COLCHICINE-DOUBLING OF GERMINATING SEEDLINGS OF INTERSPECIFIC WILDRYE HYBRIDS

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ABSTRACT
Colchicine has usually been applied to sterile clones of interspecific grass hybrids to restore fertility. However, when hybrids are partially fertile, colchicine can be applied to germinating seedlings. Four populations of Basin wildrye (*Leymus cinereus)* X beardless wildrye (*L. triticoides*) were treated with a 0.2% colchicine solution to double chromosome number from 4x=28 to 8x=56. Doubling percentage was 28, 33, 42, and 44% of all root-tips examined for the four populations. When plants without doubled sectors were discarded, doubling percentage increased to 56, 68, 78, and 78%. Plants with doubled sectors have been placed in crossing blocks to generate octoploid progeny. Octoploids identified through root-tip chromosome counts will be intercrossed to generate 8x populations.

KEYWORDS
Chromosome-doubling, colchicine, interspecific hybridization, Leymus, wildrye

INTRODUCTION
Basin wildrye (*Leymus cinereus* (Scribn. & Merr.) A. Löve) is a large-statured, grazing-susceptible bunchgrass that occurs at the tetraploid (2n=28) and octoploid (2n=56) levels. Beardless wildrye (*L. triticoides* (Buckl.) Pilger) is a medium-statured, grazing tolerant, rhizomatous grass that occurs only at the tetraploid (2n=28) level. Combining the good seed production and low seed dormancy of Basin wildrye with the grazing tolerance, rhizomatous habit, and salinity tolerance of beardless wildrye is the goal of our breeding effort. Hybrids occur naturally where the ranges of the two species overlap. Dewey (1970) reported that this hybrid exhibited 13.86 of a possible 14 bivalents and more than 10% stainable pollen, but no seed was set. Using different parent populations, we have produced sufficient hybrid seed for large-scale colchicine treatment.

Colchicine treatment is the most widely used method for chromosome doubling in crop plants. When sterile interspecific hybrid genotypes must be doubled, clonal tillers or buds are treated. Fertile doubled sectors may be distinguished and separated from sterile undoubled material. For production of autoploids from fertile populations, treatment of germinating seed in colchicine solution is the most widely used method because of its simplicity (Speckmann, 1975). While this method has been used in a variety of forage crops, 'Tetrascan' Russian wildrye (Psathrostachys junceus (Fisch.) Nevski) is the only perennial Triticeae cultivar developed using this method (Lawrence et al., 1990). Nitrous oxide has also been used to double chromosome number in Russian wildrye (Berdahl and Barker, 1991). The principal advantage of nitrous oxide is reduced chimera frequency. However, because the treatment is performed in a pressurized tank, the number of plants which can be treated is limited. This discourages generation of large populations desirable for plant breeding (Speckmann, 1975). Our objective was to chromosomally double interspecific *Leymus* populations by exposing germinating seeds to colchicine. This approach may be useful in developing large populations of chromosome-doubled material from semi-fertile hybrid populations without making controlled pollinations and isolating doubled sectors.

MATERIALS AND METHODS
We applied colchicine to double chromosome number in two pairs of reciprocal populations, L4PX-3 (Acc:636 Basin wildrye of Lethbridge, Alberta, Canada X Acc:641 beardless wildrye of Jamieson, Oregon, USA), its reciprocal (L4PX-3R), L4PX-5 ('Trailhead' Basin wildrye of Roundup, Montana, USA X Acc:641 beardless wildrye), and its reciprocal (L4PX-5R), using a modified ryegrass (*Lolium* spp.) procedure (Arnold J.P. vanWijk of D.J. van der Have B.V. [Rilland, The Netherlands], pers. comm.). Seedlings with radicles just emerging were placed into a 5-cm petri dish lined with a single disk of Whatman #2 filter paper soaked with 0.3 ml of 0.2% colchicine solution. Seedlings were arranged to ensure radicle contact with the filter paper. After 2 h in the dark at 30°C, seedlings were gently rinsed twice with water and plated on moist blotter paper before planting in the greenhouse.

Three root-tips were harvested from surviving seedlings at least 3 months old for determination of somatic chromosome number. Root-tips were placed on Whatman #2 filter paper soaked in colchicine solution (50 ml deionized water, 50 mg colchicine, 25 mg hydroquinoline 10 ml dimelthylsulfoxide) and kept in the dark at a room temperature for 3 h. Root-tips were transferred to a vial containing aceto-orcein (100 ml 45% acetic acid, 2 g certified orcein stain), incubated for 2 d at 4°C, and squashed for chromosome counts.

RESULTS AND DISCUSSION
High rates of chromosome doubling were achieved for each of the four populations (Table 1). Averaged across the four populations, over one-half of the plants had at least one of three examined root-tips doubled from 28 to 56 chromosomes. Nearly one-quarter of the plants had all of three examined root-tips doubled. After discarding undoubled plants, percentage of doubled root-tips for the four populations ranged from 56 to 78% (mean=70%).

This method (Fig. 1) is an adoption of Lawrence's et al., (1990) approach for partially fertile interspecific hybrids instead of fertile material of nonhybrid origin. While treatment of germinating seeds produces chimeras, as does the treatment of clonal tillers and buds, the proportion of the plant that is doubled is increased. Furthermore, because of the high frequency of success, nurseries can be established to produce sectorless seeds, many of which are chromosome doubled. Because the seed treatment procedure requires quantities of seed, it is not feasible for use with sterile interspecific hybrids. However, where sterility is only partial, seed treatment may be more successful than treating vegetative material because doubled and undoubled sectors are difficult to distinguish and laborious to separate.

Our doubled hybrid Leymus populations may be used for four purposes:

1) Use directly as octoploid breeding populations. Fertility may be increased if cryptic structural hybridity (Stebbins, 1971) is responsible for partial sterility of the tetraploid interspecific hybrids.
2) Hybridization with naturally occurring octoploid Leymus populations to introduce new traits.
3) Hybridization with naturally occurring octoploid *Leymus* populations to assess relationships between genomes of tetraploid and octoploid Leymus populations as proposed by Dewey (1970).
4) Hybridization with chromosome-doubled *Elymus* populations for *de novo* synthesis of octoploid *Pascopyrum* germplasm. Dewey (1975) suggested an alternative approach of making

tetraploid *Leymus X Elymus* hybrids and producing fertile amphiploids with colchicine.

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Table 1
Percentage (number) of seedlings of four Leymus interspecific hybrid populations with 0, 1, 2, or 3 root-tips doubled in chromosome number by colchicine treatment.

Population	No. 2n=56 root-tips (maximum=3)			
	0	1	2	3
	--------% of seedlings (no.) ----------			
L4PX-3	50 (52)	28 (29)	9 (9)	13 (13)
L4PX-3R	46 (54)	9 (11)	16 (19)	28 (33)
L4PX-5	43 (39)	12 (11)	14 (13)	31 (28)
L4PX-5R	43 (50)	21 (24)	12 (14)	23 (27)
Mean	46 (195)	18 (75)	13 (55)	24 (24)

Figure 1
The procedure being used to chromosome-double L4PX populations.