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Effect of mutagens on imprinting expression in apomicts

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It has been long believed that apomixis is determined by two components – apomeiosis and parthenogenesis. The current work shows the considerable role of imprinting in viable seed formation of pseudogamic asexual species. Of significance to note, in apomicts the embryo sacs develop out of diploid cells, and the central cell is tetraploid until fertilization. In the initial endosperm cell development, the ratio of female to male genomes will be 4F:1M in its nucleus. Such a combination of male and female genomes in the cell nuclei of grain storage tissue is abnormally different from that of the regular 2F:1M necessary for viable seed formation. Thus, only 20% of asexual *Tripsacum* (gamagrass) florets actually give rise to viable seeds. Despite a considerable deviation of maize genomes in endosperm cells from that of regular 2F:1M, we obtain up to 50% viable seeds in the apomictic maize x *Tripsacum* hybrids produced.

In this case, grain development was connected with a suppression of imprinting dosage effect manifestations in the presence of *Tripsacum* chromosomes. In addition, grain size and weight dependence on the pollinator's ploidy and its quality was also revealed. Grains produced with one pollinator have a large range of weight and viability. This observation can be explained by a complex interaction of genes imprinted on male and female types in endosperm cell nuclei and environmental effects on their expression. As the average weight of grains is reduced in the presence of a large number of *Tripsacum* parent chromosomes, an attempt was made at using mutagens to effectively modify their expression.

For this purpose, aerated dry seeds of 39-chromosome apomictic maize x *Tripsacum* hybrids (30 Zm + 9 Td) were soaked in a chemical mutagenic solution, e.g., ethyl methanesulfonate, 5-azacytidine, natriumbutyrate, or trichostatine A; and they were also gamma-radiated. Comparative studies of grain weight in M2 allowed us to determine that only in the 5-azacytidine-treated (0.33 μM solution) families resulted in variants having a considerable average seed weight increase. The 5-azacytidine treatment was carried out twice--in the greenhouse (2002-2003) and the hothouse (2003) of the ICG SB RAS. In 2004, the control and M2 were grown in the experimental field of Krasnodar, Russia; M3 and M4 were grown at the Kuban VIR experimental station in 2005 and 2006, respectively. Di- and tetraploid maize--Mangelsdorf (2n=2x=20) and Tester purple (2n=4x=40)--were used as pollinators in M1 (greenhouse 2002-2004 and hothouse 2003); only tetraploid pollinator C-435 was used in M2-M4.

The results of these experiments are presented in Tables 1, 2, and 3, (also Figure 1: bronze = seed from 39-chromosome hybrid, white = 4n maize parent). As is seen from the data presented in Table 1, about 30% of the germs die from the 5-azacytidine treatment. We observed significant weight differences of the seeds produced (Table 2) in the pollination of 39-chromosome lines with di- and tetraploid maize. Only the seeds of the second experiment were used for further research of the effects of 5-azacytidine treatment in M1 and subsequent generations; observations are

Table 1. Effect of 5-azacytidine on treated grain viability.

	Experiment 1 (Greenhouse, 2002-2003)		Experiment 2 (Hothouse, 2003.)	
	K	AZ	K	AZ
Grains set	24	37	20	33
Grains germinated	24	36	20	33
Died in Petri dishes	0	6	0	3
Died after planting	0	6	1	7
Died total	0	12 (33.3%)	1	10 (30.3%)
Adult plants	24	24	19-3B_{III}	23-1B_{III}
Unpollinated (late)	8	7	1	7
Total of pollinated plants	16	17	15	15

Table 2. Grain weight in M1 of 5-azacytidine-treated (AZ) apomictic plant line 4x-6 (30Zm+9Td) as compared to Control (K), Experiment 1 (AZ), Greenhouse, 2002-2003.

Line	Pollinator (2n)	Grain weight (x ± m)	min	max	n		Pollinator (4n)	Grain weight (x ± m)	min	max	n
39 (K)	Mangelsdorf (K)	0.020±0.0031	0.005	0.095	30		TP (K)	0.074±0.0052	0.040	0.135	18
	Mangelsdorf (AZ)	0.029±0.0037	0.002	0.085	44		TP (AZ)	0.083±0.0039	0.005	0.130	64
							TSh(AZ)	*0.081±0.0032*	*0.010*	*0.125*	*74*
39 (AZ)	Mangelsdorf (K)	0.036±0.0035	0.010	0.105	54		TP(K)	0.069±0.0064	0.030	0.120	15
	Mangelsdorf (AZ)	0.033±0.0027	0.005	0.105	75		TP(AZ)	0.079±0.0055	0.015	0.135	32
							TSh(AZ)	*0.064±0.0025*	*0.005*	*0.130*	123

Table 3. Average grain weight of apomictic plant line 4x-6 (30Zm+9Td) treated with 5-azacytidine at 0.33 μM (AZ) in pollination with tetraploid maize (2n=4x=40).

Family No.	M1 (AZ)			M2 (AZ)			M3 (AZ)			M4 (AZ)		
	Grain weight	min-max	n	Grain weight	min-max	n	Grain weight	min-max	n	Grain weight	min-max	n

	(g)	(mg)		(g)	(mg)		(g)	(mg)		(g)	(mg)	
87	0.094±0.007	7-155	36									
88	0.084±0.006	3-140	37	0.092±0.004***	4-170	167	0.067±0.005	2-130	42	0.086±0.005***	2-165	116
90	0.113±0.006***	15-165	34	0.093±0.003***	2-195	279	0.065±0.003	5-115	92	0.069±0.005	2-140	74
94	0.061±0.006	4-115	26	0.069±0.002	2-175	345	0.052±0.003	2-110	109			
95	0.091±0.007	10-145	33	0.081±0.002***	2-175	472	0.061±0.013	10-100	47			
97	0.097±0.005	4-140	43	0.075±0.004	3-175	119	0.063±0.010	10-110	11			
98	0.081±0.005	3-130	59	0.032±0.002***	1-125	199						
99	0.084±0.005	3-135	39									
100	0.116±0.005***	3-155	56	0.048±0.005***	5-155	77						
103	0.080±0.006	7-140	60	0.048±0.002***	2-185	448						
106	0.088±0.006	3-145	45	0.075±0.002	2-175	403	0.057±0.007	3-100	20			
108	0.088±0.005	6-125	61	0.058±0.003**	3-130	125						
114	0.067±0.006	3-125	45	0.061±0.004**	2-140	112						
115	0.090±0.004	3-145	73	0.091±0.003***	4-165	164	0.070±0.003	4-140	126	0.092±0.004***	2-175	144
116	0.100±0.006*	3-150	58	0.088±0.003***	2-170	204	0.040±0.004***	10-85	22			
Control	0.088±0.002	1-170	578	0.069±0.002	2-170	414	0.060±0.004	6-130	55	0.066±0.003	2-135	167

Figure 1. Bronze = seed from 39-chromosome hybrid, white = 4n maize parent. (For full color, see p. 34.)

presented in Table 3. Three families were studied in M4, and two families showed a significant gain in grain weight.

The mechanism of the 5-azacytidine effect has been connected with methyl-cytosine demethylation, one way of imprint marking. Therefore, grain weight increase can be explained due to signal amplification changes. The results obtained in these experiments allow us to dwell on the 5-azacytidine effect on imprinting expression, the latter being revealed both in M1 and the following generations. However, due to complicated interactions, its expression is most likely to be unstable.

It is necessary to emphasize that, in earlier data, when pollinating with hybrid pollen, the weight of produced seeds was almost equal to that of seeds in the pollination with the tetraploid. Thus, it is possible to conclude that imprinting expression and its strict dosal dependence is different from that observed in diploid maize of apomictic maize x *Tripsacum* hybrids in the presence of some of the wild parent *ipsacum*) chromosomes. The presence of *psacum* chromosomes is likely, in some way, to ect the imprinting signal-setting and modify its pression. In this connection, we tried to influence expression experimentally with 5-azacytidine atment of germinating seeds. Therefore, the DNA olved in replication in the presence of the chemical ent results in a decrease in the degree of thylation of cytosine. Thus, the elimination of the rker signal and modification by imprinting pression proceeds. Such changes can be traced in and, possibly, in the following generations by mparing average grain weight to that of the control d experimental variants. In this present ntribution, we are now left to dwell on the results of investigation and consider those of the future (Figure 2, plant height modification) in using 5-azacytidine for modification of imprinting expression in apomictic maize x *Tripsacum* hybrids.

Figure 2. Plant height modification. (For full color, see p. 34.)