

## Karyotypic Analysis of N-Banded Chromosomes of Diploid Alfalfa:

### *Medicago sativa* ssp. *caerulea* and ssp. *falcata* and Their Hybrid

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Chromosomes of two diploid ( $2n = 2x = 16$ ) subspecies of *Medicago sativa*, ssp. *caerulea* and ssp. *falcata*, and their hybrid were studied using an N-banding technique. This study was undertaken to develop an N-banded karyotype of ssp. *caerulea* and ssp. *falcata*, and determine if the same technique could be used to identify parental chromosomes in hybrids. The chromosomes of ssp. *falcata* have only centromeric N bands and thus individual chromosomes could not be identified. However, chromosome-specific bands were observed in ssp. *caerulea* enabling the identification of each of the eight pairs of chromosomes and the development of an idiogram. All chromosomes have a centromeric band and a telomeric band in the short arm. All of the chromosomes, except chromosomes 7 and 8, have interstitial bands in their short arms. Chromosomes 1, 2, and 3 each have one interstitial band in their long arms and chromosome 5 has two faint interstitial bands in its long arm. The satellited chromosome is easily distinguished due to the secondary constriction and characteristic band at the nucleolar organizer region. The differences in banding patterns between these subspecies makes it possible to distinguish chromosomes from each other in their hybrids. This is the first report of the use of N banding to identify the diploid chromosomes of *M. sativa* ssp. *caerulea* and ssp. *falcata*.

The location of the nucleolar organizer region (NOR) in animal and plant chromosomes using the Giemsa N-banding technique was first described by Funaki et al. (1975). Gerlach (1977) was the first to apply this technique in wheat, *Triticum aestivum* L., and related species. Since then there have been many reports on the application of this technique to identify the chromosomes of wheat (reviewed by Endo and Gill 1983; Gill et al. 1991) and other grass species such as *Aegilops* (Jewell 1979), barley (*Hordeum vulgare* L.) (Singh and Tsuchya 1982), *Elymus* (Morris and Gill 1987), and rye (*Secale cereale* L.)

(Schlegel and Gill 1984). However, reports of N-banding of dicotyledonous plant chromosomes are scarce. *Cestrum parqui*, a wild Solanaceous plant, was cold treated, and N-banding and karyotypic analysis was accomplished (Berg and Greilhuber 1992). N-banding in *Glycine max* (L.) Merr. and its wild relatives was used to identify the NOR in these species (Yanagisawa et al. 1991), however, other bands that were detected were located at the centromeres and thus were not helpful in identifying individual chromosomes.

This study was undertaken to apply the N-banding technique to the chromosomes of diploid alfalfa, develop an N-banded karyotype of ssp. *caerulea* and ssp. *falcata*, and determine if the same technique could be used to identify parental chromosomes in hybrids.

## Materials and Methods

One accession of diploid ( $2n = 2x = 16$ ) ssp. *caerulea*, PI 212798 (collected in Iran) and the cultivar CADL (Cultivated Alfalfa at the Diploid Level) (provided by E. T. Bingham, University of Wisconsin, Madison, Wisconsin), and three accessions of ssp. *falcata*, PI 262532 and PI 263154 (collected in the former Soviet Union); and UAG 1806 (collected in the Hungary) (provided by the Karl Lesins Collection, University of Alberta, Edmonton, Canada) were studied. The plant introductions (PI) seeds were obtained from the U.S. Plant Introduction Station in Pullman, Washington. Hybrid seeds were obtained from the cross between ssp. *falcata* (UAG1806) and ssp. *caerulea* (CADL) using vacuum suction hand-emasculatation and hand-pollination.

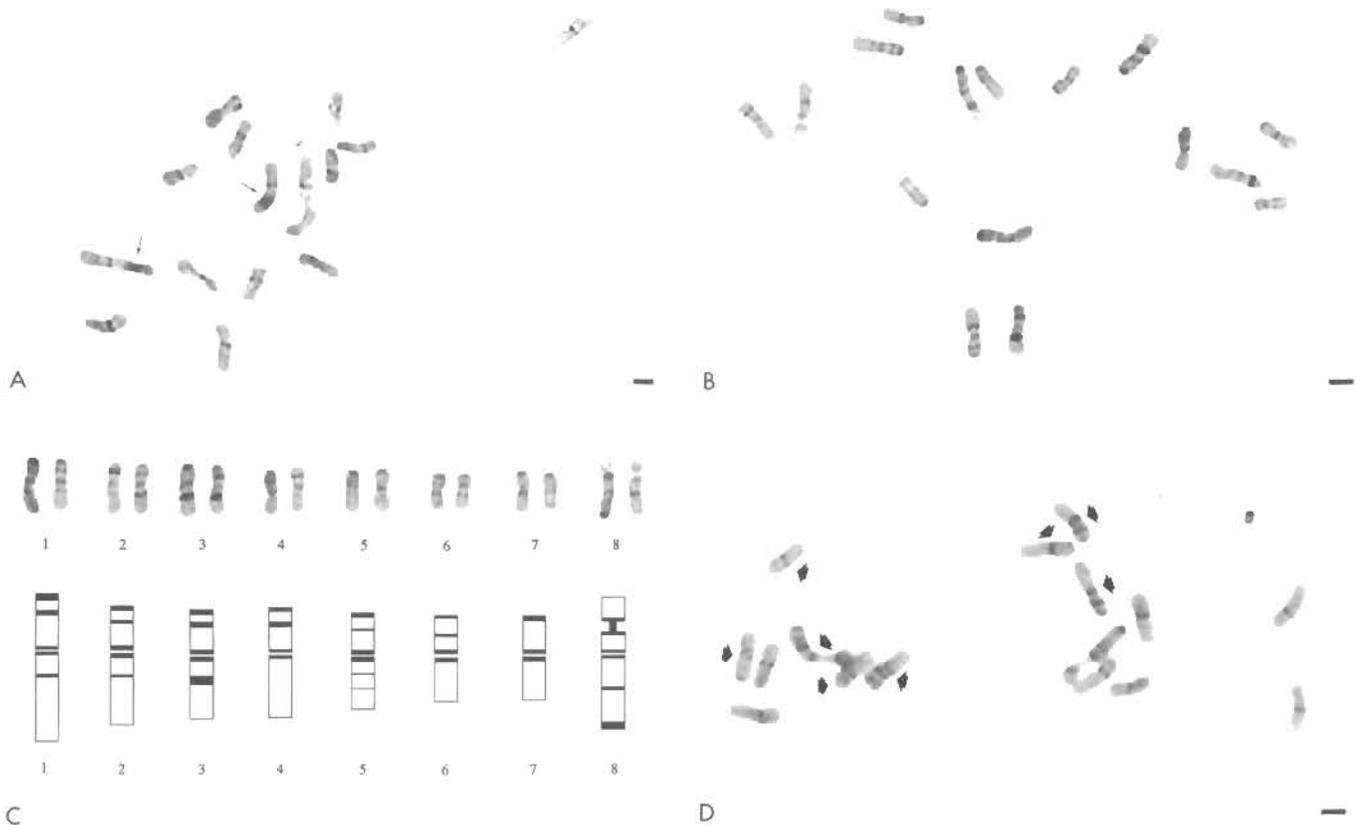
Seeds were scarified and germinated in petri dishes at room temperature on filter paper. Root tips were obtained 3 days after germination was initiated and pretreated in an ice bath for 20 h before fixation in Farmer's fixative (3:1, v/v, 95% ethanol:glacial acetic acid) for at least 30 min. The Giemsa N-banding technique of Endo and Gill (1984) was modified as follows. A single fixed root tip was placed in a drop of 45% acetic acid for 2 to 3 min on a microscope slide. Dissecting out of dividing root tip cells was performed using needles under a dissecting microscope. Slides were gently warmed and squashed under a coverslip. The coverslip was removed following freezing the slide with liquid nitrogen. The slides were immersed in 45% acetic acid at 45°C for 20 to 25 min and rinsed in distilled water for 1 to 2 min. After rinsing

the slides were dried at room temperature for 18 h. The dried slides were immersed in 1 M  $\text{NaH}_2\text{PO}_4$  for 2.5 to 3 min at 91°C–92°C. Slides were briefly rinsed in distilled water and stained in 7.3% Giemsa stain (Sigma) in pH 6.8 phosphate buffer (1 M  $\text{NaH}_2\text{PO}_4$ ) for 35 to 40 min. The stained slides were briefly rinsed in distilled water, air dried using a hair dryer, and coverslips affixed using Permount. Twenty cells from 20 different individuals containing well-spread N-banded chromosomes were observed from each accession, cultivar, and hybrid. Photomicrographs were taken using a Zeiss Axiophot Microscope with Kodak Technical Pan 2415 film. Photographs were printed on Agfa multigrade paper using high-contrast filtering. An idiogram was developed based on length, position of the centromere, and banding pattern using an image analysis system (Bauchan and Campbell 1994).

## Results

*Medicago sativa* ssp. *falcata* N-banded chromosomes have bands only at the centromeric regions with the exception of the satellited (SAT) chromosome (chromosome 8) which also has a large band at the NOR (Figure 1A). Occasionally an interstitial band was observed in the middle of the long arm of the SAT chromosome. Without diagnostic terminal or interstitial N-bands it is very difficult to karyotype the chromosomes of ssp. *falcata*. However, *M. sativa* ssp. *caerulea* has several more bands than ssp. *falcata* in the accessions that were observed (Figure 1B). All of the chromosomes have centromeric bands and telomeric bands in their short arm. All of the chromosomes except chromosome 7 have interstitial bands in their short arms. Chromosomes 1, 2, and 3 each have an interstitial band in their long arms (Figure 1B) and chromosome 5 has two interstitial bands in the long arm.

A brief description of each individual chromosome N-banding pattern is as follows: Chromosome 1: The largest chromosome without an NOR is submetacentric; has a terminal band and an interstitial band in its short arm; in addition to the centromeric band one interstitial band is located fairly close to the terminal band in the long arm. Chromosome 2: A submetacentric chromosome which is smaller than chromosome 1 has an interstitial band located near the terminal end of the short arm and one interstitial band located near the centromere in the long arm. Chromosome 3: A submetacentric chromosome



**Figure 1.** (A) N-banded chromosomes of *M. sativa* ssp. *falcata*. Arrows indicate the SAT chromosomes. The bar represents 1  $\mu$ m. (B) N-banded chromosomes of *M. sativa* ssp. *caerulea* and karyotype. The bar represents 1  $\mu$ m. (C) Karyotype and idiogram of N-banded chromosomes of *M. sativa* ssp. *caerulea*. (D) N-banded cell of a hybrid between *M. sativa* ssp. *falcata* and ssp. *caerulea*. Arrows indicate the ssp. *caerulea* chromosomes. The bar represents 1  $\mu$ m.

with a terminal band and an interstitial band in the short arm and a prominent, much amplified interstitial band in the middle of the long arm. Chromosome 4: A submetacentric chromosome with an interstitial band in the short arm and no interstitial band in the long arm. Chromosome 5: A metacentric chromosome with a terminal band and a faint interstitial band in the short arm of the chromosome and two very faint staining interstitial bands located in the long arm. Chromosome 6: A short metacentric chromosome with a small terminal band and an interstitial band in the short arm. There are no interstitial bands on its long arm. Chromosome 7: The smallest metacentric chromosome in the complement with only a centromeric band and a telomeric band in the short arm of the chromosome, no interstitial bands. Chromosome 8: The SAT chromosome which is submetacentric with two bands flanked by the NOR and the centromere; a large terminal band and an interstitial band is located in the long arm of the chromosome. Based on the banding pattern, a karyotype and idiogram is presented in Figure 1C.

Due to the distinctive differences in the banding pattern of the two subspecies,

*ssp. caerulea* having characteristic bands and *ssp. falcata* having only centromeric bands, it was possible to identify the parental chromosomes of *ssp. caerulea* from *ssp. falcata* chromosomes in the hybrids (Figure 1D).

### Discussion

This is the first report of the use of N-banding to identify chromosomes in the genus *Medicago*. N-bands were only observed at the NOR and the centromeres of *ssp. falcata* in the accessions observed, thus it was impossible to develop a karyotype of this subspecies. However, *ssp. caerulea* chromosomes have unique banding patterns, thus it is possible to identify the individual chromosomes. There is some banding pattern similarity of chromosomes 1 and 2, however, chromosome 1 is a larger chromosome than chromosome 2.

We found that the pretreatment time of the root tips in the ice bath was critical for obtaining the suitable size of the chromosomes for banding alfalfa chromosomes with interstitial bands. Chromosomes that are too contracted will fuse their bands together and a fewer number of bands than are actually present will be

detected. The critical step in the N-banding technique is the temperature (91°C–92°C) for the 1 M  $\text{NaH}_2\text{PO}_4$  in a water bath. Our experience in observing N-bands of *Medicago* species is that there is little contrast between the darkly stained heterochromatin and the lighter stained euchromatin and thus the chromosome bands are clearer when observing them in the microscope than in the photomicrographs.

The distinctive N-banding pattern of *ssp. caerulea* chromosomes enabled us to develop a standard karyotype which may be helpful in studying cytogenetic and evolutionary relationships among species of *Medicago*. The differences we observed in the banding patterns of these two subspecies makes it possible to identify parental chromosomes in the hybrids.

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## References

- Bauchan GR and Campbell TA, 1994. Use of an image analysis system to karyotype diploid alfalfa (*Medicago sativa* L.). *J Hered* 85:18-22.
- Berg C and Greilhuber J, 1992. Cold-sensitive chromosome regions and their relation to constitutive heterochromatin in *Cestrum parqui* (Solanaceae). *Genome* 35: 921-929.
- Endo TR and Gill BS, 1983. Identification of wheat chromosomes by N-banding. In: Proceedings of the Sixth International Wheat Genetics Symposia. Kyoto, Japan; 355-359.
- Funaki K, Matsui S, and Sasaki M, 1975. Location of nucleolar organisers in animal and plant chromosomes by means of an improved N-banding technique. *Chromosoma* 62:49-56.
- Gill BS, Friebe B, and Endo TR, 1991. Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). *Genome* 34:830-839.
- Gerlach WL, 1977. N-banded karyotypes of wheat species. *Chromosoma* 62:49-56.
- Jewell DC, 1979. Chromosome banding in *Triticum aestivum* cv. Chinese Spring and *Aegilops variabilis*. *Chromosoma* 71:129-134.
- Morris KLD and Gill BS, 1987. Genomic affinities of individual chromosomes based on C- and N-banding analyses of tetraploid *Elymus* species and their diploid progenitor species. *Genome* 29:247-252.
- Schlegel R and Gill BS, 1984. N-banding analysis of rye chromosomes and the relationship between N-banded and C-banded heterochromatin. *Can J Genet Cytol* 26: 765-769.
- Singh RJ and Tsuchiya T, 1982. An improved Giemsa N-banding technique for the identification of barley chromosomes. *J Hered* 73:227-229.
- Yanagisawa T, Tano S, Fukui K, and Harada K, 1991. Marker chromosomes commonly observed in the genus *Glycine*. *Theor Appl Genet* 81:606-612.

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