U.S. PLANT, SOIL, AND NUTRITION LABORATORY
Tower Road, Ithaca, New York

1) Cotyledon culture.

A procedure for aseptically culturing immature soybean cotyledons has been developed to study the synthesis of seed storage proteins. Experiments were carried out so that one cotyledon from an embryo was compared to the second cotyledon. Cotyledons were normally incubated for 6 days at 25 C in light with gentle shaking. The medium was modified from Linsmaier and Skoog (1965) by omitting any auxin and by replacing NH_4NO_3 and KNO_3 with glutamine (30-120 mM).

Under these conditions, cotyledons grew better than on the plant and produced more protein. Both major groups of storage proteins (7S and 11S) increased throughout the culture period. The relative amount of these two storage proteins was similar to that of cotyledons developed on the plant.

Using this culture method in pulse-chase experiments with tritiated glycine, the turnover of storage proteins was found to be very slow while the half life of the nonstorage proteins was 1 to 2 weeks.

Reference

Linsmaier, E. M. and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18: 100-127.

John F. Thompson—USDA
James T. Madison—USDA

UNIVERSITY OF WISCONSIN
Department of Agronomy
Madison, Wisconsin

1) Progress in obtaining soybean haploids 2n = 20.

Male sterility gene $\underline{ms_1}$ from North Carolina was transferred to maturity groups I, II, and III over the last few years to facilitate the use in Wisconsin of the twinning and haploidy phenomena associated with $\underline{ms_1}\underline{ms_1}$ plants. In 1975 we had an extended fall growing season and seed was obtained from several hundred male sterile $\underline{ms_1}\underline{ms_1}$ plants, representing maturity groups I, II, III,

IV, and V. Honey bees were used as pollinators. Seed set was lowest for Group I (1-2 seeds/plant) and highest in Group V (20-60 seeds/plant). It is not known whether seed set was affected simply by less bee pollination of the earlier flowering steriles or by greater female sterility in the early background.

About 8000 seeds have been screened for twins and haploids. The frequency of twinning is about 2% and the frequency of twin sets containing a haploid member is about 2%. Thus far, four viable haploids $2n = 20$ have been isolated. We are still screening at this writing and these figures may change slightly in the final analysis.

The intended use of the haploids is in production of aneuploids, particularly deficiency aneuploids. Two major unknowns at the moment are: (1) whether or not ms_1 haploids will produce deficiency gametes (they may produce only numerically unreduced gametes), and (2) whether or not deficiency gametes and aneuploids will be viable. Viability or lack of it will provide a test of the hypothesis that the cultivated soybean $2n = 40$ is a tetraploid.

E. T. Bingham
W. D. Beversdorf
G. L. Cutter

2) Histology of the embryo sac of male sterile ms_1ms_1 soybeans.

The fact that ms_1ms_1 plants in maturity ranges I to V were producing haploids, triploids, and even higher ploidy levels along with the predominant normal diploids, indicated the female gametophyte was at least occasionally functioning abnormally. Histological sections of 92 male sterile pistils from plants about Groups III and IV, indicated only about 28% of the ovules had a normal embryo sac, by our interpretation. The remainder most commonly had extra nuclei in the regions of the secondary nucleus (endosperm mother cell) and/or the egg apparatus. The haploids may occur as twin members when one of these extra nuclei develops parthenogenetically. The polyploids may be the result of $2n$, $3n$, etc., gametes being formed by fusion of the extra nuclei, followed by union of these female gametes with reduced or unreduced male gametes.

G. L. Cutter
E. T. Bingham