The Maize–Gamagrass Hybrids (2*n* = 56, 20*Zm* + 36*Td*) Carrying Genomes of the Lines Used for Obtaining Heterosis F1 (

P. A. Panikhin[1], E. A. Abdyrakhmanova[1, 3], C. A. Blakey[2], and V. A. Sokolov[1, 3]

1 Cytology and Apomixis Laboratory, Institute of Molecular and Cellular Biology, pr. Akad. Lavrentieva 8/2, Novosibirsk, 630090 Russia
2 Department of Biology, Ball State University, Muncie, IN 47306-0440, USA
3 Vavilov Institute of Plant Industry, ul. Bol'shaya Morskaya 44, St. Petersburg, 190000 Russia

Continuing our study of apomixis in the hybrids between the maize (*Zea mays*, 2*n* = 2*x* = 20) and gamagrass (*Tripsacum dactyloides*, 2*n* = 4*x* = 72), we set the challenge to obtain a new set of 56-chromosome hybrids (2*n* = 56 = 20*Zm* + 36*Td*). Note that the main goal of this work was to combine in their genomes two haploid sets of the maize lines used for obtaining heterosis hybrids. The background for setting such goal was the earlier studies demonstrating that at least nine chromosomes of the wild parent were necessary for maintaining an apomictic reproduction in maize–gamagrass hybrids (Sokolov et al., 1998; Sokolov and Khatypova, 2000). This quantity of the gamagrass genetic material is able to considerably alter the expression of heterosis; in this case, its fixation via adopting the elements of apomixis from the wild relative should be regarded as groundless.

The 573MV and Kubanskaya 611SV lines (analogs of B73 and Mo17, respectively, utilized in breeding commercial heterosis hybrids) were used as cultivated parents. To obtain the F1 progeny, the chosen lines were pollinated with the pollen of tetraploid gamagrass earlier used to produce apomictic hybrids (Fig. 1).

The seed setting rate in the crosses where maize lines were pollinated with gamagrass was rather high, allowing for producing a considerable number of F1 hybrids (Figs. 2 and 3). However, this rate is somewhat lower as compared with that observed in the case of gamagrass self-pollination (Table 1). Note that a low percentage of set seeds observed in the years of 2009 and 2013 was associated with adverse weather conditions, which prevented an optimal pollination. Then the produced F1 seeds were planted in fields of the Kuban' experimental station (Institute of Plant Industry); the grown plants (Figs. 4 and 5) were used in two variants of hybridization, namely, (1) pollination with the maize line giving heterosis F1 hybrids with the cultivated parent (Table 3) and (2) backcrossing with the cultivated parent (Table 2). The hybrids obtained in the first variant (2*n* = 56 = 20*Zm* + 36*Td*) will be used for assessing the expression of heterosis and the hybrids of the second variant analogous in their ploidy will serve as the control. When commencing this study, we expected to obtain segregation for the genes controlling apomixis, since the apomictic plants are heterozygous for this character. Thus, only part of the produced 46-chromosome hybrids (2*n* = 10*Zm* + 36*Td*) will further display this type of progeny production. Correspondingly, only the apomictic F1 plants will massively give functional caryopses after pollination with the maize. The remaining plants (non-apomictic) will develop sexual embryo sacs with the chromosome sets unbalanced as a result of meiosis and will mainly give inviable seeds.

This particular pattern was observed in the progenies of F1 hybrids pollinated with maize lines 573 and 611 (Tables 2 and 3).

Two classes of hybrids contrasting in the seed setting rate are evident in tables, namely, with a rate less than 5% and exceeding 20%.

The intermediate class with a seed setting rate of about 10% (7.7% in Table 2 and 11.5% in Table 3) is likely to result from concurrent development of sexual and diplosporic embryo sacs in a pollinated line. These data require further verification by hybridization with tester lines and cytological analysis.

The fact is that an *Antennaria*-type apomixis, observed in the gamagrass, is a genetically complex trait. We have earlier demonstrated that non-reduction (diplospory) and parthenogenesis in it are controlled in an independent manner (Sokolov, 2000) and the latter is likely to be subject to a polygenic control (Blakey et al., 2007). That is why the used gamagrass pollen may carry different combinations of the major and minor genes involved in the control of apomixis. As a result, this gives a fuzzy pattern of the well-filled grains in BC1 generation. Backcrossing of the apomictic F1 plants gives BIII hybrids ($2n = 56 = 20Zm + 36Td$), necessary for our further studies, at a rate of approximately 3–5% (Fig. 6). Therefore, their reliable production requires several hundreds of F1 plants. Currently, the available amount of seeds is sufficient for producing the necessary number of BIII hybrids of BC1 generation (Table 1).

References

1. Sokolov, V.A., Kindigir, B, and Khatypova, I.V., Apomictically reproducing 39-chromosome maize–*Tripsacum* hybrids, *Genetika,* 1998, vol. 34, no. 4, pp. 499–506 [in Russian].

2. Sokolov, V.A. and Khatypova, I.V., The development of apomictic maize: update, problems and perspective, *Acta Biol. Yugoslavica, Series F. Genetika,* 2000, vol. 32, no. 3. pp. 331–353.

3. Sokolov, V.A.. Independent inheritance and expression of apomeiosis and parthenogenesis in maize–gamagrass hybrids, *Dokl. Ross. Akad. Nauk,* 2000, vol. 374, no. 2, pp. 280–282 [in Russian].

4. Blakey, C.A., Costich, D., and Sokolov V.A., *Tripsacum* research: a perspective from observation along a river to molecular genomics, *Maydica,* 2007, vol. 52, pp. 81–99.

FIGURE SUMMARY

Figure 1. Ears of maize lines, their F1 reciprocal hybrids, and 46-chromosome F1 maize–gamagrass hybrids (line 611 × *T. dactyloides* and line 573 × *T. dactyloides*).

IN FIGURE:
F1 Kubanskaya 611SV × *T. dactyloides,* 46 chromosomes (10*Zm* + 36*Td*)
self pollinated line Kubanskaya 611SV
F1 Kubanskaya 611SV × 573MV
F1 573MV × Kubanskaya 611SV
self pollinated line 573MV
F1 573MV × *T. dactyloides,* 46 chromosomes (10*Zm* + 36*Td*)

Figure 2. Seeds of 46-chromosome F1 maize–gamagrass hybrids (line 611 × *T. dactyloides* and line 573 × *T. dactyloides*).

Figure 3. Seeds of line 611 and 46-chromosome F1 maize–gamagrass hybrid (line 611 × *T. dactyloides*).

Figure 4. Inflorescences of *T. dactyloides* (left) and 46-chromosome F1 maize–gamagrass hybrid (right).

IN FIGURE:
Inflorescence of *T. dactyloides* (2*n* = 4*x* = 72)
Ear of F1 hybrid line 573 × *T. dactyloides,* 46 chromosomes (10*Zm* + 36*Td*)

Figure 5. A plant of 46-chromosome F1 maize–gamagrass hybrid (line 573 × *T. dactyloides*).

IN FIGURE:
46-chromosome F1 maize–gamagrass hybrid line 573 × *T. dactyloides,* plant no. 4.8

Figure 6. A plant of 56-chromosome maize–gamagrass hybrid with a genomic composition of 10*Zm* (line 573) + 10*Zm* (line 611) + 36*Td.*

IN FIGURE:
BC1 of 46-chromosome F1 maize–gamagrass hybrid (line 573 × *T. dactyloides*) × line 611, plant no. 37.2

TABLES.

Table 1.
Seed setting rates of the maize lines 573 and 611 pollinated with *T. dactyloides* and self-pollinated gamagrass plants (2*n* = 4*x* = 72)

Pollination variants	Year	Number of pollinated ears	Number of set ears	Number of unset ears	Number of flowers	N g
573 x *T. dactyloides*	2010	5	5	0	3040	
611 x *T. dactyloides*		1	1	0	580	
573 x *T. dactyloides*	2011	11	11	0	6688	
611 x *T. dactyloides*		17	17	0	9945	
573 x *T. dactyloides*	2012	70	70	0	42589	
611 x *T. dactyloides*		18	18	0	10442	
T. dactyloides self-pollination	2009	93	28	65	852	
T. dactyloides self-pollination	2010	186	173	13	2076	
T. dactyloides self-pollination	2012	154	154	0	1719	
T. dactyloides self-pollination	2013	65	42	23	763	

Table 2.

BC1 variants	Year	Number of pollinated ears	Number of flowers	Number of set grains	Number of filled grains	Seed setting rate %
F1 no. 1.9 x 573	2012	2	16	7	6	43.8
F1 no. 3.8 x 573		3	34	17	13	50
F1 no. 3.15 x 573		3	26	2	1	7.7
F1 no. 4.8 x 573		6	88	1	0	1.1
F1 no. 3.8 x 573	2013	15	193	39	29	20.2
F1 no. 3.9 x 573		2	25	0	0	0
F1 no. 3.15 x 573		2	29	0	0	0

Seed setting rates of the 46-chromosome F1 maize–gamagrass hybrids line 573 (*Zea mays*) × *T. dactyloides* backcrossed with line 573

Table 3.
Seed setting rates of the 46-chromosome F1 maize–gamagrass hybrids line 573 (*Zea mays*) × *T. dactyloides* backcrossed with line 611

BC1 variants	Year	Number of pollinated ears	Number of flowers	Number of set grains	Number of filled grains	Seed setting ra %
F1 no. 1.9 x 611	2012	8	81	31	24	38.3
F1 no. 3.8 x 611		12	159	78	58	49.1
F1 no. 3.9 x 611		1	25	1	0	4
F1 no. 3.15 x 611		5	76	1	0	1.3
F1 no. 4.8 x 611		12	192	0	0	0
F1 no. 1.9 x 611	2013	24	275	105	83	38.2
F1 no. 3.8 x 611		44	603	212	161	35.2
F1 no. 3.9 x 611		11	139	16	9	11.5
F1 no. 3.15 x 611		10	115	3	3	2.6
F1 no. 4.8 x 611		58	672	3	2	0.5

Figure 1. Ears of maize lines, their F1 reciprocal hybrids, and 46-chromosome F1 maize–gamagrass hybrids (line 611 × *T. dactyloides* and line 573 × *T. dactyloides*).

F1 Kubanskaya 611SV × T. dactyloides, 46 chromosomes (10Zm + 36Td)	*self pollinated line Kubanskaya 611SV*	*F1 Kubanskaya 611SV × 573MV*	*F1 573MV × Kubanskaya 611SV*	*self pollinated line 573MV*	*F1 573MV × T. dactyloides, 46 chromosomes (10Zm + 36Td)*

Figure 2. Seeds of 46-chromosome F1 maize–gamagrass hybrids (line 611 × *T. dactyloides* and line 573 × *T. dactyloides*).

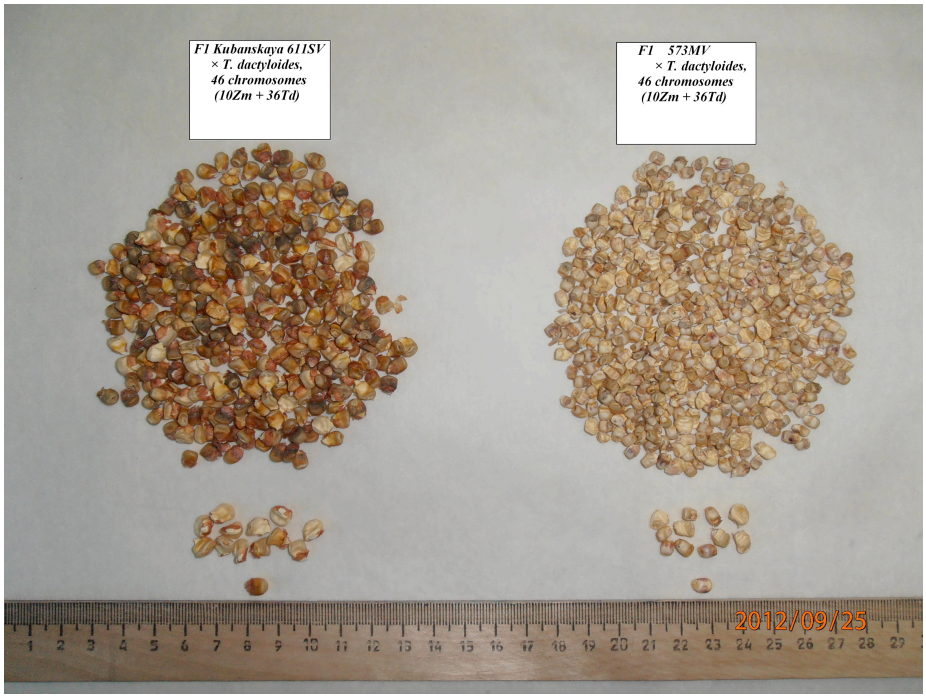

Figure 3. Seeds of line 611 and 46-chromosome F1 maize–gamagrass hybrid (line 611 × *T. dactyloides*).

Figure 4. Inflorescences of *T. dactyloides* (left) and 46-chromosome F1 maize–gamagrass hybrid (right).

Figure 5. A plant of 46-chromosome F1 maize–gamagrass hybrid (line 573 × *T. dactyloides*).

Figure 6. A plant of 56-chromosome maize–gamagrass hybrid with a genomic composition of 10*Zm* (line 573) + 10*Zm* (line 611) + 36*Td.*