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.

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To determine whether maize or sorghum pollen could germinate on stigmas of the other genus (i.e. sorghum ♀ maize ♂ and maize ♀ x sorghum ♂), we conducted in vitro pollen growth studies. The first experiment was conducted during summer, 1971. We used silks from MS214 and Argentine Popcorn and maize pollen from MS214, Argentine Pop., and Japanese Hulless Popcorn. We collected sorghum pollen from Tx7078, Redbine 60, Kafir 60B, Sooner Milo, Durra, Kaoliang, Hegari, (Martin x 4N Bulk II), and 4N Bulk II. Ears, tassels, and heads were removed from field-grown plants during early morning and transported to the laboratory in vials of water. Microscope slides were prepared by affixing fresh pieces of silk to the slides, dusting the silks with appropriate pollen, covering the preparations with cover glasses, and placing them in moist chambers for 3-4 hours. We prepared two replications of 10 slides per maize ♀ x sorghum ♂ pollination, and on each day a new set (two reps of 10) of control slides (maize ♀ x maize ♂) was prepared. Slides were examined under 400X magnification, and pollen grains counted as definitely germinated were those with pollen tubes which had grown into the silk and which displayed protoplasmic streaming. Grains considered questionable showed stunted pollen tube growth and no protoplasmic streaming. Data in Table 1 are percentages of microscope slides containing at least one germinated or questionable pollen grain.

With two exceptions, sorghum pollen displayed only questionable germination when placed on maize silks. No maize ♀ x maize ♂ pollination showed questionable germination. MS214 x Kaoliang showed definite pollen germination. Positive results with Argentine Pop. x Sooner Milo may have been an artifact since the frequency of slides showing germination was low (both slides were from the same rep.) and MS214 x Sooner Milo pollinations showed only questionable responses. Germination was not influenced by amount of pollen on a silk.

During winter, 1971 we conducted a second pollen growth study using greenhouse-grown plants. Maize cultivars used were MS214, SynB 4N, and Japanese Hulless Popcorn. Sorghums studied were Kafir 60B, Kaoliang, Feterita, and 4N Bulk II (pollen sources) and Kafir 60A (male-sterile stigma source).

Table 1. Pollen grain germination in maize ♀ x maize ♂ and maize ♀ x sorghum ♂ *in vitro* pollinations (Field).

Pollination	% Microscope slides		Average No. grains/silk
	Definite germination	Questionable germination	
MS214 ⊗ [a]	52.5	0.0	31.6
Argentine Pop. ⊗	90.0	0.0	85.1
MS214 x Japanese Hulless	85.0	0.0	155.7
MS214 x Redbine 60	0.0	15.0	18.8
MS214 x (Martin x 4N Bulk II)	0.0	30.0	24.0
MS214 x Kafir 60B	0.0	5.0	14.0
MS214 x Sooner Milo	0.0	15.0	40.7
MS214 x Durra	0.0	15.0	52.2
MS214 x 4N Bulk II	0.0	30.0	37.0
MS214 x Kaoliang	65.0	0.0	96.0
MS214 x Tx7078	0.0	20.0	114.3
Argentine Pop. x Sooner Milo	10.0	0.0	89.4
Argentine Pop. x Hegari	0.0	55.0	108.8

[a]Four reps - 10 slides per rep.

Slides were prepared by affixing two fresh maize silks and two fresh male-sterile stigmas to a slide and dusting each with appropriate pollen (e.g. MS214 silks and Kafir 60A stigmas were dusted with MS214 pollen or pollen from one of the male-fertile sorghums), covering with cover glasses, and placing in a moist chamber (room temperature) for 3-4 hours. This procedure permitted examination of maize ♀ x maize ♂, maize ♀ x sorghum ♂, sorghum ♀ x maize ♂, and sorghum ♀ x sorghum ♂ pollinations each day. The number of replications varied.

Another technique, used on a limited basis, involved making *in vivo* pollinations and preparing microscope slides (silks and stigmas) four hours after pollination. Results of this technique are listed as *in vivo* in Table 2.

Table 2. Pollen grain germination in various combinations of maize and sorghum pollinations. (Greenhouse)

Pollination	Total obs.	% Microscope slides		% Pollen grains		Total pollen grains
		Definite germination	Questionable germination	Definite germination	Questionable germination	
				IN VITRO		
MS214 ⓧ	26	100.0	100.0	4.5	10.1	3860
Kafir 60A x MS214	24	12.5	83.3	0.4	3.3	1632
Syn B4N ⓧ	4	75.0	100.0	0.2	7.9	470
Kafir 60A x Syn B4N	4	0.0	100.0	0.0	3.8	105
Jap. Hull. ⓧ	8	62.5	100.0	0.4	9.6	1617
Kafir 60A x Jap. Hull.	8	0.0	62.5	0.0	1.9	372
MS214 x Kaoliang	8	0.0	100.0	0.0	8.8	1016
Kafir 60A x Kaoliang	8	0.0	100.0	0.0	2.7	843
MS214 x Feterita	8	0.0	100.0	0.0	6.3	1483
Kafir 60A x Feterita	8	0.0	100.0	0.0	2.0	1223
MS214 x Kafir 60B	18	0.0	100.0	0.0	7.1	7279
Kafir 60A x Kafir 60B	16	5.0	94.4	0.1	2.4	2951
MS214 x 4N Bulk II	16	0.0	87.5	0.0	4.6	2660
Kafir 60A x 4N Bulk II	14	0.0	85.7	0.0	2.9	1630
				IN VIVO		
MS214 ⓧ	6	0.0	50.0	0.0	2.2	182
Kafir 60A x MS214	6	0.0	33.3	0.0	0.8	241
Syn B4N ⓧ	12	0.0	83.3	0.0	6.2	732
Kafir 60A x Syn B4N	12	0.0	16.7	0.0	1.1	179
Kafir 60A x Jap. Hull.	10	0.0	10.0	0.0	1.1	95

In the first study we recorded only the number of slides having at least one pollen grain showing definite or questionable germination. In the second experiment, we not only recorded the number of slides, but also the number of pollen grains showing each type of germination.

Results from this experiment were similar to those obtained in the first experiment; i.e., maize ♀ x maize ♂ pollinations displayed definite germination and pollen tube growth, and maize ♀ x sorghum ♂ pollinations showed only questionable germination (Table 2). Unlike the previous results, maize ♀ x maize ♂ also showed questionable germination; and Kaoliang pollen did not germinate on MS214 silks. MS214 pollen germinated on Kafir 60A stigmas but pollen from SynB 4N and Japanese Hulless did not. Only Kafir 60B pollen germinated on Kafir 60A stigmas (Table 2). Percentages of pollen grains showing either type of germination were low for all pollinations (Table 2). This may be a consequence of *in vitro* conditions.

Several times during the second experiment slides were kept in the moist chamber for 24 hours before being examined for pollen germination. Neither germination percentages nor type of germination (i.e. definite or questionable) had changed after 24 hours. If pollen germination in maize x sorghum pollinations requires more than 24 hours, it is doubtful that the pollen would be viable enough to effect fertilization. Longer time intervals were not studied.

Pollen (both maize and sorghum) placed on maize silks gave better germination responses than when placed on male-sterile sorghum stigmas (Table 2). Apparently, maize silks provided a better *in vitro* environment for pollen germination than did sorghum stigmas. However, maize silks may lack germination "stimulators" normally present in sorghum stigmas and, therefore, sorghum pollen could not definitely germinate. The absence of germination in sorghum ♀ x sorghum ♂ pollinations may have been a consequence of the *in vitro* conditions we used. However, since pollen from Kafir 60B, which is the isogenic fertile counterpart of Kafir 60A, germinated on Kafir 60A stigmas, the phenomenon may be a manifestation of incompatibility between less adapted sorghums (Kaoliang, Feterita, and 4N Bulk II) and adapted Kafir 60A.

Generally *in vivo* pollinations resulted in poor germination. These results may be misleading, however, since the *in vivo* technique was used at the close of the greenhouse study and pollen viability during this period was low. Alternatively, poor germination may be a result of techniques not being perfected. The method deserves further study.

In vitro studies of pollen germination on liquid nutrient media also were conducted. All possible combinations of four concentrations (0.00, 0.03, 0.06, and 0.10%) of calcium nitrate, two concentrations (0.00 and 0.02%) of boric acid and four concentrations (10.0, 20.0, 30.0, and 40.0%) of sucrose were tested. Sorghum pollen did not germinate on media which were optimal for pollen from two maize lines, but it germinated on a medium containing a lower sucrose concentration and no boric acid (Table 3). These studies were repeated using 0.10% yeast extract as a medium supplement but no significant effects were observed.

Table 3. Optimal conditions for *in vitro* germination and growth of maize and sorghum pollen.

	Sucrose	Calcium	Boron
Maize			
MS214[a]	20%	0.06%	0.02%
Syn 4N[b]	30%	0.10%	0.02%
Sorghum			
Kafir 60B[c]	10%	0.06%	0.00%

[a] 6 replications
[b] 5 replications
[c] 4 replications

Although these data are preliminary, they parallel results from the microscope pollen growth studies (i.e., abnormal sorghum pollen germination on maize silks, and vice versa). Perhaps stigmatic surfaces of maize and sorghum are chemically different and pollen from the other genus cannot germinate and grow when placed on them.

Although we did not correlate in vitro with in vivo pollen growth, our results suggest that maize or sorghum pollen, when placed upon stigmas of the other genus, was incapable of sufficient pollen tube growth to effect fertilization. Therefore, embryoless seeds from maize x sorghum pollinations may have resulted from parthenogenesis. Further research on this possibility and on physiology of pollen germination and growth is being conducted.

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3. The distribution of meiotic stages in the developing tassel.

Although the progression of microspore mother cell development in the developing tassel is well known to the maize cytogeneticist, there have been few attempts to quantify this process with respect to the interrelationships of each region.

If one divides the developing tassel into four regions (I, II, III and IV) as shown in this diagramatic mature tassel and then standardizes each of the stages numerically one can quantify the relation between the areas and stages of maturation. The stages and their numerical assignment beginning with Leptotene and ending with the immediate post-quartet microspore are:

Stage	No.	Stage	No.
Leptotene	1	Anaphase I	5
Zygotene	2	Meiosis II	6
Pachytene	3	Quartet	7
Metaphase I	4	Microspore	8