RFLP Analyses of Chloroplast DNA of the Crested Wheatgrasses

K. P. Vogel
USDA-ARS

D. J. Lee
University of Nebraska

C. A. Caha
University of Nebraska

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K.P. Vogel[1], D.J. Lee[2] and C.A. Caha[2]
[1]USDA-ARS, 344 Keim Hall, Univ. of Nebraska, PO Box 830937, Lincoln, NE 68583-0937; [2]Dept. Agronomy & Horticulture, Univ. of Nebraska, Lincoln, NE -68583-0915, USA. Email:kpv@unlserve.unl.edu

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Introduction The crested wheatgrasses (*Agropyron* spp.) are widely distributed Eurasian grasses that have been used to re-vegetate millions of hectares of land in the northern plains and intermountain areas of North America. The genus consists of about 10 species that are based on the 'P' genome and includes diploids ($2n$ = 14), tetraploids ($2n$ = 28) and octoploids ($2n$ = 42) (Dewey, 1984, Vogel *et al*., 1999). The two main agronomic species used in North America are *A. cristatum* (L.) Gaertner and *A. desertorum* (Fischer ex Link) Schultes. Chloroplast genomes are predominately maternally inherited through the cytoplasm in angiosperms. Chloroplast DNA (cpDNA) variation can provide information on the evolutionary development of plant species. The objective of this study was to determine if chloroplast DNA polymorphisms occur within and among ploidy levels of these two main species of the *Agropyron* complex.

Materials and methods Cultivars, strains, or germplasms of *A. cristatum* diploids (Fairway, Ruff, NE AC1, NE AC2) and hexaploids (PI 222955, PI 401077) and *A. desertorum* tetraploids (Hycrest, NE AD1) and octaploid (E1 990) were grown in the greenhouse. Plants represented germplasm from the range of the species. Restriction fragment length polymorphisms (RFLP) procedures were used to assay for chloroplast DNA polymorhphism using the methods and probes described by Hultquist *et al*. (1996). Four restriction enzymes (*Bam*HI, *Eco*RI, *Eco*RV, and *Hind*III) and 20 chloroplast DNA probes were used in the RFLP analyses. Technical assistance of Risa Cohen and Sherry Hulquist is acknowledged with appreciation.

Results A total of 72 different bands were produced by the 80 probe-enzyme combinations of the RFLP analyses. All evaluated crested wheatgrass strains produced similar bands with the probe-enzyme combinations that were used. No chloroplast DNA polymorphisms were detected among the diploid, tetraploid, and octaploid *A. cristatum* and *A. desertorum* strains. See example in Figure 1.

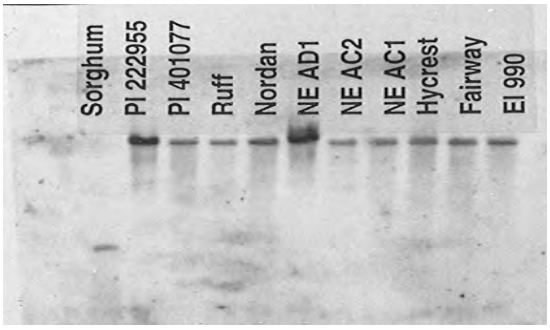

Figure 1 Crested wheatgrass and sorghum (control) restriction fragments produced by *Hind*III on a Southern blot hybridized with chloroplast DNA probe pLD 7

Conclusions The chloroplast DNA of the evaluated species of *Agropyron* complex appears to be relatively uniform which indicates that this DNA is conserved within the genus. The evaluated *Agropyron* species apparently have a common ancestral cytoplasm. This suggests that the primary genetic variation in the genus is due to nuclear DNA variation including variation due to polyploidy.

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