Table 3. The effect of polyamines added to plant regeneration medium on maize plant regeneration.[1]

Polyamine Treatments	Genotype		
	H99 (6 mo. old)[3]	H99 (54 mo. old)	Pa91 (6 mo. old)
	Shoots gfw^{-1}		
No additions	41.3 ± 8.75	0.0	26.1 ± 8.7
1.0mM Putrescine	28.6[2]	0.0	47.4 ± 10.3
0.1mM Putrescine	45.5 ± 5.5	0.0	37.0 ± 2.2
1.0mM Spermidine	47.1 ± 15.1	0.0	33.6 ± 1.7
0.lmM Spermidine	45.7 ± 18.8	0.0	29.6 ± 3.7

[1]Treatments made of H medium containing 3.5 mg l^{-1} 6BA (Duncan and Widholm 1988, Plant Cell Reports 7:452-455) and H Medium (Duncan et al., 1985, Planta 165:322-331) plus appropriate concentration of polyamines. Treatments were randomly inoculated with callus maintained on a 14-21 d subculture routine. Initial inoculum size was 0.01 gfw callus^{-1} pieces, with 20 pieces per petri dish^{-1} and two replicates per treatment.
[2]Only one replicate, the other was contaminated.
[3]Time after culture was initiated.

These accumulated results suggest that, although inhibitor studies indicate that polyamine biosynthesis is required for plant regeneration, under typical culture and regeneration conditions dark-grown maize callus seems to have an adequate supply of polyamines, except in cases where the untreated control plant regeneration capability is low, as with H99 in Table 2 and Pa91 in Table 3. Variability in response to polyamines may possibly be due to variability of the polyamine content of callus prior to regeneration. Also, the variable response to polyamines could possibly be due to uneven and uncontrolled loss of the volatile polyamines from the culture system. Unlike the studies with light-grown maize callus where lower polyamine concentrations stimulated plant regeneration, the dark-grown maize callus seemed in most cases to adequately produce polyamines, and the addition or removal of polyamines to regeneration medium did not increase plant production.

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Induction of maternal haploids in maize

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Recently, haploidy has been used widely in both maize breeding and genetics. Moreover, the technology of chromosome doubling of haploids (DH) is the main method of producing homozygous lines in most maize breeding companies. The creation of inducers of maternal haploids, based on the Stock 6 line (Coe, 1959), has allowed many maize breeders to use haploid methods. However, the mechanism of haploid induction has not been explained, so far. The induction of maternal haploids is considered to occur due to a single fertilization, i.e., one of the sperms fertilizes the central nucleus of an embryo sac and the formation of an endosperm provokes the development of an unfertilized (haploid) egg cell (Enaleeva et al., 1990). Some morphological differences among sperm of a haploid inducer have been revealed (Bylich and Chalyk, MNL 70, 1996). The results of that study have been connected with the hypothesis of single fertilization. This kind of mechanism is known in apomictic development--pseudogamy. However, this ability is specific for female genotypes and, as a rule, results in the development of diploid embryos.

In this work, we would like to present some results which have some contradictions with the mechanism of haploid induction mentioned above. Earlier, it was assumed that the frequency of haploids could be decreased by heterofertilization (Rotarenco and Eder, MNL 77, 2003). Studying the influence of heterofertilization on the induction of maternal haploids was one purpose of this work.

Two inbred lines, A464 and A619, were crossed with two males, a haploid inducer and the X28C line (not a haploid inducer). The males possessed a dominant marker gene *R1-nj* which causes a purple scutellum and a "purple crown" of the aleurone (Nanda and Chase, 1966). Different kinds of pollinations were performed: simple pollinations of females with the pollen of males, pollinations with pollen mixtures (made of the pollen of the males and females in a 50/50 mixture), and repeated pollinations after 24, 48 and 72 hours with the pollen of females (self-pollination). No fewer than 10 ears were used for each kind of pollination.

The following four types of kernels were obtained (Fig. 1): 1) yellow kernels (female type); 2) kernels with the full expression of the *R1-nj* gene; 3) kernels with colored aleurone (endosperm); and 4) kernels with colored scutellum (embryo). The kernels of the third and fourth groups are the results of heterofertilization. However, among the kernels of the third group there were kernels with haploid embryos. All of the kernels of the third group were planted and haploids were identified.

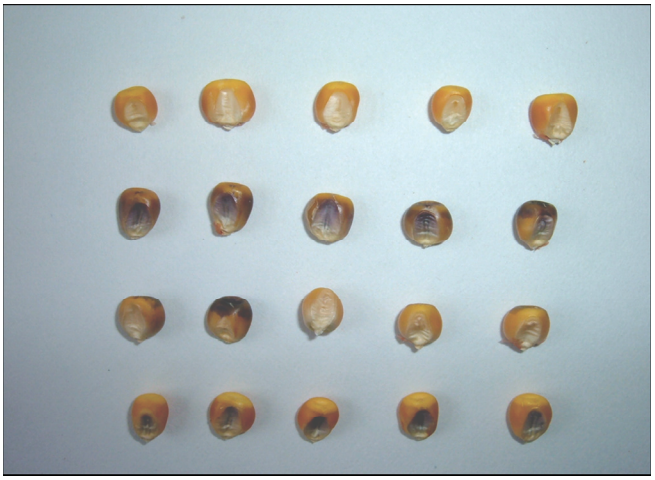

Figure 1. Four types of kernels produced by different methods of pollination.

The results of heterofertilization were more often revealed in the pollinations where the pollen of the inducer was used. In the pollinations with pollen mixtures, 3.9% of such kernels were noticed in the A464 line, and 2% in the A619 line, whereas applying the mixtures with the pollen of the X28C line resulted in 0.32% and 0.42%, respectively. The frequency of haploids in these pollinations decreased from 12.6% to 10.6% in the A464 line, and from 11.8% to 3.9% in the A619 line (Table 1).

The frequency of haploids was almost twice as low in the repeated pollinations after 24 hours. In the A464 line haploid frequency was 5.2% and in the A619 line it was 6%. There were relatively high frequencies of heterofertilization when the pollen of the inducer was used for the first pollination (Fig. 2).

Table 1. Results of haploid induction and heterofertilization.

Pollinators	A464		A619	
	%, haploids	%, heterofert.	%, haploids	%, heterofert.
Haploid inducer	12.6	-	11.8	-
X28C line	-	-	-	-
Haploid inducer (pollen mixture)	10.6	3.9	3.9	2.0
X28C (pollen mixture)	-	0.32	-	0.42
Haploid inducer/selfed after 24h	5.2	1.5	6.0	1.3
X28C/selfed after 24h	-	0.54	-	-
Haploid inducer/selfed after 48h	12.5	0.35	9.5	0.3
X28C/selfed after 48h	-	0.2	-	-
Haploid inducer/selfed after 72h	10.0	0.68	8.5	0.4
X28C/selfed after 72h	-	0.33	-	-

Figure 2. Ears of maternal genotypes in repeated pollinations (first pollination--the haploid inducer or the X28C line; second pollination--self-pollination).

These results indicate that during the induction of haploids a delay of fertilization occurs, and this was noticed both for the egg cells and for the central nuclei. In our opinion, the delay of fertilization was the reason for the high frequencies of heterofertilization in the pollinations where the haploid inducer was used.

Producing haploids under the conditions of repeated pollinations at an isolated plot leads to a high level of heterofertilization or to a complete replacement of early penetrating sperm (carriers of haploid induction) by normal sperm. Thus, the frequency of haploids is reduced in comparison with manual (artificial) pollinations (Rotarenco, MNL 76, 2002). However, the frequency of haploids varies in manual pollinations as well. In this case, a delay of pollination leads to a decrease in the frequency which could also be caused by the phenomenon of heterofertilization (Rotarenco and Mihailov, MNL 81, 2007). The highest yield of haploid kernels per ear was obtained in the manual pollinations of ears with three-day-old silks. In this case, there was a combination of a maximal frequency of haploids and a good seed set (unpublished).

Whatever the reason for the variation in the frequency of haploids, there is a cardinal difference between the induction of maternal haploids and apomictic development--the frequency of maternal haploids is higher in early pollinations, whereas a delay of pollination leads to an increase in the frequency of apomixis (according to the literature).

While creating new inducers, it was noticed that the haploid induction was accompanied by the formation of embryoless and endospermless kernels. It was revealed that the frequency of haploid induction of an inducer positively correlated with the frequencies of both embryoless and endospermless kernels resulting from its self-pollination. By means of the following experiment, it has been found that only the inducers' pollen possessed the ability to induce both embryoless and endospermless kernels, whereas their female inflorescences (ears) were mostly able to form normal kernels.

An inducer with a frequency of haploid induction of about 10% was self-pollinated and crossed with the A464 line. The frequency of embryoless kernels was 10.2% in the self-pollinated ears, and 0.4% as a result of pollination with the A464 line (Fig. 3).

Figure 3. Ears of a haploid inducer. The two ears on the left are the result of self-pollination, the other two ears are the result of pollination with the A464 line.

There were a large number of endospermless kernels in the self-pollinated ears (more than 30%). There were a variety of endospermless kernels in the pollinations with haploid inducers--completely endospermless kernels, kernels with reduced endosperms and endospermless kernels with viable embryos.

Significant variation has been revealed among haploids of an inbred line developed by different inducers. The haploids differed by the speed of germination in a thermostat and phenotype at the early stages of growth (up to 4 leaves). One of the possible explanations for those differences could be the influence of hybrid endosperms. A similar effect was observed by Haskell (1960) in pseudogamous *Rubus* species and called pseudogamous heterosis.

Our next goal was to compare adult haploids of inbred lines produced by different inducers. The A464 line was crossed with three inducers: two of them were homozygous lines (#1 and #2), and the third one was the hybrid between them (1x2). The A619 line was crossed with the second inducer (#2) and the hybrid inducer (1x2).

Haploids were cultivated in three-row plots. By the flowering phase, there were 60 to 80 haploids in each plot. Three plant traits (plant height, leaf length, leaf width) and two ear traits (ear

length and number of kernel rows) have been measured in the haploids. There were significant differences in all the traits among the haploids of the A464 line. The haploids of the A619 line differed significantly in plant height (Table 2). Besides the differences in quantitative traits, it was noticed that the haploids differed in time of flowering.

Table 2. Parameters of quantitative traits of haploids produced by different inducers.

Traits	(n) A464			(n) A619	
	Inducer #1	Inducer #2	Hybrid Inducer 1x2	Inducer #2	Hybrid Inducer 1x2
Plant height, cm.	142.1±3.1	126.2±2.2**	120.2±2.1***	133.4±1.3	125.5±2.0**
Leaf length, cm.	53.6±0.8	51.0±0.6	46.9±0.7***	49.2±0.4	47.7±0.6
Leaf width, cm.	6.7±0.2**	6.7±0.1**	7.1±0.1	7.3±0.6	6.9±0.8
Ear length, cm.	10.2±0.3	8.9±0.2**	9.7±0.2	10.2±0.3	9.2±0.4
Number of kernel rows, no.	13.3±0.2	11.6±0.2***	11.8±0.2***	12.3±0.2	11.7±0.3

It seems unlikely that differences between haploids could have been caused by the influence of their hybrid endosperms. Most likely the reason for the variation was partial hybridization with the inducers, and perhaps, each inducer had a certain degree of partial hybridization. The phenomenon of partial hybridization has been described in rice and found in sunflower (Faure et al., 2002). Every year we find aneuploid plants among haploids (Chalyk et al., MNL 77, 2003). There is much variation among the aneuploids in their phenotype; also, they might be sterile or partially fertile and usually possess the inducer's marker genes. Traces of the marker genes are being revealed in plants considered haploids, too. Some researchers believe that such plants are androgenic haploids. However, these haploids have nothing in common with haploids usually obtained after self-pollinations of inducers, either in phenotype or in the way the marker genes are expressed.

The observations mentioned above can be applied to confirm partial hybridization during the induction of maternal haploids. However, based on all the results presented, we cannot be sure yet that the development of embryos (considered haploid embryos) occurs due to partial hybridization and that maternal haploids actually are aneuhaploids, i.e., possess some genetic information from inducers.

Overall, the results show that the induction of maternal haploids is a rather complex and, at the same time, interesting phenomenon.

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Map locations of the telomeres

--Coe, EH

Tentative positions of the telomeres have been added to the Genetic 2008 maps in MaizeGDB. The names assigned to these loci are Telomere1S, Telomere1L, etc. Each short-arm end is assigned a zero coordinate, while long-arm ends are approximated from available evidence. Wherever possible, placement is inferred in an anchored contig by evidence in silico for localization of telomere-specific sequences (e.g., pMTY9ER, pBF266) at one end of a contig that is oriented correctly. Firmly mapped contigs at the ends often match well by this criterion, but some contigs do not. Telomere9S, in particular, is ambiguously placed because of conflicts in contig order and relationship to knob probes, presumably at K9S. Comments with each telomere locus identify the basis of its placement.

Note: these positions are based on (1) the July 2005 FPC build; (2) sequenced BACs as of October 2008; and (3) genetic mapping of other loci in the same contig, e.g., on IBM2 or NAM maps.

I look forward to receiving feedback on these placements, and hope that a next-generation placement will be possible for persons with direct interest in the telomeres when the sequencing project is completed and a rebuild is done. If matched with a high-density, high-resolution genetic map – e.g., an enhanced NAM, the cross-reinforcement between the physical and the cytological and the genetic map will be substantial.

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Pollen shed delay, silking anthesis interval (SAI), occurred in a cool, late season

--Troyer, AF

Corn has an imperfect flower. The female flower becomes the ear, and the male flower is the tassel. Darwin pointed out that silk delay after pollen shed is normal in corn varieties to facilitate cross pollination, which increases plant vigor. The term silk delay also explains what happens during drought (moisture stress) at flowering time. Fresh corn silks are 90% water; thus, they are sensitive to water availability. Corn breeders' selection against silk delay at high plant densities has been useful to increase hybrid corn's drought stress tolerance. Growing degree heat units to pollen shed is normally very stable with a much lower coefficient of variation than heat units to silk. Upright leaves have become more popular in commercial hybrid corn during the last 40 years. I noticed some very unusual flowering of corn while pollinating in my nursery during this late, cool, 2008 season in northern Illinois. This is about heat units and flowering in corn.

My breeding starts are typically backcrosses of related, elite inbred lines. I grew 1600 plants each of six backcross pedigrees involving four different elite inbred backgrounds at 60,000 plants per acre, including alleys. I self-pollinated the earliest, strongest silking 10% of the plants. This year many plants silked strongly, and I had to wait a day or two or three or more days for the tassel to shed before pollinating. That's very unusual. The pollen shed delay plants had their tassels tightly encased in the uppermost two leaves of the plant. All of my plants had ligules. When I "unwrapped" these tassels, the tassels felt cool and damp; they were water cooled by plant transpiration. I've never seen plants and felt tassels like these before. This year I saw and handled about a thousand plants with delayed pollen shed.

My nursery was planted April 24 and emerged evenly in about 10 days. We were 30% short of heat units in May and June was normal. We never caught up. We had a cool, late season with timely, ample rainfall. The July 7, 2008 Illinois Weather & Crops,