Table 1. Chemical composition of single-cross hybrids determined by NIRT.

Hybrid	Type	%Oil	%Protein	%Starch.	Density
HC5	HQP	5.2	11.0	70.3	1.32
HC25	HQP	5.1	12.3	69.7	1.30
HC26	HQP	5.6	11.5	69.4	1.31
HC27	HQP	5.9	11.9	68.9	1.31
HC28	HQP	6.0	12.5	68.0	1.29
HC29	HQP	5.4	11.6	69.7	1.32
HC30	HQP	6.1	11.0	69.4	1.33
HC14	DR	4.3	10.9	71.6	1.30
HC15	DR	4.6	10.9	70.9	1.31
HC8	DR	5.2	11.4	69.8	1.33
HC16	DR	5.4	11.2	69.7	1.32
HC17	DR	5.8	10.4	70.4	1.31
HC18	DR	5.7	10.5	70.7	1.31
HC19	DR	5.2	10.2	70.9	1.30
HC20	DR	5.6	10.5	70.7	1.32
HC21	DR	5.8	10.5	70.1	1.32
HC22	DR	6.3	11.7	68.8	1.32
HC23	DR	6.0	11.1	69.2	1.34
HC24	DR	5.8	10.9	69.9	1.31
HC1	WAXY	4.7	11.7	70.4	1.29
HC31	WAXY	5.1	11.7	69.4	1.28
HC32	WAXY	4.6	10.9	70.6	1.29
HC33	WAXY	5.2	9.9	71.5	1.30
HC34	WAXY	5.3	11.0	70.0	1.29
HC35	WAXY	5.3	11.0	70.0	1.30
HC36	WAXY	4.9	11.1	70.5	1.29
HC37	WAXY	5.1	11.9	70.0	1.31
ACA2000	TESTER	6.0	10.3	69.4	1.32
	Env. avg.	5.4	11.2	69.9	1.31
	CV%	9.5	5.7	1.1	0.8
	Min.	4.3	9.9	68.0	1.28
	Max	6.3	12.5	71.6	1.34

9.5% (estimated on 109 genotypes grown in the provinces of Buenos Aires and Córdoba between 1999 and 2001). This value is in agreement with others published in the Argenfoods database (Universidad Nacional de Lujan, 2002). The HC hybrids showed an average protein content of 11.2% (range = 9.9% to 12.5%) (Table 1). 63% of the single-crosses averaged 11% to 12.5% kernel protein content, exceeding the tester protein content in 7.0% to 21.4%. In addition, kernel starch content averaged 69.9% for the HC hybrids with values ranging from 68.0% (HC28) to 71.6% (HC14). Twenty HC single-crosses exceeded the average starch content found for the tester ACA 2000. The average starch content of HC hybrids corresponds to values published by MAIZAR Association in Argentina after testing 48 commercial hybrids grown in the Argentine Corn Belt or ZMT during the growing season of 2004/05 by NIRT. Significant differences for protein and starch content were only found between HQP and DR single-crosses, as well as between HQP and waxy hybrids (Table 2).

Table 2. Significance test for NIRT values among groups of hybrids.

Contras	%Oil	%Protein	%Starch	Density
QPM- DR	0.51 ns	2.73*	2.34*	0.00 ns
QPM-Wx	1.36 ns	2.26*	2.29*	0.00 ns
DR- Wx	1.00 ns	0.36 ns	0.31 ns	0.00 ns

ns = non significant; * = significant at 0.05

The results of the ANOVA showed highly significant differences among genotypes for oil content ($F_{27\text{-}27}$: *7.77; p: 0.01*), for protein content ($F_{34\text{-}34}$: *2,26; p: 0,01*) and also for starch content ($F_{27\text{-}27}$: *3.09; p: 0.01*). Only significant differences among hybrids were detected for kernel density ($F_{27\text{-}27}$: *1.84; p: 0.05*). When Fisher´s LSD test was used to compare the average oil content, 14 homogeneity groups were distinguished (*D: 0.74; p: 0.01)*. Using the same methodology, 8 homogeneity groups were found for protein content (*D: 0.74; p: 0.01)*, 6 homogeneity groups for starch content (*D: 1.74; p: 0.01)* and only 3 groups for kernel density (*D: 0.03; p: 0.01).* Pearson´s correlation coefficients among starch, protein, oil content and kernel density were estimated (Table 3). All genotypes showed only significant but negative correlations between oil and starch content, as well as between protein and starch content.

Table 3. Relation among the different kernel chemical components.

	Pearson´s simple correlation coefficient (r)			
	%Oil	%Protein	%Starch	Density
%Oil		-0.04 ns	-0.69**	0.17 ns
%Protein			-0.60**	0.05 ns
%Starch				-0.04 ns

ns = non significant;** = significant at 0.01

The data in Table 4 show that the inbreds' oil content averages 5.61% and 5.75% via NIRT and Soxhlet, respectively. The Soxhlet results indicate that oil content varies from 4.4% (CIG29) to 7.7% (CIG6). Six inbreds yielded more than 6.0% oil and could be considered HOC genotypes and used as male progenitors in future crosses. A very high correlation between the results obtained by NIRT and Soxhlet (*r=0.921*) was found.

Table 4. Kernel oil content of inbreds measured by destructive and non-destructive methodologies.

Inbred	Type	NIRT*	Soxhlet*
CIG81	waxy	5.1	4.8
CIG 28	waxy	6.3	6.0
CIG 30	waxy	4.9	5.0
CIG 36	HQP	6.5	6.5
CIG15	waxy	6.8	6.4
CIG35	waxy	3.9	4.5
CIG13	waxy	4.6	5.3
CIG1	waxy	6.1	6.6
CIG6	waxy	7.3	7.7
CIG34	waxy	6.4	7.0
CIG9	waxy	5.5	5.0
CIG29	DR	4.2	4.4
CIG37	HQP	5.4	5.5

* = expressed in percentage on the basis of dry weight.

The results obtained demonstrate that the protein, starch and oil content in most of the HC hybrids analyzed equal or exceed the average values of the commercial hybrids actually grown in Argentina. This fact, along with their grain yield, implies that these genotypes are able to produce important amounts of these components per unit area. As a result, they are very competitive for use in diverse industrial processes. In addition, all these materials carry in their genetic background one or two of the recessive genes *wx*, *o2*, *o5* and *o12* that promote a better response when used in animal or human feeding.

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Polyamine biosynthesis is required for normal plant regeneration from maize callus cultures

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Studies examining the polyamine concentration of light-grown maize callus (Tiburcio et al., Plant Tissue and Organ Cult. 27:27-32, 1991; Torne et al., Plant Cell Physiol. 34:371-374, 1993; Boget

et al., Plant Cell Tissue Organ Cult. 40:139-144, 1995) suggest that plant regeneration is highest from tissues with low levels of polyamines either endogenously present or induced by inhibitors of polyamine biosynthesis. This increased regeneration is greatest when the tissues are pretreated with a polyamine biosynthesis inhibitor prior to, but not during, plant regeneration (Tiburcio et al., Plant Tissue Organ Cult. 27:27-32, 1991; Torne et al., Plant Cell Physiol. 34:371-374, 1993). The most commonly used inhibitors of polyamine biosynthesis are α-difluoromethylornithine (DFMO) and α-difluoromethylarginine (DFMA), irreversible inhibitors of ornithine decarboxylase and arginine decarboxylase, respectively.

Immature embryo-derived maize callus is often grown in the dark and only regenerated in the light (Armstrong and Green, Planta 164:207-214, 1985; Duncan et al., Planta 165:322-331, 1985). Since the previous studies were done using light-grown callus, we examined the role of polyamines in plant regeneration from dark-grown maize callus.

To determine if de novo polyamine biosynthesis is required for plant production from dark-grown callus cultures, type I callus was initiated from immature embryos of the self-pollinated maize *(Zea mays L.)* inbred lines Pa91 and H99. Calluses were maintained in the dark at 28 C on D medium (Duncan et al., Planta 165:322-331, 1985) and sub-cultured at 14 to 28-d intervals.

Plants were regenerated by placing 20 pieces of callus per petri plate (0.01 gfw/piece and 2 to 3 plates per treatment) on H medium (Duncan et al., Planta 165:322-331, 1985) containing 3.5 mg l^{-1} 6-benzylaminopurine (6BA) for 3 d. Calluses were then transferred to H medium without 6BA for an additional 21 d (Duncan and Widholm, Plant Cell Reports 7:452-455, 1988), after which the number of individual regenerated plants were counted. The regenerating cultures were incubated at 28 C in continuous light (approximately 80 μmole photons $m^{-2}s^{-1}$).

Experimental modification of the above media consisted of adding filter sterilized putrescine, spermidine, spermine, DFMO or DFMA to the cooled, autoclaved medium prior to pouring into Petri dishes. Concentrations of medium additives are listed in the Tables. The DFMO and DFMA were obtained courtesy of Merrill Dow Research Institute, Cincinnati, Ohio.

Since it had earlier been shown by Tiburcio et al. (Plant Tissue Organ Cult. 27:27-32, 1991) and Torne et al. (Plant Cell Physiol. 34:371-374, 1993) that polyamines could be synthesized in maize by both arginine decarboxylase and ornithine decarboxylase, both DMFO and DMFA were added simultaneously to the culture media. In the present study, these inhibitors reduced plant production by 4-month-old H99 and Pa91 cultures approximately 62% and 91%, respectively, as compared to untreated controls (Table 1).

Table 1. The effect of polyamine biosynthesis inhibitors added to plant regeneration medium on maize plant regeneration from cultures initiated 4 months earlier.[1]

	Genotype	
Treatment	H99	Pa91
	Shoots gfw^{-1}	
No additions	28. 9 ±10. 2	25.8 ± 6.8
lmM each DFMO and DFMA[2]	11.1 ± 5.5	2.4 ± 1.8

[1]Inhibitors were added to both H medium containing 6-benzyladenine (Duncan and Widholm, Plant Cell Rep. 7:452-455, 1988) and H medium without 6-benzyladenine (Duncan et al., Planta 165:322-331, 1985). The cultures were 4 months old and were subcultured at 28 d intervals. Initial inoculum size was 0.01 gfw $callus^{-1}$ pieces with 20 pieces per petri $dish^{-1}$ and three replicates per treatment.
[2]DFMO = α-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase; DFMA = α-difluoromethylarginine, an irreversible inhibitor of arginine decarboxylase.

Regeneration inhibition by DFMO and DFMA was consistent throughout all the experiments; however, the degree by which the combination inhibited plant regeneration was variable. For example, using 8-month-old callus, maintained on a 28 d subculture cycle, H99 and Pa91 were inhibited by the combination of 1 mM DFMO and DFMA approximately 81% and 33%, respectively, as compared to untreated controls (Table 2). Variation in the quantity of preexisting polyamines may explain the variable degree of plant regeneration ability as well as the variation in the inhibition caused by DFMO and DFMA (Tables 1 and 2).

Table 2. The effect of polyamine biosynthesis inhibitors added to plant regeneration medium on maize plant regeneration from cultures initiated 8 months earlier.[1]

	Genotype	
Treatment	H99	Pa91
	Shoots gfw^{-1}	
No additions	4.25 ± 0.75	49.5 ± 2.28
1 MM each DFMO and DFMA[2]	0.83 ± 0.83	33.05 ± 3.32
1 mM each DFMO, DFMA and 1 mM PUT	7.81 ±1.14	46.67 ± 0.95
1 mM PUT	12.52 ± 0.467	40.95 ± 7.62

[1]Inhibitors were added to both H medium containing 6-benzyladenine (Duncan and Widholm, Plant Cell Rep. 7:452-455, 1988) and H medium without 6-benzyladenine (Duncan et al., Planta 165:322-331, 1985). The cultures were 8 months old and subcultured at 28 d intervals. Initial inoculum size was 0.01 gfw $callus^{-1}$ pieces with 20 pieces per petri $dish^{-1}$ and three replicates per treatment.
[2]DFMO = α–difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase; DFMA = α–difluoromethylarginine, an irreversible inhibitor of arginine decarboxylase; PUT = putrescine.

To determine if the inhibitory effect of DFMO and DFMA actually resulted from polyamine biosynthesis inhibition, putrescine (the first polyamine whose synthesis is inhibited by DFMO and DFMA) was added to the DFMO and DFMA treatment medium in an attempt to reverse the inhibition of plant regeneration. In the case of Pa91, putrescine restored plant regeneration to the same level as the untreated control, whereas DFMO and DFMA alone suppressed plant production by about 33% (Table 2). In the case of H99, putrescine alone or in combination with DFMO and DFMA stimulated plant production by about 91% and 81%, respectively, as compared to the untreated control (Table 2). The DFMO and DFMA treatment alone, however, reduced plant production by about 81% as compared to the control (Table 2). These results further demonstrate that de novo polyamine biosynthesis is needed for normal plant regeneration from dark-grown maize callus cultures, especially in the case of H99 where the plant regeneration capacity was low.

Considering the stimulatory effect of putrescine on plant regeneration from H99 callus noted in Table 2, further experiments were conducted to determine if the addition of polyamines to the regeneration protocol would increase plant production. When either putrescine or spermidine were added to regeneration medium, plant production by Pa91 callus was increased compared to the untreated control, with the greatest increase (about 82%) resulting from the 1 mM putrescine treatment (Table 3). No treatment, however, showed a significant effect on plant production of H99 (Table 3). Furthermore, 54-month-old H99 callus which had lost its capacity to regenerate plants did not respond to any polyamine treatment by producing plants (Table 3).

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.

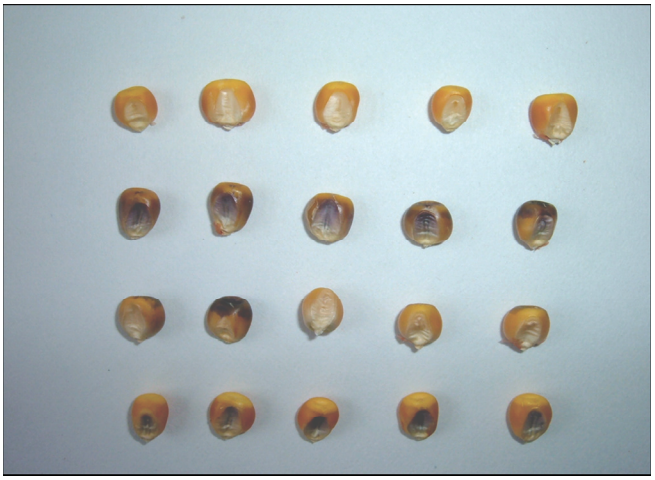

Figure 1. Four types of kernels produced by different methods of pollination.

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).