Theor Appl Genet (1989) 78: 721–727

Embryo-callus-regenerated hybrids and their colchicine-induced amphiploids between *Elymus canadensis* and *Secale cereale*

C.H. Park and P.D. Walton

Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

Received January 25, 1989; Accepted June 27, 1989
Communicated by K. Tsunewaki

Summary. Intergeneric hybrids recovered through plant regeneration from embryo callus culture and their colchicine-induced amphiploids were obtained from a cross of *Elymus canadensis* with *Secale cereale* (cv 'Gazelle'). The embryo-callus-regenerated F_1 plants grew vigorously to maturity and regrew well after clipping, while the embryo-rescued F_1 died of hybrid necrosis before maturity. Most of the morphological characters of the sterile F_1 hybrids were intermediate between the parents, but tiller number and dry matter yield were higher than the parents. Amphiploids from these F_1 plants had improved fertility but were less vigorous than the F_1 plants. The predominance of univalents in the F_1 and bivalents in the amphiploids indicated that the genomes S, H, and R were distinct. However, the occasional occurrence of multivalents reflected a random, intergenomic or intragenomic pairing. The mean chromosome associations of the F_1 ($2n=21$, SHR), the C_0 amphiploids ($2n=42$, SSHHRR), and the C_1 amphiploid ($2n=40$) at metaphase I (MI) were $16.51_{I}+2.05_{II}+0.06_{III}+0.02_{IV}+0.02_{V}$, $2.20_{I}+19.87_{II}+0.02_{IV}$, and $7.10_{I}+16.37_{II}+0.04_{III}+0.02_{IV}$, respectively. These amphiploids could be exploited as new germplasm for forage crop improvement by controlled introgression and backcrosses to the parents.

Key words: Canada wild rye – Spring rye – Intergeneric hybrids – Callus culture – Plant regeneration

Introduction

Canada wild rye (*Elymus canadensis* L.) is a North American native perennial grass with agronomic characteristics of high forage yield and moderate quality (Walton 1983). Spring rye (*Secale cereale* L.) is an annual species used as a reliable cereal crop, forage crop, or cover crop over a wide geographic range (Stoskopf 1985). From the standpoint of forage production, the poor tillering and foliage of spring rye make it unsuitable for hay or pasture production. Canada wild rye decreases forage quality due to its coarsed leaves as it matures, but it is winter-hardy and drought-resistant (Walton 1983). In an attempt to combine the advantages of both species, we endeavored to produce synthetic hybrids and amphiploids. Previously, Canada wild rye was hybridized with wheat (Mujeeb-kazi and Bernard 1985; Yen and Liu 1987), barley (Mujeeb-kazi and Rodriguez 1982), and rye (Hang and Franckowiak 1984) for the purpose of breeding disease-resistant cereal species. Those intergeneric hybrids were produced by the embryo rescue of hybrid embryos. Callus formation and plant regeneration from immature hybrid embryos have been regarded as an alternative way to develop hybrid plants (Larkin 1985; Merkle et al. 1988). In this study, the undifferentiated hybrid embryos in conventional embryo rescue were subjected to the callus formation and plant regeneration.

This paper describes the morphological and cytological characterization of those F_1 hybrids and amphiploids between Canada wild rye and spring rye.

Materials and methods

Canada wild rye (*Elymus canadensis* L., $2n=4x=28$, SSHH) accession, 'Montana', which was provided by Dr. Aung, University of Alberta, Edmonton, was emasculated and then hand-pollinated with pollen of spring rye (*Secale cereale* L., $2n=2x=14$, RR) cv 'Gazelle'. At 24 h and 48 h before and after pollination, the pollinated florets of Canada wild rye were sprayed with GA3 (50 ppm). Fifteen days after pollination, the immature embryos were excised, sterilized in 4%–6% sodium hypochlorite solution for 5 min, rinsed three times with sterilized water, and then cultured on MS medium (GIBCO) in

15 mm × 180 mm test tubes. The seedlings differentiated from the cultured embryos were transplanted into soil in the greenhouse at the three-leaf stage. Undifferentiated embryos were transferred to MS callus culture medium containing 2 mg 2,4-D per liter. Calli were initiated in dark conditions for the first month and then subcultured monthly on fresh MS callus culture medium in a growth chamber at 24° ± 1 °C and 110 $\mu Em^2/s$ fluorescent light. Three-month-old calli were transferred to hormone-free MS medium for plant regeneration. The regenerated plantlets under the same culture conditions as for callus culture were moved to soil in the greenhouse at the three-leaf stage. At maturity, the parents and F_1 hybrids from both procedures were studied for such quantitative characters as plant height, tiller number, leaf size (length and width), leaf area, spike length, dry weight, leaf/stem ratio, and fertility. Pollen fertility was determined from pollen grains stained with 1.5% acetocarmine. Seed fertility was considered to be the numbers of seeds per spike. The regenerated hybrid plants were split into the individual tillers to double their somatic chromosome complements by colchicine treatment. Four-hundred-and-eight tillers were trimmed and immersed in the mixture of 0.2% colchicine and 4% DMSO for 24 h. After 6-h rinse in tap water, the tillers were potted in the greenhouse and the surviving plants were examined for somatic chromosome numbers, dehiscent anthers, dusty pollen, and seeds set in florets. Doubled sectors (C_0) were separated from the mixoploid clones. Open-pollinated seeds (C_1) were collected from the C_0 clones and the C_1 seedlings were grown in the greenhouse. The same morphological characters and fertility as for the parents and F_1 hybrids were studied for the amphiploid plants (C_0 and C_1).

Somatic chromosome numbers and karyotypes were determined for root-tip cells which were treated in cold water at 2 °C for 24 h and then fixed in acetic alcohol (1:3). Pollen mother cells (PMCs) were fixed in Carnoy's (6:3:1) solution to observe chromosome behavior at metaphase I (MI), anaphase I (AI), and the quartet stages. Squashed preparations for mitosis and meiosis were made in 1.5% acetocarmine solution.

Results

Production of F_1 hybrids and amphiploids

The pollination of 70 florets of *E. canadensis* with pollen from *S. cereale* cv 'Gazelle' yielded 28 embryos (40%), of which 5 germinated on MS culture medium; 2 plants grew and were identified as hybrids (triploids). The 2 hybrid plants necrotized at seedling and heading stage, respectively. Twenty-three embryos which did not germinate in embryo culture were transferred to MS callus culture medium. From these, 1 embryo produced white and compact calli (Fig. 1a). Four hybrid plants were regenerated via organogenesis from the calli which had been subcultured for 3 months (Fig. 1b). These grew vigorously to maturity. Of the 480 clones which were treated with colchicine and DMSO, 7 had doubled sectors, from which 4 amphiploid plants (C_0) were obtained. Nineteen C_1 plants were obtained from the open-pollinated seeds (28 plump seeds) of the C_0 plants.

Plant morphology and fertility

The morphological characteristics and dry weight of the parents, F_1 hybrids (both embryo-rescued and embryo-callus-regenerated), and amphiploids (C_0 and C_1) are shown in Table 1. The data of the embryo-rescued hybrid which died at the heading stage were obtained prior to necrosis. Most of the characters of the callus-regenerated hybrids were intermediate between the parents, but the hybrids produced more profuse tillers than the parents. As a result, dry weight of the hybrids was 64% and 335% higher than that of *E. canadensis* and *S. cereale*, respectively. However, the superiority of the hybrids over the parents in dry matter yield tended to decline during regrowth followed by clipping (data not shown). The leaf/stem ratio of the hybrids was significantly higher than that of the parents. There was no significant difference in morphology among the four callus-regenerated hybrids. Most of the hybrid pollen were empty and non-stainable. All of the hybrids were completely sterile. Amphiploids (C_0 and C_1) showed a substantial loss in vigor compared to the F_1 hybrids and *E. canadensis* (Fig. 1c–g).

Table 1. Morphological characteristics of parents, F_1 hybrids and amphiploids (C_0 and C_1) from crosses between *E. canadensis* and *S. cereale*

Genotype	No. of plants	Plant height (cm)	No. of tillers	Leaf length (cm)	Leaf width (cm)	Spike length (cm)	Leaf area (cm^2)	Dry weight (g)	Leaf/stem ratio
E. canadensis	4	117.0	27.0	36.0	1.0	20.2	7.16	28.9	1:1.8
		(7.62)[a]	(4.08)	(1.43)	(0.08)	(0.71)	(0.36)	(2.52)	(0.09)
S. cereale	4	99.8	14.3	25.0	1.3	9.2	4.21	10.9	1:2.1
		(7.52)	(4.50)	(2.50)	(0.08)	(0.42)	(0.28)	(1.19)	(0.18)
Hybrid (embryo-rescued)	1	43.8	41.0	23.8	0.9	–	3.67	7.2	1:1.6
Hybrids (regenerated)	4	115.6	86.5	25.8	1.1	15.7	5.29	47.4	1:3.2
		(5.12)	(3.11)	(2.12)	(0.08)	(1.69)	(0.62)	(2.15)	(0.37)
Amphiploids (C_0)	4	103.9	28.3	19.8	0.8	15.4	4.98	15.3	1:1.4
		(7.89)	(7.27)	(1.37)	(0.05)	(0.90)	(0.67)	(0.88)	(0.13)
Amphiploids (C_1) ($2n=42$)	2	94.9	7.0	26.6	1.0	19.3	3.52	5.5	1:1.5
		(18.80)	(2.83)	(7.07)	(0.14)	(0.28)	(0.48)	(0.99)	(0.28)

[a] Standard errors in parentheses

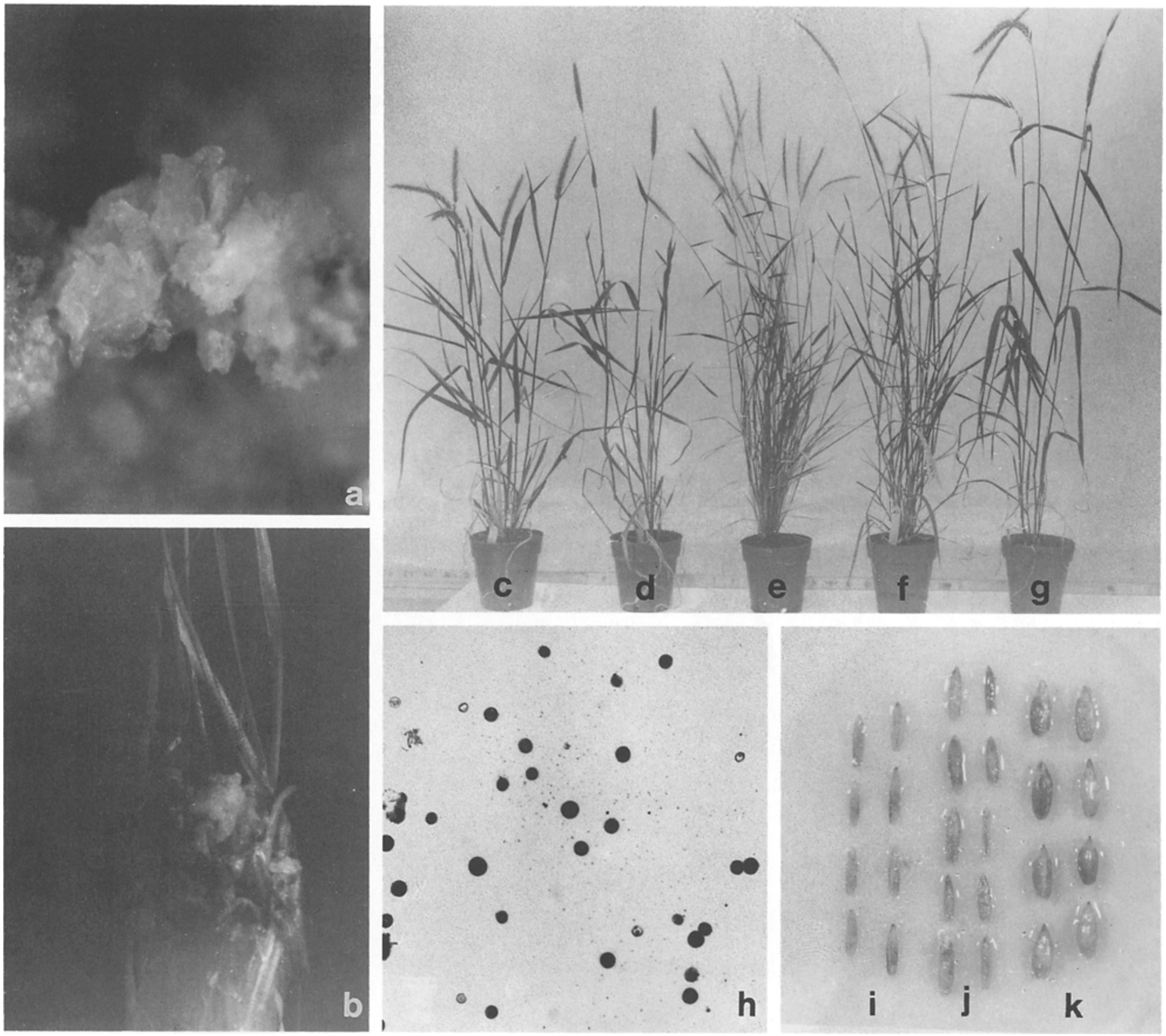

Fig. 1. **a** Hybrid embryo-derived callus; **b** plant regenerated from the callus; **c–g** plant morphology; **c** *E. canadensis;* **d** *S. cereale;* **e** F_1 hybrid; **f** C_0 amphiploid; **g** C_1 amphiploid ($2n=42$); **h** stained pollen of C_0 amphiploid; **i–k** seed morphology; **i** *E. canadensis;* **j** C_0 amphiploid; **k** *S. cereale*

C_0 amphiploid plants had smaller leaves than did their parents and the F_1 hybrids. Leaf area and dry weight of the C_0 plants were, respectively, 18% and 40% higher than those of *S. cereale,* but 30% and 47% lower than those of *E. canadensis.* The bursting pollen of the C_0 plants showed an average 56% of pollen stainability (Fig. 1 h) and set an average 4.6 seeds per spike. However, more than 90% of the seeds were shriveled and did not germinate. Twenty-eight plump seeds (9.3%) in all germinated of which sizes were intermediate between the parents (Fig. 1 i–k), and from these, 19 C_1 seedlings developed at irregular intervals. Of the C_1 seedlings, 3 early-maturing plants were examined for morphological characters and fertility. In comparison with C_0 plants, the C_1 plants had increased leaf length and leaf width but reduced tiller numbers (Fig. 1 g). Pollen stainability of the C_1 plants averaged 38% at euploid level ($2n=42$) and 12% at aneuploid level ($2n=40$). One seed per spike set in the C_1 plant ($2n=42$), while the C_1 aneuploid was completely infertile.

Cytological behavior

A majority of the F_1 hybrid somatic cells were triploids ($2n=21$) and those of the C_0 amphiploids were hexaploid ($2n=42$) (Fig. 2 a and b). However, two hexaploids and one aneuploid ($2n=40$) were observed among the three C_1 plants examined. Chromosome numbers of the other

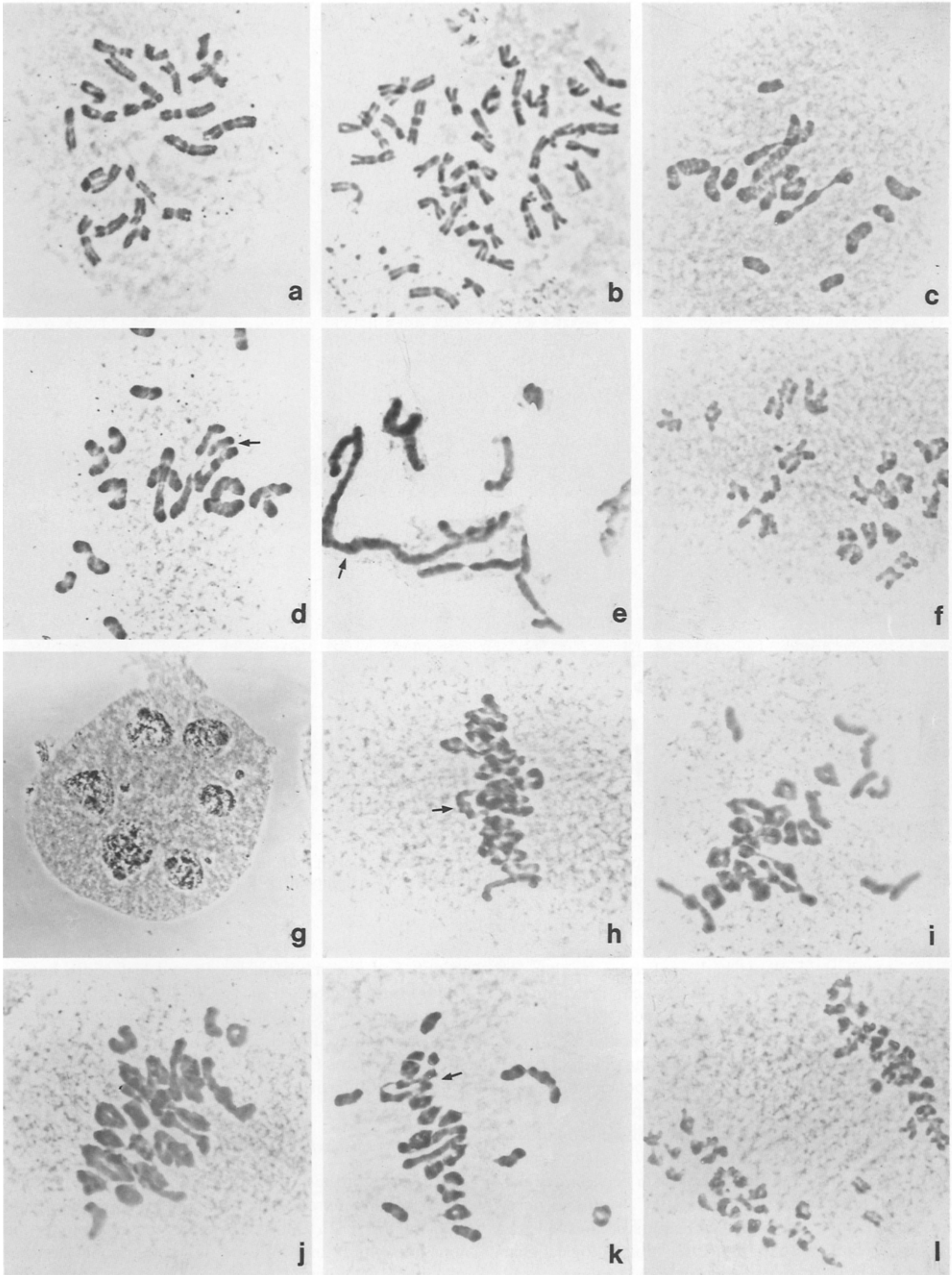
a
b
c
d
e
f
g
h
i
j
k
l

Table 2. Chromosome associations at MI of parents, F_1 hybrids and amphiploids (C_0 and C_1) between *E. canadensis* and *S. cereale*

Genotype	2n	Genome constitution	No. of plants	No. of cells	Mean chromosome associations (range)						X̄ta
					I	Rod II	Ring II	III	IV	V	
E. candensis	29	SSHH	2	98	0.12 (0–4)	0.71 (0–3)	13.23 (11–13)	–	–	–	27.16
S. cereale	14	RR	2	113	0.23 (0– 2)	0.87 (0–6)	6.01 (1–7)	–	–	–	12.03
Hybrid (embryo-rescued)	21	SHR	1	117	17.98 (11–21)	1.28 (0–4)	0.19 (0–2)	0.01 (0–1)	0.02 (0–1)	–	0.58
Hybrids (regenerated)	21	SHR	4	417	16.56 (7–21)	1.77 (0–7)	0.28 (0–4)	0.06 (0–2)	0.02 (0–1)	0.02 (0–1)	0.95
Amphiploids											
C_0	42	SSHHRR	2	119	2.20 (0–14)	6.61 (5–20)	13.26 (1–15)	–	0.02 (0–1)	–	32.94
C_1	42	SSHHRR	2	86	3.40 (0–14)	5.56 (2–10)	13.82 (6–19)	0.02 (0–1)	–	–	33.15
C_1	40	?	1	106	7.10 (0–20)	6.00 (1–12)	10.37 (5–17)	0.04 (0–1)	0.02 (0–1)	–	26.74

16 C_1 plants have not been determined at present. *S. cereale* chromosomes were readily distinguished from *E. canadensis* chromosomes by their larger size. There were no differences in karyotypes between the embryo-rescued hybrids and the embryo-callus-regenerated hybrids. Table 2 shows the chromosome associations of the parents, F_1 hybrids (both embryo-rescued and callus-regenerated), and amphiploids (C_0 and C_1) at MI. *E. canadensis* and *S. cereale* genotypes used in this study showed, respectively, 13.9 and 6.9 bivalents per cell at MI. Ninety-five percent of bivalents were ring-types in *E. canadensis*, as were 87% of bivalents in *S. cereale*.

All hybrids showed low chromosome pairing ranging from 1.47 to 2.52 in bivalent frequency per cell. The callus-regenerated hybrids exhibited a higher frequency of bivalents than those of the embryo-rescued hybrids. More than 85% of bivalents in all hybrids were loosely or well-connected rod-types (Fig. 2c). Multivalents such as trivalents, quadrivalents, and pentavalents were not commonly observed in the hybrid PMCs (Fig. 2d and e). The callus-regenerated hybrids showed quadrivalents in 1.2% of PMCs and pentavalents in 0.5% of PMCs. The most frequent chromosome association of all hybrids at MI was 2 bivalents and 17 univalents. Most of the hybrid PMCs showed unequal chromosome disjunction at AI (Fig. 2f) and some of them contained occasional laggards and bridges. Abnormal cytokinesis and micronuclei of variable numbers and sizes were occasionally observed in the quartet stage (Fig. 2g). Meiotic chromosome behavior of the amphiploids was characterized by the predominance of bivalents (Fig. 2h and i). Sixty-seven percent of bivalents in the C_0 amphiploids were well-connected rod-types ranging from 1 to 15 per cell. In the C_0 amphiploid PMCs, the frequent chromosome configurations at MI were 21 bivalents (34%) and 20 bivalents with 2 univalents (36%). Univalents averaged 2.20 per cell ranging from 0 to 14, and quadrivalents were observed in only two cells. Mean chiasma frequency per cell was 32.9. The chromosome distributions at AI were predominantly unequal (19:23 or 20:22) (Fig. 2l), and only 15% of the AI cells showed equal chromosome disjunctions (21:21). Laggards and bridges were occasionally observed in the anaphase stage as were micronuclei ranging from one to six in the quartet stage. The chromosome pairing of the C_1 amphiploids (2n = 42) at MI was almost the same in the C_0 plants (Fig. 2j). However, the aneuploid plant having two chromosomes less than hexaploid showed a higher frequency of univalents and trivalents than the hexaploid plants of C_0 and C_1 (Fig. 2k). The irregular chromosome behavior of the C_1 plants at AI and in the quartets were of the same general nature as in the C_0 plants.

◄

Fig. 2a–l. Somatic chromosome complements and meiotic chromosome behavior of *E. canadensis* × *S. cereale* hybrids and amphiploids. **a** F_1 hybrid (2n = 21); **b** amphiploid (2n = 42); **c–e** MI in the F_1 PMCs; **c** $5_{II}+11_{I}$; **d** 1_{III} (*arrow*)$+2_{II}+14_{I}$; **e** 1_{V} (*arrow*)$+1_{III}+2_{II}+9_{I}$; **f** AI in the F_1 PMC showing irregular chromosome disjunction; **g** quartet in the F_1 PMC with micronuclei; **h–k** MI in the amphiploid PMCs; **h** 1_{IV} (*arrow*)$+19_{II}$ (C_0); **i** $17_{II}+8_{I}$ (C_0); **j** $19_{II}+4_{I}$ (C_1, 2n=42); **k** 1_{IV} (*arrow*) $+1_{III}$ $+13_{II}+7_{I}$ (C_1, 2n=40); **l** AI in the C_0 amphiploid PMC showing unequal chromosome distribution

Discussion

F_1 hybrids of *E. canadensis* with *S. cereale* (cv 'Prolific') was first obtained through the conventional embryo rescue by Hang and Franckowiak (1984), but this study is the first report of the regenerants from the callus culture of *E. canadensis* × *S. cereale* (cv 'Gazelle') hybrid embryo and their colchicine-induced amphiploids. It is interesting that hybrid necrosis was not expressed in the regenerants, while the embryo-rescued hybrids died of hybrid necrosis. This might be a result of genetic modification during the process of tissue culture. Hybrid necrosis, which was found in some hybrids of wheat (Hermsen 1963) and *Agropyron* (Wang 1987a), was assumed to be controlled by complementary genes. In spite of hybrid vigor of the F_1, the reduction in tillering capacity and leaf sizes resulted in a decrease of dry matter yield in the amphiploids (C_0 and C_1). Although fertility was restored to some extent in the C_0 amphiploid plants, it declined in the C_1 plants. A substantial loss in vegetative vigor and a decline in fertility have been reported previously in high-ploidy-level (8x or 6x) amphiploids of *Elymus* × *Agropyron* hybrids (Asay and Dewey 1976; Dewey 1977) and *E. canadensis* × *Psathyrostachy juncea* hybrids (Park and Walton 1989). However, *E. canadensis* × *Agropyron libanoticum* amphiploids (SSSSHH) showed some very desirable characteristics and cytological stability (Dewey 1974). The vigorous regrowth of the F_1 hybrids and amphiploids reported here indicates a perennial growth habit. However, the potential of increasing forage yield by the repeated regrowth and improving forage quality were not studied.

E. canadensis (SSHH) × *S. cereale* (RR) hybrids were allotriploid of genome formular SHR and the amphiploids were allohexaploid (SSHHRR) or near-hexaploid ($2n = 40$). The low chromosome pairing of the F_1 hybrids and the predominance of bivalents (diploidization) of the amphiploids indicate the distinctness among the genomes (S, H, and R). However, the occasional appearance of multivalents reflects a randomy, intergenomic or intragenomic pairing, even though it occurs at a low frequency. A few reports have recently been documented on the genomic relationships between the genomes: SH (Asay et al. 1987), SR (Wang 1987b), HR (Gupta and Fedak 1985, 1987a, b), and SHR (Hang and Franckowiak 1984). There was a remote phylogenetic relationship between the genomes in each cross, and some capability of intragenomic pairing was found in H and R genomes (Gupta and Fedak 1987a) and in S and H genomes (Asay et al. 1987).

If this is true, regardless of genotypes used in hybridization, multivalents in this study may have resulted from the intragenomic pairing in H, S, and R genomes. On the other hand, multivalent association may be due to the intergenomic pairing between chromosomes structurally rearranged by tissue culture and their intact homologous counterparts. This hypothesis would explain the higher frequency of chromosome pairing in the callus-regenerated hybrids than in the embryo-rescued hybrids. Induction of structural chromosome rearrangements accompanied by increasing chromosome associations were reported in the regenerants from the cultured inflorescences or embryos of *Triticum* × *Hordeum* hybrids (Fedak and Grainger 1986) and *Hordeum vulgare* × *H. jubatum* hybrids (Orton 1980). Sterility in the *E. canadensis* × *S. cereale* amphiploids appears to be due to genetically unbalanced gametes resulting from heterogenetic pairing and meiotic irregularity. Although the performance of *E. canadensis* × *S. cereale* amphiploids in the greenhouse has fallen short of expectations, the opportunity for introgression between two species may still be possible by backcrossing to the parents and repeating somatic cell and tissue culture of the amphiploids.

Acknowledgements. We wisk to thank the Natural Sciences and Engineering Research Council of Canada and the Alberta Wheat Pool for the financial support that made this work possible.

References

Asay KH, Dewey DR (1976) Fertility of 17 colchicine-induced perennial *Triticeae* amphiploids through four generations. Crop Sci 16:508–513

Asay KH, Hsiao C, Dewey DR (1987) Intergeneric hybrids and amphiploids between *Pseudoroegneria spicata* and *Critesion violaceum*. Bot Gaz 148:123–129

Dewey DR (1974) Hybrids and induced amphiploids of *Elymus canadensis* × *Agropyron libanoticum*. Am J Bot 61:181–187

Dewey DR (1977) Morphology, cytology, and fertility of F_1 and induced-amphiploid hybrids of *Elymus canadensis* × *Agropyron subsecundum*. Crop Sci 17:106–111

Fedak G, Grainger J (1986) Chromosome instability in somaclones of a *Triticum crassum* × *Hordeum vulgare* hybrid. Can J Genet Cytol 28:618–623

Gupta PK, Fedak G (1985) Meiosis in seven intergeneric hybrids between *Hordeum* and *Secale*. Z Pflanzenzuecht 95:262–273

Gupta PK, Fedak G (1987a) Preferential intragenomic chromosome pairing in two new diploid intergeneric hybrids between *Hordeum* and *Secale*. Genome 29:594–597

Gupta PK, Fedak G (1987b) Meiosis in two new intergeneric hybrids between *Hordeum* and *Secale*. Plant Breed 99:159–163

Hang A, Franckowiak JD (1984) Cytological analysis of an *Elymus canadensis* × *Secale cereale* hybrid. J Hered 75:235–236

Hermsen JH (1963) The genetic basis of hybrid necrosis in wheat. Genetica 33:245–287

Larkin PJ (1985) In vitro culture and cereal breeding. In: Bright SWJ, Jones MGK (eds) Cereal tissue and cell culture. Nijhoff, Dortrecht, pp 273–296

Merkle OG, Guenzi AC, Veal RA, Baker CA (1988) Comparison of embryo rescue with callus induction and plant regeneration for recovering interspecific wheat hybrids. Agronomy Abstracts, Madison/WI

Mujeeb-kazi A, Bernard M (1985) Cytogenetics of intergeneric *Elymus canadensis* × *Triticum aestivum* hybrids ($n = 5x = 35$, SHABD) and their backcross progenies with *T. aestivum*. Z Pflanzenzuecht 95:50–62

Mujeeb-kazi A, Rodriguez R (1982) Cytogenetics of hybrids of *Elymus canadensis* × *Hordeum vulgare*. J Hered 73: 77–79

Orton TJ (1980) Chromosomal variability in tissue cultures and regenerated plants of *Hordeum*. Theor Appl Genet 56: 101–112

Park CH, Walton PD (1989) Intergeneric hybrids and an amphiploid between *Elymus canadensis* and *Psathyrostachys juncea*. Euphytica (in press)

Stoskopf NC (1985) Cereal grain crop. Reston, Virginia, pp 403–404

Walton PD (1983) Production and management of cultivated forages. Reston, Virginia

Wang RRC (1987a) Progenies of *Thinopyrum elongatum* × *Agropyron mongolicum*. Genome 29: 738–743

Wang RRC (1987b) Diploid perennial intergeneric hybrids in the tribe *Triticeae*. III. Hybrids among *Secale montanum*, *Pseudoroegneria spicata*, and *Agropyron mongolicum*. Genome 29: 80–84

Yen Y, Liu D (1987) Production, morphology, and cytogenetics of intergeneric hybrids of *Elymus* L. species with *Triticum aestivum* L. and their backcross derivatives. Genome 29: 689–694