a hybrid enzyme pattern. That each seed possesses its own enzyme variant suggests that different loci, controlling catalase synthesis, function in each seed.

The comparison of endosperm catalase with that of the leaves and seedlings within a single line has shown that the enzyme is tissue specific. The catalase of these tissues differs in the electrophoretic mobility of the enzyme and in the number of zones of enzyme activity.

From the studies on the catalase of maize endosperm, leaves and seedlings it may be inferred that an oligogenic system functions to control the synthesis of multiple molecular forms of the enzyme. The presence of the catalase variant is determined by the type of tissue and its stage of development.

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1. Attempted maize x sorghum hybridization.*

Materials used in our 1971 maize x sorghum crossing nursery are listed in Tables 1 and 2. Three dates of planting (delayed approximately 10 days) were used. To reduce contamination from stray pollen, maize stocks used as females were planted in isolation from their male counterparts and were detasseled. Also, male-sterile sorghums were isolated from male-fertile sorghums. A total of 1,293 reciprocal, controlled pollinations (727 using maize ♀ and 566 using male-sterile sorghum ♀) were made by conventional methods and by a "vial method." The latter technique involved attaching plastic vials (filled with water) to the maize or male-sterile sorghum plants at the bases of the ears or heads. Tassels or male-fertile sorghum heads were inserted into the vials and all components were placed under pollinating bags. Bags were tapped periodically to release pollen onto the stigmatic surfaces. Observations indicated that the "vial method" supplied viable pollen for 3-7 days.

*Research supported by Rockefeller Foundation Grant.

Table 1. Maize cultivars grown in 1971 maize x sorghum nursery.

Cultivar	Ploidy
A619[a]	2N
B14A[a]	2N
MS214[a]	2N
(A619xA632)[a]	2N
(B37xB70)[a]	2N
(Mo17xN28)[a]	2N
Argentine Popcorn[b]	2N
Japanese Hulless Popcorn	2N
Maiz Chapalote[b]	2N
Zapalote Chico[b]	2N
Syn B 4N (Late)[c]	4N
Syn B 4N (Early)[c]	4N
Syn B 4N (Isol. Bulk)[c]	4N
4N wx[d]	4N
4N su[d]	4N
4N gl_1[d]	4N
Gourdseed[d]	2N
Papago Flour Corn[d]	2N
Tama Flint[d]	2N
Northern Flint[b]	2N

[a]From W. A. Russell, Iowa State University.

[b]From Wm. L. Brown, Pioneer Hi-Bred Corn Company.

[c]From J. W. Dudley, University of Illinois.

[d]From Maize Genetics Cooperative, University of Illinois.

Table 2. Sorghum cultivars grown in 1971 maize x sorghum nursery.

Cultivar	Ploidy
Male-sterile	
Martin A[a]	2N
Kafir 60A[a]	2N
Wheatland A[a]	2N
979 Sorghum x Sudangrass[b]	2N
990 Sorghum x Sudangrass[b]	2N
PS2A Sudangrass[b]	2N
Male-fertile	
Plainsman[a]	2N
Tx7078[a]	2N
Redbine 60[a]	2N
Caprock[a]	2N
Kafir 60B[a]	2N
Sooner Milo[a]	2N
Durra[b]	2N
Kaoliang[a]	2N
Feterita[a]	2N
Hegari[a]	2N
Shallu[b]	2N
Sorghum virgatum[b]	2N
PS2B Sudangrass[b]	2N
PS1R Sudangrass[b]	2N
4N Bulk II[c]	4N
Martin x 4N Bulk II[c]	4N
5AD x 198/12/1[d]	4N
5AD x 270/4/4[d]	4N
3D x 2[d]	4N
Tet. Pop. 72[d]	4N

[a]From R. E. Atkins, Iowa State University.

[b]From Wm. L. Brown, Pioneer Hi-Bred Corn Company.

[c]From Wm. Ross, University of Nebraska.

[d]From L. V. Peters, Serere Research Station, Uganda, East Africa.

The method was more valuable for pollinating sorghum heads, which flower over a period of 7-10 days, than it was for pollinating maize ears.

Also, we cut, to ½-inch length, the silks of approximately one-half the maize ears pollinated. To reduce contamination, a new isolation bag was used for each day's cutting and the knife blade was sterilized with 95% ethanol before each ear was cut.

Ears were removed from maize plants 15 days post-pollination, examined in a portable, sterile chamber in the field, and any with seeds were taken into the laboratory where seeds were examined and embryos were excised and transferred to the culture medium described in Table 3. Approximately 30 maize ears and all pollinated male-sterile sorghum heads were sampled at maturity.

Table 3. Synthetic nutrient medium used to culture seeds and embryos.

Component	Concentration (mg/ml)
Sucrose	20,000.0
Agar	7,500.0
$Ca(NO_3)_2 \cdot 4H_2O$	237.0
KNO_3	85.0
KCl	65.0
$NaH_2PO_4 \cdot H_2O$	16.5
$MgSO_4 \cdot 7H_2O$	36.0
Fe Citrate (Ferric)	30.0
$MnSO_4 \cdot 4H_2O$	0.4
Adenine	0.2
Nicotinic Acid	0.1
Pantothenic Acid	0.05
Pyridoxine Hydrochloride (Vitamin B_6)	0.2
Ascorbic Acid (Vitamin C)	17.8
Thiamine Hydrochloride	0.1
Flavin Mononucleotide	0.2
Myo - Inositol	10.0
Succinic Acid	25.0
Glycine	3.0

Conclusions drawn from analyses of numbers of maize ears setting seed should be viewed with caution since we had no estimate of normal amounts of foreign-pollen contamination for these materials. A few trends, however, should be mentioned. Highest frequency of pollinated ears setting seed was from tetraploid materials ($\bar{x}$ = 36%), whereas lowest frequency ($\bar{x}$ = 24%) was from "unadapted" maize cultivars (Argentine Pop., Japanese Hulless, Zapalote Chico, Gourdseed, Papago Flour Corn, Tama and Northern Flints). Twenty-nine percent of the pollinated ears from adapted materials produced seed. Adapted single-crosses set seed on 37% of their pollinated ears, whereas adapted inbreds set seed on 19%.

The apparent influence of vigor on seed set may be a consequence of more vigorous materials (tetraploids and single-crosses) extruding silks from ear shoot bags or pollinating bags and, therefore, being more prone to contamination. Alternatively, vigorous materials may be capable of overcoming physical and (or) physiological barriers to pollination and fertilization. There was no influence of male-fertile sorghum genotype (used as pollen parents) on frequency of maize ears setting seed.

All seeds recovered from male-sterile sorghum plants came from cultivated sorghums. Maize pollen parent genotype was not correlated with frequency of pollinated heads producing seed.

When compared with the 11,000 seeds obtained in 1970, the 780 seeds recovered in 1971, represent a significant reduction in contamination by foreign pollen. Precautionary measures taken at planting (isolated plantings) and during crossing (detasseling, vial pollinations, and sterile cutting procedures) were effective.

Data concerning presence or absence of embryos in seeds from maize and sorghum females are presented in Table 4. Only 34 seeds were recovered from sorghum females. These seeds were dissected and the embryos were cultured. Five sorghum female seeds (from three pollinations involving Wheatland A x Maize Chapalote) were small and wrinkled; embryos from these seeds were small and failed to develop in culture. From the 29 "normal" seeds, 17 embryos germinated, developed normally, and are growing in the greenhouse. Four embryos from "normal" seeds germinated,

developed abnormally, and are being maintained in culture in hopes of stimulating further development.

Table 4. Data on seeds and embryos recovered from maize and sorghum females.

Maize ears with seeds	208
Seeds recovered from maize ears	746
Seeds dissected 15 days after pollination	627
Seeds with embryos	354
Seeds with embryos $\leq$ 1.0 mm	114
Seeds with neither embryo nor endosperm	273
Seeds recovered from maize ears-mature	119
Mature maize seeds cultured	42
Male-sterile sorghum heads with seeds	20
Seeds recovered from male-sterile sorghum heads-mature	34
Sorghum embryos cultured from mature seeds	33
Mature sorghum seeds cultured	1

Of the maize seeds dissected 15 days post-pollination, 43% were devoid of embryos (Table 4). Many embryoless seeds displayed various degrees of endosperm development. If these seeds were hybrid seeds, the 3N ploidy level (2N maize and 1N sorghum) of the endosperm may have buffered genetic imbalances and permitted this tissue to develop, whereas the 2N (1N maize and 1N sorghum) nature of the embryo could not overcome genetic imbalance and resulted in embryo abortion at an early stage of development. Alternatively, pollination may have stimulated seed and endosperm-like development without fertilization. Distinguishing between these alternatives would be difficult, requiring either that chromosome counts be made in the developing endosperm tissue or that quantitative measurements of DNA content of endosperm nuclei be made.

Seeds containing embryos were in various stages of development. Fifty-two percent of the embryos we dissected were $\leq$ 1.0 mm in length and 15% were only slightly developed (usually $<$ 0.1 mm long).

Success in culturing embryos was dependent upon their size. Generally, embryos $<$ 0.2 mm long would not develop in vitro (several nutrient media and environmental conditions were used). Although these very small embryos failed to germinate, nearly all remained alive for

several months. Most larger embryos (0.2 - 1.0 mm in length) grew but many of them developed abnormally, either forming an undifferentiated callus or only very small roots or shoots. The majority of embryos >1.0 mm developed normally and were eventually transferred to soil. These results suggest that the younger the embryos at the time of excision, the more heterotrophic they were, and therefore they were more dependent upon a complex nutrient medium. Either the media we used lacked constituents required for normal development or they contained incorrect concentrations of essential growth factors.

Our data showed no association of embryo size with pollination method, eliminating the possibility that many of the smaller embryos resulted from use of the "vial method" which could have caused pollination to occur several days after crosses were made. Therefore, small embryos may have developed slowly in vivo because of either genetic weaknesses or failure of the endosperm to provide them with optimum nutrients; i.e. they may have been hybrids. Therefore, further work to develop nutrient media which will stimulate and sustain development of extremely small embryos is needed. Only when such embryos can be grown to seedling stages which will permit chromosome counts and observations of seedling morphology will we be capable of presenting conclusive evidence on the feasibility of maize x sorghum hybrids.

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2. Pollen growth in in vitro maize x sorghum pollinations.*

Many seeds recovered from maize x sorghum pollinations were devoid of embryos but contained endosperm tissue (see report in this Newsletter and MGCNL 45:78-80). These seeds may be the result of either parthenogenic seed and endosperm development (possibly stimulated by pollination), embryo abortion after fertilization, or restricted embryo development. The first of these possibilities suggests that pollen germination and gamete union may be the limiting step in maize x sorghum hybridization.

*Research supported by Rockefeller Foundation Grant.