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1. A nutrient medium for in vitro culture of maize and sorghum embryos.*

Recently, as part of a project investigating the possibility of obtaining a hybrid between maize and sorghum, we grew a large number of immature embryos in vitro. This was done because we felt that an endosperm-embryo incompatibility may have been responsible for the lack of success in previous attempts to obtain the intergeneric hybrid. To determine the optimum conditions for immature embryo development in vitro we tested the effects of a number of different levels of hormones, vitamins, and other supplements. Our intent was to develop a medium that would ensure the survival and subsequent normal development of as many potentially hybrid progeny as possible. The medium we finally adopted allowed us to grow routinely 10- to 12-day-old embryos, as small as 0.5 mm, and occasionally 6- to 7-day-old embryos, as small as 0.1 mm (Table 1). Embryos smaller than 0.1 mm could not consistently be excised without damage. Similar results were obtained with embryos from sorghum ♀ x maize ♂ pollinations.

The medium, modified from that of Murashige and Skoog (1962), contained the following components (concentrations in mg/l): NH_4NO_3 (1650), KNO_3 (1900), $CaCl_2 \cdot 2H_2O$ (440), $MgSO_4 \cdot 7H_2O$ (370), KH_2PO_4 (170), H_3BO_3 (6.2), $MnSO_4 \cdot 4H_2O$ (22.3), $ZnSO_4 \cdot 7H_2O$ (8.6), KI (0.83), $NaMoO_4 \cdot 2H_2O$ (0.125), $CuSO_4 \cdot 5H_2O$ (0.025), $CoCl_2 \cdot 6H_2O$ (0.025), thiamine · HCl (0.5), sucrose (60,000), Difco agar (10,000). Iron was added as 5 ml/l of a stock solution containing $FeSO_4 \cdot 7H_2O$ (5.57 gm/l) and disodium EDTA (7.45 gm/l). All components were autoclaved together. Optimum pH for normal growth was 5.0, adjusted with KOH or HCl as needed. This mixture of inorganic components was highly superior to other mixtures which have been used for culture of various species of embryos (Dure, 1960; Norstog and Smith, 1963; Raghavan and Torrey, 1963; White, 1963). Most hormones and vitamins at the levels tested were either toxic or had no effect on growth. Only thiamine was essential. Of the amino nitrogen

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Table 1

Growth and development of embryos of various sizes recovered from maize ♀ x sorghum ♂ pollinations*
(From Mock and Loescher, 1973)

Embryo size (mm)	Number of embryos	Percentage of embryos displaying		
		No development	Abnormal** development	Normal development
<0.1	23	39	56	5
0.1 - 0.2	8	25	16	63
0.2 - 0.4	8	13	0	87
0.4 - 0.6	18	0	11	89
0.6 - 0.8	5	0	0	100
0.8 - 1.0	8	0	0	100
1.0 - 1.2	4	0	0	100
1.2 - 1.4	3	0	0	100
1.4 - 1.6	3	0	0	100
1.6 - 1.8	2	0	0	100

* All progeny grown to maturity possessed maize phenotypes.

** Embryos displaying this type of development displayed only very small roots and (or) shoots.

sources tested, glutamine and casein hydrolysate both appeared to enhance growth but not survival rate of very small embryos; glycine and asparagine were toxic. Addition of coconut milk did not significantly promote growth, even of very small embryos. Yeast and malt extracts appeared to retard growth.

Except for very large embryos which began root and shoot growth within 24 hr after excision, most in vitro embryo development consisted of scutellar enlargement followed by root and shoot development. Once roots were well-developed in vitro, the plants could be transferred without difficulty to sterile soil and a large number were grown to maturity.

References:

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W. H. Loescher
J. J. Mock

2. Variegation associated with controlling element systems in tribal maize from Colombia.

The appearance of color variegated kernels in accessions of maize conjures up visions of r-mottling, B-B-F, and controlling element systems. Such variegation appeared in an accession of maize grown by the Cuna Indians from the remote village of Unguia in the western part of Colombia, S.A. Some preliminary findings will be reported on crosses with this Colombia line.

Hypothesis for r-allele-factor interaction: For purposes of adapting these lines to North American conditions, plants arising from variegated kernels were crossed to an early maturing $\underline{A}_1$ et/$\underline{A}_1$ et stock. The F_1 was pollinated by all the color testers (rr; cc; $\underline{a}_1\underline{a}_1$; $\underline{a}_2\underline{a}_2$). Plants resulting from the series of crosses to the r tester were subsequently backcrossed to r/r; five ear cultures were obtained and the kernels classified as variegated and non-variegated (Table 1). The non-variegated kernels contained both colorless and a subclass designated incomplete color.

It is hypothesized that variegation is dependent on a factor (temporarily designated, F-co*) segregating independently of the r locus, which acts on the Colombia line r allele that has been designated r-co. In the absence of the factor the r-co allele gives rise to the incomplete color subclass previously alluded to. This subclass contains kernels ranging from very faintly colored to possibly completely colorless.

A X^2 test was done for each of the crosses to test the hypothesis of the r-co, F-co interaction. None of the X^2 values was significant at the 0.05 level of probability. Since the X^2 test for heterogeneity was not significant, the data were pooled over all crosses and a non-significant X^2 value of 0.007 was obtained.

r-co + F-co = variegation r-co + f-co = colorless