

maximum frequency of 8% of the pods being 3-seeded. Thus, in two diverse environments, the UMS and T260 are phenotypically distinguishable, based on the frequency of 3-seeded pods.

These results indicate that the UMS is controlled by a single recessive gene at the same locus as the T260 gene (ms₁) but that it may be a different allele in a multiple allelic series. Definitive crosses have been made and segregating populations will be grown in the field in 1975.

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1. Soybean tissue culture studies.

Regeneration of whole plants from cell cultures has been a primary objective. Several cultivars cultured in a liquid medium (Miller, 1965) with 0.5 mg/liter IAA and kinetin and 1.0 mg/liter 2,4-D have developed compact spherical structures, 0.5 to 2.0 mm in diameter, composed of vascular elements enclosed in a compact sheath of parenchyma cells. These structures readily develop roots but not shoots. Altered levels and combinations of plant growth regulators have not promoted shoot differentiation in these cultures.

Fifty-six cultivars of soybeans were screened for the capacity to regenerate whole plants from cell cultures. Callus cultures were initiated from natural, immature embryos 1.0 to 2.0 mm in length on each of four media. Of the cultivars tested, 'NK 9447', 'Hisoy 225', 'SRF 100', and 'Wayne' appeared to demonstrate superior regeneration capacities by developing leaf-like and/or embryo-like structures from callus. These structures, however, deteriorated when subcultured to lower auxin, higher cytokinin media. Callus cultures of these four cultivars initiated from hypocotyl and cotyledon sections of germinated seedlings failed to produce similar organized structures.

The influence of reduced osmotic potentials on callus cultures was studied to determine if large, irregularly shaped callus cells could be modified to conform more closely to typical somatic cells. Reduced osmotic potentials were generated by the addition of mannitol, sorbitol, glucose, and sucrose to the culture medium (Miller, 1965) with 0.5 mg/liter kinetin and IAA. Development of large, irregularly shaped cells was inhibited and callus growth increased when -8 to -12 bars of osmotic tension beyond that of the standard medium was supplied. However, reduction of osmotic potential has not enhanced regeneration of whole plants.

Reference

- Miller, Carlos O. 1965. Evidence for the natural occurrence of zeatin and derivatives: compounds from maize which promote cell division. P.N.A.S. 54: 1052-1058.

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2. Soybean embryo culture studies.

To obtain information of potential usefulness in promoting the embryos from callus to develop into whole plants, experiments were conducted to find an improved technique and medium for the culture of young natural embryos. Embryos as small as 0.3 mm in diameter across the cotyledons from the embryonic axis were successfully cultured, germinated, and grown into vigorous plants which were eventually transplanted to the greenhouse.

Small embryos were placed beneath the surface of an agar nutrient medium in a loosely capped glass vial. The medium used was a modification of the B5 medium described by Gamborg, Miller and Ojima (1968); the 2,4-D was omitted, 32 mg/liter sodium ferric ethylenediammetetra-acetate was substituted for the sequestrene 330 Fe, the pH was adjusted to 5.8 instead of 5.5, and 8 grams of Difco Bacto-agar was added per liter of medium. The embryos did not germinate on this initial medium but increased in size. After 30 days, the embryos were removed and placed on the surface of a second medium of modified B5, or modified B5 supplemented with 5 mg/liter gibberellic acid.

After 1 to 2 months on this second medium, approximately one-half of the embryos germinated and developed a leafy shoot. The embryos on the gibberellic acid medium developed roots and shoots slightly earlier than those on the basal medium and thus seemed more vigorous. The plantlets were transferred to "Jiffy 7" peat pellets and kept in a humid environment under a tent of cellophane wrap for 2 to 3 weeks under fluorescent lights and a photoperiod of 16 hours, after which they could be handled as natural seedlings.

Reference

Gamborg, O. L., R. A. Miller and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* 50: 151-158.

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3. The search for haploid soybeans by using male sterility.

The main objective of the work with the ms_1 male-sterile gene of soybeans is to obtain haploid plants for genetic and cytogenetic research. A haploid individual was found by Kenworthy, Brim and Wernsman (1973) as a member of a set of twins which was observed among the progeny of ms_1ms_1 male-sterile plants. Seed with the ms_1 gene was obtained from Brim at North Carolina. Cultivars 'Altona', 'Chippewa 64', 'Corsoy' and 'Hark' were used in crosses and backcrosses to achieve maturity suitable for Wisconsin.

Hand pollinations of male-sterile (ms_1ms_1) plants were made in the greenhouse in the summers of 1973 and 1974. Among 30 seeds obtained and germinated in 1973, one twin set was observed which was a triploid : haploid set. In 1974, 840 crosses were made and 361 seeds were obtained. All except 5 seeds germinated and 20 sets of twins were obtained. To date, 18 of these sets have been examined cytologically and 15 were found to be diploid : diploid, 1 was diploid : triploid, and 2 were diploid : lost. Thus, no haploids have yet been found in 1974.

References

Brim, C. A. and M. F. Young. 1971. Inheritance of a male-sterile character in soybeans. *Crop Sci.* 11: 564-566.

Kenworthy, W. J., C. A. Brim and E. A. Wernsman. 1973. Polyembryony in soybeans. Crop Sci. 13: 637-639.

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4. Soybean aneuploid studies.

We have isolated a group of aneuploid plants from the cultivar 'Dunn'.

A plant with 43-44 chromosomes produced four progeny which had chromosome numbers of 40, 40, 41, and 42 respectively. One of the 40-chromosome plants had full fertility and normal morphology. The other 40-chromosome plant was moderately compressed in stature and had lower fertility. This plant produced 27 seeds, of which 13 were shriveled. Analysis of this material has just begun. Two progenies thus far evaluated each have 42 chromosomes. Among the normally-shaped seeds of this 40-chromosome plant, 3 progenies which have been evaluated all have 41 chromosomes.

The 41-chromosome plant of the previous generation was also reduced in stature and highly sterile, producing only 6 small shriveled seeds. All of these seeds have germinated and the progenies are currently being evaluated. The 42-chromosome plant of the previous generation was greatly reduced in size and produced only 1 seed. The resulting progeny is currently under study. It is not yet known if all the trisomics carry the same extra chromosome or if the 42 chromosomes are tetrasomics or double trisomics.

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