806 ^b	Canada

^a The plant introductions (PI) were obtained from the U.S. Plant Introduction Station in Pullman, Washington.

^b UAG 1806 provided by E. Small from the Karl Lesins Collection, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada.

the use of a computerized image analysis system as described by Bauchan and Campbell (1994).

Results and Discussion

Three individual seedlings from two different accessions contained a B chromosome. The interphase nucleus of a normal C-banded cell contains small darkly stained heterochromatin blocks located on either side of the centromere. In the aneuploid plant a single, condensed heterochromatic structure in addition to normal centromeric blocks of heterochromatin was detected (Figure 1). The Giemsa banding pattern of the A chromosome complement in the aneuploid cells was not altered from the banding patterns in normal cells.

There have been two apparently erroneous reports of B chromosomes in the genus *Medicago*. The first was in *M. grandensis* Willd. in plate 1 in Heyn's (1963) monograph. The second was in *M. ciliaris* All., *M. intertexta* Mill., *M. littoralis* Rohde, and *M. murex* Willd. (Agarwal and Gupta 1983). From the photomicrographs given in these two reports, it appears that the structures reported as B chromosomes were the detached satellites of satellited chromosomes. Many *Medicago* species have chromosomes that have large nucleolus organizer regions (NOR) which can cause the satellited portion of the chromosome to be located away from the main body of the chromosome. The satellites of the species *M. rugosa* Desr. and *M. scutellata* (L.) Mill. were incorrectly identified as whole chromosomes until Bauchan and Elgin (1984) determined that they were satellites and thus corrected the chromosome number for these species to $2n = 30$.

In conclusion, this is the first report of the existence of a B chromosome in the genus *Medicago*. We are uncertain about the origin of the B chromosomes as the A-chromosome complement does not appear to have a reduction in DNA content as observed by the normal C-banding pattern of the A chromosomes. We isolated two plants, one from each accession, from a total of 500 seedlings possessing a B chromosome. However, both of the plants died before flowering, thus we have not been able to study meiosis in these plants. We are unsure if the plants died due to genetic load, environmental stresses, or both.

From the U.S. Department of Agriculture, Agricultural Research Service, Plant Sciences Institute, Soybean and Alfalfa Research Laboratory, Beltsville, MD 20705-2350 (Bauchan) and the University of Maryland, Natural Resources and Landscape Architecture Department, College Park, Maryland (Hossain). Mention of a trademark or proprietary product does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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Corresponding Editor: Prem Jauhar