IDENTIFICATION OF ALFALFA CHROMOSOMES USING GIEMSA BANDING AND IMAGE ANALYSIS TECHNIQUES

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ABSTRACT

Chromosomes of two diploid (2n=2x=16) subspecies of *Medicago sativa*, ssp. *caerulea* and ssp. *falcata*, their hybrid and tetraploid (2n=4x=32) cultivated alfalfa (M. *sativa*, ssp. *sativa)* were studied. Feulgen's staining, C- and N-banding techniques and an image analysis system were used. The chromosomes of ssp. *falcata* have only centromeric bands, however, a multitude of bands were observed in both the C- and N-banding pattern of *ssp. caerulea* and ssp. *sativa* enabling the precise identification of each of the eight sets of chromosomes and development of a karyotype. The differences in banding patterns between the diploid subspecies makes it possible to identify hybrids between these subspecies. Karyotypic analysis of tetraploid alfalfa revealed that alfalfa has four nearly identical sets of chromosomes based on their identical chromosome morphology and C-banding patterns, thus providing support that alfalfa is an autotetraploid.

KEYWORDS

Alfalfa, C-banding, N-banding, image analysis, karyotype, cytogenetics

INTRODUCTION

Cytogenetic research on alfalfa has lagged far behind other crops mainly due to: alfalfa chromosomes are very small, ranging from 2-3 µ in length in root tip cells; the chromosomes are morphologically very similar; cultivated alfalfa has a relatively high number of chromosomes (2n=32); and alfalfa is an autotetraploid.

Analysis of somatic chromosomes of alfalfa has been conducted (Agarwal and Gupta, 1983; Falistocco, 1987; Sclarbaum et al., 1988). Somatic chromosomes of ssp. *caerulea* have been analyzed through the use of an image analysis system (Bauchan and Campbell, 1994) using chromosome morphological measurements. There are reports of successfully banding diploid *ssp. caerulea* cv. CADL. chromosomes (Masoud et al., 1991) and tetraploid *M. sativa* ssp. *sativa* (Falistocco et al., 1995), however there are no reports of N-banding in the genus *Medicago*.

This study was undertaken to develop C- and N-banded karyotype of the *ssp. caerulea*, ssp. *falcata*, and ssp. *sativa* and determine if these banding techniques could be useful to identify individula chromosomes in hybrid cells.

MATERIALS AND METHODS

Seeds were scarified and germinated in petri dishes at room temperature of six accessions of diploid (2n=2x=16) *ssp. caerulea*, PI 206453, PI 212798, PI 243225, PI 299046, PI 440507, PI 464720, and PI 299046 plus cv. CADL (provided by Ted Bingham, University of Wisconsin, Madison, WI), eight accessions of ssp. *falcata*, PI 115365, PI 262332, PI 263154, PI 307398, PI 405064, PI 464728, PI 467970, and UAG 1806 (provided by the Karl Lesins Collection, University of Alberta, Edmonton, Canada); and cv. 'Saranac' and PI 536539 of *M. sativa* ssp. *sativa*. The plant introductions (PI) seeds were obtained from the U.S. Plant Introduction Station in Pullman, WA. Hybrid seed was obtained from the cross between ssp. *falcata* (UAG1806) and *ssp. caerulea* (CADL). Modified improved Giemsa C- and N-banding techniques (Hossain, 1985 and Endo and Gill, 1984, respectively) were utilized. Twenty cells containing well spread C-and N-banded chromosomes were observed from each accession, cultivar, and hybrid. Photomicrographs were taken using a Zeiss Axiophot Microscope using Kodak® Technical Pan 2415 film. Photographs were printed on Agfa® multi-grade paper using high contrast filtering.

A Loats Associates® (Loats Associates, Inc. Westminster, MD) image analysis system with Karyotyper© software was utilized. This image analysis system utilizes a personal computer and a black and white video camera mounted on a Zeiss® Axiophot Microscope, the software was developed with the cooperation of Dr. Gary Bauchan. The chromosomes from each cell were analyzed using the image analysis system and chromosomes were paired to develop a karyotype.

RESULTS AND DISCUSSION

Medicago sativa ssp. *falcata* C- and N-banded chromosomes have bands only at the centromeric regions thus it is difficult to karyotype this subspecies without diagnostic bands. *M. sativa ssp. caerulea* and ssp. *sativa* C-banded chromosomes have several more bands than ssp. *falcata* in the accessions which were observed. In addition to the centromeric bands all of the chromosomes have telomeric bands in their short arms, all of the chromosomes except chromosome 7 have interstitial bands on their short arms and chromosomes 1, 2 and 3 each have one prominent interstitial band on their long arms. An idiogram of a standard C-banded karyotype is presented in figure 1.

The N-banding pattern of ssp. *caerulea* is similar to the C-banding pattern of the subspecies. Chromosomes 1, 2, 4, 7 and 8 are similar to the C-banding patterns, however, slight differences exist in the location and intensity of some of the interstitial bands. Chromosome 3 has a very prominent interstitial N-band in the long arm. The faint double interstitial N-bands which exist in the long arm of chromosome 5 is unique to N-banding. An idiogram of a standard N-banded karyotype is present in Figure 2.

Karyotypic analysis of tetraploid alfalfa (ssp. *sativa*) revealed that alfalfa has four nearly identical sets of chromosomes which resemble the banding pattern of ssp. *caerulea* this provides support that alfalfa is an autotetraploid.

The image analysis system with the karyotyping software is an efficient method of obtaining quality images of alfalfa chromosomes due to its enhancement capability. Enhancement of the chromosomes by pseudocoloration and enlargement of the images enable the edges of the chromosomes and the heterochromatic bands to be distinguished for easy identification. The image analysis system is also a rapid method of obtaining large amounts of data on chromosome morphology.

Due to the distinctive differences in the banding pattern of the two diploid subspecies, *ssp. caerulea* having multiple bands and ssp. *falcata* having only centromeric bands, it was possible to identify the individual chromosomes of *ssp. caerulea* from ssp. *falcata* chromosomes in hybrid cells. Using C-and N-banding techniques

in conjunction with image analysis it may be useful in studying cytogenetic and evolutionary relationships among species of *Medicago*.

REFERENCES

Agarwal, K. and P.K. Gupta. 1983. Cytological studies in the genus *Medicago* L. Cytologia **48**: 781-793.

Bauchan, G.R. and T.A. Campbell. 1994. Use of an image analysis system to karyotype diploid alfalfa (*Medicago sativa* L.) J Hered **85**: 18-22.

Endo, T.R. and B.S. Gill. 1983. Identification of wheat chromosomes by N-banding. *in* G. Kimber, ed. Proceedings of the Sixth International Wheat Genetics Symposia. Kyoto, Japan, pp 355-359.

Falistocco, E. 1987. Cytogenetic investigations and karyological relationships of two *Medicago*: *M. sativa* L. (alfalfa) and *M. arborea* L. Caryologia **40**: 339-346.

Falistocco, E., M. Falcinelli, and F. Veronesi. 1995. Karyotype and C-banding pattern of mitotic chromosomes in alfalfa, *Medicago sativa* L. Plant Breeding **114**: 451-453.

Gillies, C.B. 1968. The pachytene chromosomes of a diploid *Medicago sativa*. Can J Genet Cytol. **10**: 788-793.

Gillies, C.B. 1970. Alfalfa chromosomes. I. Pachytene karyotype of a diploid *Medicago falcata* L. and its relationship to *M. sativa*. Crop Sci **10**: 169-171.

Hossain, M.A. 1985. An improved Giemsa C-banding technique. Bangladesh J Bot **14**: 37-40

Masoud, S.A., B.S. Gill, and L.B. Johnson. 1991. C-banding and alfalfa chromosomes: Standard karyotype and analysis of a somaclonal variant. J Hered **82**: 335-338.

Schlarbaum, S.E., L.B. Johnson, and D.L. Stuteville. 1988. Characterization of somatic chromosome morphology in alfalfa, *Medicago sativa* L.: comparison of donor plant with regenerated protoclone. Cytologia **53**: 499-507.

Figure 1
Ideogram of C-Banded chromosomes of *Medicago sativa* ssp.*caerulea*

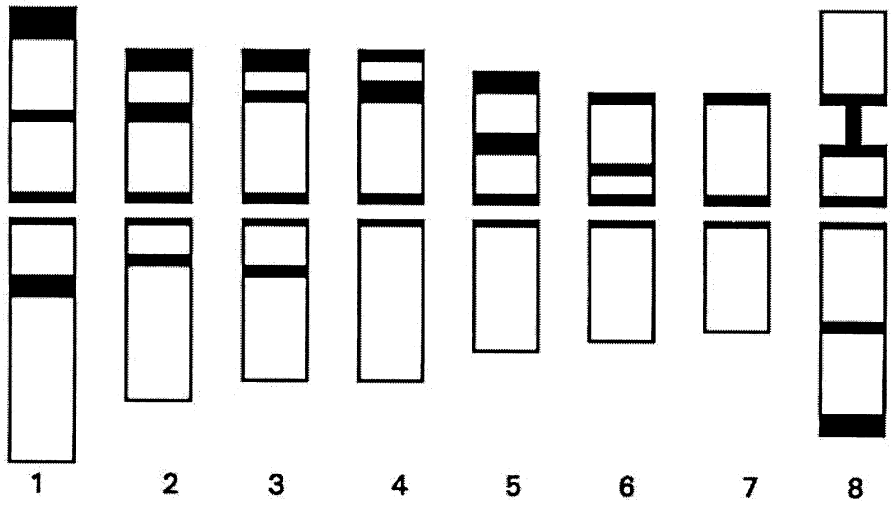

Figure 2
Ideogram of N-Banded chromosomes of *Medicago sativa* ssp. *caerulea*