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Hybrids and Amphiploids of *Aegilops cylindrica* with *Triticum aestivum* L.; Production Morphology and Fertility

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Abstract: Wheat (*Triticum aestivum* L.) and jointed goatgrass (*Aegilops cylindrica* Host) are genetically related. Both species share the D-genome, allowing hybrids between them to easily form under natural and controlled environments. These hybrids are 100% male sterile but partial female fertile. The objective of this study was to produce hybrids and amphiploids of *Ae. cylindrica and T. aestivum* and test them for male and female fertility. Hybrids between wheat and jointed goatgrass were produced in the greenhouse. Of the 24 plants only sixteen germinated. All germinated wheat × jointed goatgrass hybrids were treated with colchicine to produce amphiploids. Root-tip chromosome counts in the treated plants showed 70 chromosomes in 6 (37.5%) plants and chimeric numbers (35 and 70 chromosomes) in 3 (18.7%) plants while the remaining 7 plants had 35 chromosomes. Amphiploids of *Ae. cylindrica* × *T. aestivum*, produced by colchicine treatment, were 100% male and female sterile and did not produce any seeds. Moreover, phenotypically amphiploids of wheat × jointed goatgrass looked similar to normal wheat×jointed goatgrass hybrids with 35 chromosomes. Possible reasons for the sterility in amphiploids could be due to cytological instability and/or genome dosage.

Key words: *Triticum aestivum* L., *Aegilops cylindrica*, host, amphiploids, gene transfer

INTRODUCTION

Amphiploidy is a mechanism that is used to introgress desirable traits (genes) and to restore fertility in hybrids between diverse species. For example amphiploidy was used to transfer salinity tolerance from *Aegilops speltoides* subsp. *speltoides* (SS) to *Triticum turgidum durum* (AABB) (Noori, 2005) and to transfer *Aegilops ovata* chromosomes (carrying disease resistance) into bread wheat (Landjeva and Ganeva, 1998).

Most of the perennial grass species in the tribe Triticeae are allopolyploids that originated from genome combinations of two or more species. Bread wheat (*Triticum aestivum* L.) originated from a series of natural hybridizations between the wild diploids (2n = 2x = 14) *Triticum urartu* Tumanian ex Gandilyan, A-genome (Chapman *et al*., 1976; Dvorak *et al*., 1988); a species closely related to *Aegilops speltoides* Tausch., B-genome and *A. tauschii* Coss., D-genome (Zohary *et al*., 1969). The narrow genetic base of wheat is a concern in coping with evolving disease and insect pressures and variations in environmental stresses. However, crosses with unproductive wild relatives or primitive ancestors are not common in a wheat improvement program because they do not result in highly productive cultivars. For this reason, distantly related germplasm has been considered almost exclusively as a source of improved resistance to diseases, insects and environmental stresses.

Jointed goatgrass (*Aegilops cylindrica*) is an allotetraploid species (2n = CCDD), originating from two diploid progenitor species: *Aegilops caudata* (designated as CC) and *Ae. tauschii* Coss. (Designated as DD) (Kimber and Sears, 1987). Wheat and jointed goatgrass are genetically related and both share the same progenitor (*Aegilops tauchii* Coss) for the D-genome (Kimber and Sears, 1987). Therefore, hybrids between the two species can be produced under controlled and natural field conditions (Zemetra *et al*., 1998a). Wheat × jointed goatgrass hybrids have 35 chromosomes (21 from wheat and 14 from jointed goatgrass) and have ABCDD as their genomic constitution. These hybrids are 100% male sterile (Zemetra *et al*., 1998a), but a low level of female fertility in the hybrids allows for hybrids to backcross to either wheat or jointed goatgrass (Zemetra *et al*., 1998a).

Jointed goatgrass has been identified as a useful but under-utilized source for wheat improvement and has been reported to have salt tolerance (Farooq *et al*., 1992), Hessian fly resistance (El Bouhssini *et al*., 1998), snow mold resistance and freezing tolerance (Iriki *et al*., 2001). All these traits from jointed goatgrass into wheat were

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transferred through backcross breeding, however, the use of amphiploidy has not yet been reported. The objectives of this study were to determine if amphiploids of *Ae. cylindrica* and *T. aestivum* could be produced and to determine if the self-fertility was restored due to genome duplication.

MATERIALS AND METHODS

Plant material: Wheat and jointed goatgrass hybrids were produced in the greenhouse at University of Idaho, Moscow Idaho, USA in 2003 using the approach method (Curtis and Croy, 1958). A local (Moscow, Idaho) jointed goatgrass collection was used to cross to the wheat cultivar Brundage 96 (Zemetra *et al*., 1998b). Twenty-four F_1 seeds were planted in peat pellets (Jiffy-7, Jiffy products (NB) Ltd., Shippagan, Canada, P.O. Box 2004 Shippangan, New Brunswick E8S 3H1). The plants were grown in the greenhouse with a 16/8 light/dark photoperiod and 23/18°C day/night temperatures.

Chromosome doubling: Plants at the three to four leaf stage were removed from the peat pellets (Jiffy-7 peat pellets, Shippagan Canada) and the soil was washed from the individual plant roots and crowns. Before treating with colchicine, roots were trimmed to about 1-2 cm. Similarly, excess leaves were removed and leaf tips of the remaining leaves were further trimmed. Five milliliter test tubes were filled about 1/3 -full with 0.1% colchicine solution (Sigma Chemical Co., P.O. Box 14508, St. Louis, MO, 63178). Crowns of wheat × jointed goagrass hybrids were submerged into 0.1% colchicine containing 2% DMSO (dimethyl-sulfoxide) (Sigma Chemical Co. P.O. Box 14508 St. Lois, MO, 63178), 1% of 10 ppm GA_3 (10 mg GA_3/1 L dH_2O) and 10 drops of Tween 20 for 5 h. The colchicine treatment was done in a fume hood with lights at a temperature ranging from 16-21°C. After the colchicine treatment, plants were rinsed thoroughly in the sink under running tap water for 15 min. Plants were then transplanted into 4×4 cm pots in Sunshine mix #1 (SunGro Horticulture, Bellevue, WA). Since the plants are fragile after colchicine treatment and need to be protected from a sudden shock of light and temperature, the treated plants were covered with plastic wrap and placed in a controlled environment chamber (Environmental Growth Chambers, P.O. Box 390 510 East Washington Street Chagrin Falls, Ohio 44022-0390) for two to three weeks with 25°C, 16 h photoperiod, before being transferred to a vernalization chamber for 6 weeks with 8/16 h light/dark photoperiod at 4°C day/night temperatures. These plants were subjected to cytological analysis to confirm amphiploidy after vernalization.

Chromosome preparations: Treated plants were examined after three to four weeks for mitotic chromosome numbers. 1 to 2 cm long root tips were collected, immediately pretreated in ice water for 24 h (Tsuchiya, 1971) and fixed in Farmer's solution (95% ethanol-glacial acetic acid, 3:1) for at least one day (Singh, 1993). The fixed root tips were then transferred to 45% acetic acid for at least 2 h before use. Chromosome preparations were observed under a phase contrast light microscope (Nikon Model Labophot, Japan) and photographic images were taken with a Nikon Eclipse E1000 microscope (Meridian Instrument Company, Kent WA).

Fertility: To test if amphiploids were self-fertile, five heads from each plant were covered with glassine bag prior to anthesis to avoid any cross-pollination. To test for female fertility, two heads from each amphiploid were emasculated and were pollinated by wheat using the approach method (Curtis and Croy, 1958).

RESULTS AND DISCUSSION

Of the 24 hybrid seeds planted only 16 germinated. All germinated wheat × jointed goatgrass hybrids were treated with colchicine and the survival rate of these plants after treatment was 100%. Root-tip chromosome counts in the treated plants showed 70 chromosomes in 6 plants (37.5%), chimeric numbers (35 and 70 chromosomes) in 3 plants (18.7%) and 35 chromosomes in the remaining 7 plants (43.8%) (Table 1). Chimeric amphiploids also were reported in *Aegilops ovata* ×*Secale cereale* crosses (Barbara and Hanna, 2002).

Amphiploids derived from *Ae. cylindrica* × *T. aestivum* hybrids looked similar to normal *Ae. cylindrica* × *T. aestivum* hybrids with 35 chromosomes with regard to their leaf size, height and spike morphology. However, the plants showed more vigorous vegetative growth and good tillering ability as compared to normal *Ae. cylindrica* × *T. aestivum* hybrids (personnel observation).

Amphiploids of *Ae. cylindrica* × *T. aestivum* hybrids, produced by colchicine treatment, were 100% sterile and did not produce any seeds by selfing or when pollinated with wheat pollen. Sterility in amphiploids is common since cytological instability and low fertility are commonly associated with newly formed amphiploids (Ashman and Boyle, 1955; Hill and Buckner, 1962). The other possible

Table 1: Analysis of *Aegilops cylindrica* × *Triticum aestivum* amphiploids and their female- and self-fertility

Total plants	Germination	Amphiploids	Chimeric	F. fert.[a]	S-fert.[b]
24	16/24 (66.6%)	6	3	0	0

[a]Female fertility, [b]Self-fertility

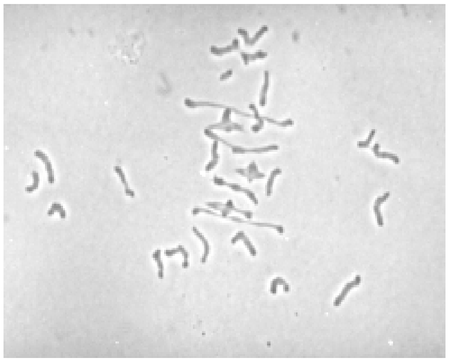

Fig. 1: Meiotic chromosomes showing univalents and bivalents of *Aegilops cylindrica* × *Triticum aestivum* hybrids

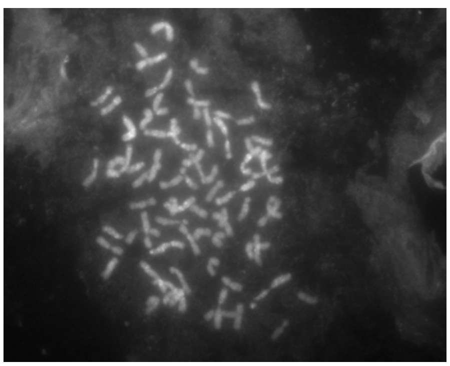

Fig. 2: Amphiploid of *Aegilops cylindrica* × *Triticum aestivum* hybrid with 70 chromosomes

reason for sterility in wheat × jointed goatgrass amphiploids could be due to the genome dosage. *Ae. cylindrica* × *T. aestivum* hybrids have 35 chromosomes with ABCDD as their genome constitution. During meiosis, 7 bivalents (between the D-genomes of wheat and jointed goatgrass) and 21 univalents are formed (Fig. 1). Due to chromosome doubling, the genome constitution is changed to AABBCCDDDD with 70 chromosomes (Fig. 2). As a result, the diploid DD is changed into a tetraploid DDDD-genome that could possibly lead towards the sterility. For example, Birchler (1993) found that the interploidy crosses in maize between diploid and tetraploid lines resulted in collapsed endosperm in both directions (2×4 and 4×2). In addition, chromosome doubling of maize diploid crosses produced by nitrous oxide treatment after fertilization or at the time of the first endosperm mitosis resulted in endosperm collapse. These endosperms are hexaploid with a normal maternal to paternal ratio within the endosperm itself, but nevertheless failed to develop correctly. These results suggested that the ratio of genomes in the female gametophyte to the fertilized endosperm is an important component of the interploidy effect.

Furthermore, male and female sterility of wheat × jointed goatgrass amphiploids also provides the basis for the fact that seeds are produced on the sterile hybrids when backcrossed to either wheat or jointed goatgrass (Zemetra *et al*., 1998a; Wang *et al*., 2001) and not through spontaneous doubling.

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