6-24-1994

Experimental Hybridization and Genome Analysis in Elymus L. Sect. Caespitosae and Sect. Elytrigia (Poaceae: Triticeae)

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ABSTRACT

Crossing experiments were performed between and within taxa of *Elymus* sect. *Elytrigia* and sect. *Caespitosae* from Iran and two taxa from Central Asia and China. The hexaploid *Elymus repens* (genomic constitution SSH) was crossed with the octoploid *E. elongatiformis*. The chromosome associations at meiosis show that *E. elongatiformis* possesses the SSH genome of *E. repens* as well as and additional genome of unknown origin. Crosses between different accessions of *E. libanoticus* (genomic constitution S) from W., NW. and N. Iran as well as crosses between *E. libanoticus* and accessions morphologically assignable to *Elytrigia gracillima* and *Elymus sosnovskyi* should therefore be merged into *E. libanoticus*. Crosses between *Elymus libanoticus* and the diploid *Elytrigia geniculata* ssp. *ferganensis* and *Elytrigia strigosa* ssp *aegilopoides* showed a high degree of meiotic pairing (c-values 0.6-0.8) confirming that these taxa have the genomic constitution S, as reported by Löve. The pollen fertility was zero in both hybrids. The configurations at metaphase I in a hybrid between *E. libanoticus* and *E. pertenuis* (genomic constitution SP) indicate that the two species may share the same version of the S genome.

INTRODUCTION

The study deals with crossing experiments within *Elymus* sect. *Caespitosae* (Rouy) Melderis and sect. *Elytrigia* (Desv.) Melderis, belonging to *Pseudoroegneria* and *Elytrigia s. str.*, respectively, in the classification based on genomic constitutions (Löve 1984). In accordance with Melderis (1980, 1985) and Assadi & Runemark (1995), a relatively broad generic concept is used.

MATERIALS AND METHODS

The species used in the crossing experiments are shown in Table 1.

Elytrigia geniculata ssp. *ferganensis* and *Elytrigia strigosa*

Table 1. Genomic constitutions of *Elymus* species used in the crosses.

Taxa	2n	Genomes	References	Origin
Elymus repens (L.) Gould	42	SSH	Assadi & Runemark 1994	Iran
—*elongatiformis* (Drobov) Assadi	56	SSHX	This study	Iran
—*libanoticus* (Hackel) Melderis	14	S	Dewey 1972	Iran
—*pertenuis* (C. A. Meyer) Assadi	28	SP	Assadi 1994a	Iran
Elytrigia geniculata (Trin.) Nevski ssp. *ferganensis* (Drobov) Tzvelev	14	S	This study	C. Asia
—*strigosa* (Bieb.) Nevski ssp. *aegilopoides* (Drobov) Tzelev	14	S	This study	C. Asia & China

ssp. *aegilopoides* are probably members of *Elymus* sect. *Caespitosae (Pseudoroegneria* according to Löve's (1984) classification based on genomic constitutions) but, since they belong to a critical species complex (Tzvelev 1976) which has not yet been satisfactorily revised, the author at present refrains from publishing new combinations under *Elymus*.

Information on the origin of the accessions used is available from the author on request. Voucher specimens are deposited in TARI and LD. For the methods used in seed germination, vernalization, mitotic and meiotic studies, crosses and pollen fertility tests, see Assadi & Runemark (1995). The c-values were calculated according to Wang (1989).

RESULTS

All successful crossing combinations are given in Table 2. The hybrid plants grew well and no hybrid weakness was observed. Table 3 shows the mean chromosome associations at meiotic metaphase I as well as chiasma frequencies and pollen fertility (percentage of stainable pollen grains) in the crossing combinations.

Elymus elongatiformis x E. repens

The parents are morphologically distinct (Assadi 1995a). *Elymus elongatiformis* is octoploid and *E. repens* is hexaploid. The hybrid was morphologically closer to *E. elongatiformis*, with lax spikes, ciliate sheaths, obtuse and mucronate glumes and lemmas, and mid spike internodes c. 7 mm long. The chromosome number was 2n=49. The anthers dehisced and the pollen fertility was 72%. A mean of 7.12 univalents, 20.68 bivalents and 0.14 multivalents and 38.18 chiasmata was observed in PMCs at metaphase I. Six to 12 lagging univalents were observed at anaphase I.

Elymus libanoticus intraspecific crosses

Elymus libanoticus is diploid and shows intraspecific variation in glume shape, stem indumentum and leaf width. Collections with narrow, 3-nerved glumes with an acuminate-subulate apex were similar to *E. sosnovskyi* and collections with thin, filiform leaves were similar to *Elytrigia gracillima* (Nevski) Nevski (e. g. accession H3729) described from the Caucasus. Accessions from N., NW., and W. Iran, representing different morphological variants, were used in the crossing experiments. All six crosses gave rise to vigorous hybrids, with pollen stainability ranging from 70-99%. A mean of 6.50 to 7.00 bivalents was observed at metaphase I. The chiasma frequency varied from 10.98 to 13.32.

Elymus libanoticus x E. pertenuis

Elymus libanoticus is a diploid with the genomic constitution S and *E. pertenuis* is a tetraploid with the genomic constitution SP. The hybrid was morphologically intermediate between the parents. The glumes were pointed, a possible influence from the P genome. The anthers did not dehisce and the pollen fertility was zero. The hybrid was triploid with an average of 6.36 univalents, 6.18 bivalents and 0.76 trivalents. Four lagging univalents were observed at anaphase I.

Table 2. Results of the crossing program in *Elymus* sect. *Elytrigia* and sect. *Caespitosae* (percentages are based on the number of flowers crossed).

Combinations	No. of combinations	No. of flowers	Seed set %	Embryo %	Plants %
Sect. *Elytrigia*					
Elymus elongatiformis x E. repens	1	18	33	-	17
Sect. *Caespitosae*					
E. libanoticus x E. libanoticus	6	135	19	15	15
E. libanoticus x E. pertenuis	1	26	77	19	23
E. libanoticus x E. sosnovskyi	1	12	17	17	8
E. libanoticus x Elytrigia geniculata ssp. *ferganensis*	1	18	67	61	44
E. libanoticus x Elytrigia strigosa ssp. *aegilopoides*	2	60	52	-	33
Elytrigia strigosa ssp. *aegilopoides* x *Elymus libanoticus*	1	20	25	-	5

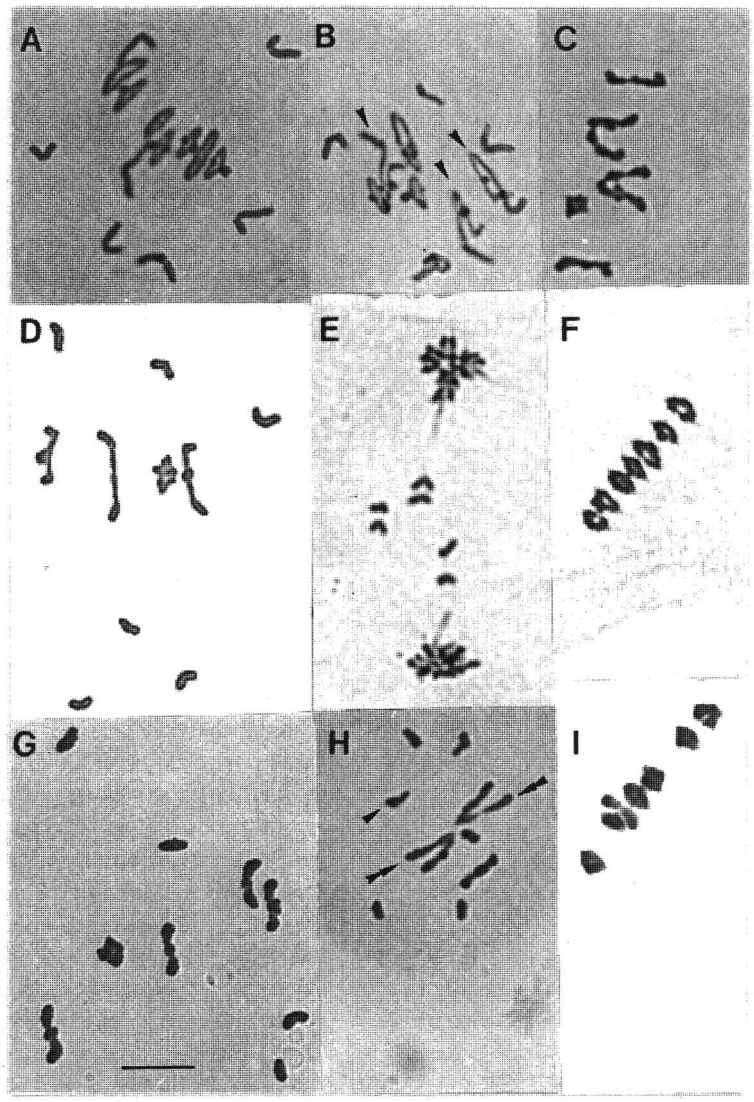

Figure 1 Meiotic configurations at metaphase I (A-D & F-I) and anaphase I (E) in *Elymus* species. (A-B): *E. libanoticus x E. pertienuis* (2n=21) with 7 univalents and 7 ring bivalents in A, and 6 univalents, 3 ring bivalents and 3 trivalents indicated by arrows in B. (C-E): *E. libanoticus x Elytrigia geniculata ssp. ferganensis* (2n+14) with 7 bivalents (6 rods and 1 ring) in C, 6 univalents and 4 bivalents (3 rods and 1 ring) in D, and 6 lagging univalents in E. (F): *Elymus sosnovskyi x E. libanoticus aegilopoides* with 4 univalents and 5 bivalents (4 rods and 1 ring) in G, 4 trivalents indicated by double arrows in H, and 7 bivalents (1 rod and 6 rings) in I. —Bar+ 10 mm.

Elymus libanoticus x E. sosnovskyi

Elymus sosnovskyi was described from material from a locality near the Iranian border in Turkey. It has been distinguished from *E. libanoticus* by its narrower 3-veined glumes with an acuminate-subulate apex (see Melderis 1985). The accession (H3741) used in the cross was collected very close to the type locality of *E. sosnovskyi*. Two hybrid combinations (representing reciprocal crosses) were produced. The hybrids were vigorous and had pollen stainability of 97 and 94%, respectively, and a mean of 6.98-7.00 bivalents (chiasma frequencies of 13.34 and 13.48) were observed at metaphase I.

Elymus libanoticus x Elytrigia geniculata ssp. *ferganensis*

The two taxa are allopatric, with *E. libanoticus* occurring in Lebanon, Turkey, Iraq, Iran and the Caucasus, while the other taxon is confined to Central Asia. Both taxa are diploids. The hybrid is morphologically closer to *Elytrigia geniculata* than to *E. libanoticus* and has scabrous culms and rachis, and lanceolate glumes with a broad membranous margin. The anthers did not dehisce and pollen fertility was zero. A mean of 2.95 univalents, 5.50 bivalents and 0.02 trivalents and a chiasma frequency of 8.62 was found at metaphase I. In 9 of the 50 cells studied 7 bivalents were observed.

Elymus libanoticus x Elytrigia strigosa ssp. *aegilopoides*

Elytrigia strigosa ssp. *aegilopoides* occurs in Siberia, Central Asia and China, far from the distibutional area of *Elymus libanoticus*. Both taxa are diploids. The hybrids were intermediate between the parents and vigorous. The anthers did not dehisce and the pollen fertility was zero. Three crossing combinations, including a reciprocal one, were made. A mean of 6.26, 5.52, and 6.52 bivalents and a chiasma frequency from 8.36 to 11.24 were observed in the hybrids.

DISCUSSION

Sect. *Elytrigia*

Elymus repens is hexaploid with the genomic constitution SSH (Assadi & Runemark 1995), while *E. elongatiformis* is octoploid. A mean of 7.12 univalents in the chromosome association of the hybrid *E. repens x elongatiformis* indicates that *E. elongatiformis* has the same genomic constitution as *E. repens* plus an additional unknown genome. Therefore, the genomic constitution of *E. elongatiformis* is designated as SSHX. The genomic configuration of the hybrid in the present study agrees with that given by Dewey (1980).

Sect. *Caespitosae*

All the taxa of the section used in the crosses belong to the genomically-defined genus *Pseudoroegneria* (cf. Löve's (1984) classification) which consists of c. 15 species in Asia and W. North America. According to Dewey (1984), hybrids between diploid species of *Elymus* have an almost complete bivalent pairing at metaphase-I but are completely sterile, indicating different versions of the same basic genome (S).

Elymus libanoticus is a morphologically variable diploid. Meiotic pairing was regular or almost regular and pollen fertility was high in the seven crosses between accessions from N., NW., and W. Iran, which included morphological variants similar to *Elytrigia gracillima* and *Elymus sosnovskyi*. The results of the present study indicate that *Elytrigia gracillima* and *Elymus sosnovskyi* should be included in *E. libanoticus* (see Assadi 1995b).

Elymus libanoticus was also crossed with the diploid *Elytrigia geniculata* ssp. *ferganensis* and *Elytrigia strigosa* ssp. *aegilopoides*. At metaphase-I means of 5.50 to 6.52 bivalents were observed and c-values ranged from 0.6 to 0.8. The complete pollen sterility of the hybrids, as well as a somewhat incomplete meiotic pairing, indicates that the *Elytrigia* species have different versions of the S genome compared to *E. libanoticus*.

Diploid *Agropyron cristatum* (L.) Gaertner, with the genomic constitution P, has been recorded from NW. Iran (Dewey and Asay 1975). *Elymus libanoticus*, with the genomic constitution S, has a relatively large distributional area from Lebanon to Turkey, Iraq, W., NW., and N. Iran and the Caucasus. *Elymus pertenuis*, with the genomic constitution SP, is confined to the Caucasus, NW., and W. Iran. From the present-day distribution pattern it seems reasonable to assume that *E. pertenuis* is an amphidiploid between equivalents to *E. libanoticus* and diploid *Agropyron cristatum*. The high number of bivalent in the hybrid *E. libanoticus x E. pertenuis* supports the suggested amphidiploid origin of *E. pertenuis*. However, this evidence is not conclusive, since the meiotic pairing may have been influenced by homoeologic pairing between chromosomes of the S and P genomes (see Wang 1989).

Table 3. Meiotic configurations and pollen fertility in the different hybrid combinations within and between *Elymus* species. Haploid genomic constitutions are given in parenthesis.

Name of parents and accessions ♀ x ♂	No. of cells	Mean and range of chromosome association and chiasmata/cell							Pollen %
		I	II			III	IV	Chiasmata	
			Total	Rods	Rings				
Elymus elongatiformis x E. repens (SSHX x SSH)									
H3727 x H3736	50	7.12 (1-13)	10.68 (19-24)	3.66 (0-9)	17.02 (11-20)	0.04 (0-1)	0.10 (0-1)	38.18 (31.43)	72
Elymus libanoticus x E. libanoticus (S x S)									
H3729 x H3751	50		7.00 (7)	1.20 (0.3)	5.8 (4.7)			12.80 (11-14)	93
H3729 x H3755	50	0.12 (0-2)	6.94 (6-7)	1.32 (0-4)	5.62 (3-7)			12.56 (10-14)	88
H3735 x H3754	50	0.12 (0.2)	6.94 (6-7)	0.68 (0-3)	6.26 (4-7)			13.20 (11-14)	89
H3755 x H2729	50		7.00 (7)	0.78 (0-3)	6.22 (4-7)			13.22 (11-14)	70
H3778 x H3735a	50		7.00 (7)	0.68 (0-3)	6.32 (4-7)			13.32 (11-14)	93
H3778 x H3787	50	1.00 (0-6)	6.50 (4-7)	2.02 (0-4)	4.48 (1-7)			10.98 (10-14)	99
Elymus libanoticus x E. pertenuis (S x SP)									
H3755 x H3733	50	6.36 (3-9)	6.18 (3-8)	1.16 (0-3)	5.02 (3-8)	0.76 (0-3)		12.74 (10-16)	0
Elymus libanoticus x E. sosnovskyi (S x S)									
H3755 x H3741	50		7.00 (7)	0.66 (0-3)	6.34 (4-7)			13.34 (11-14)	94
Elymus sosnovskyi x E. libanoticus (S x S)									
H3741 x H3751	50	0.04 (0-2)	6.98 (6-7)	0.48 (0-3)	6.50 (4-7)			13.48 (11-14)	97
Elymus libanoticus x Elytrigia geniculata ssp. *ferganensis* (S x SP									
H3751 x H10230	50	2.94 (0-8)	5.50 (3-7)	2.42 (0-6)	3.08 (0-7)	0.02 (0-1)		8.62 (5-14)	0
Elymus libanoticus x Elytrigia strigosa ssp. *aegilopoides* (S x S)									
H3755 x H10094	50	1.14 (0-8)	6.26 (3-7)	2.34 (0-6)	3.92 (1-6)	0.06 0-2)	0.04 (0-1)	10.42 (5-13)	0
H3754 x H7712	50	2.84 (0-8)	5.52 (1-7)	2.76 (0-5)	2.76 (0-5)	0.04 (0-2)		8.36 (3-11)	0
Elytrigia: strigosa ssp. *aegilopoides x Elymus libanoticus* (S x S)									
H10094 x H3729	50	0.96 (0-4)	6.52 (5-7)	2.80 (0-7)	4.72 (0-7)			11.24 (5-14)	0

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