2. Further experiments in mechanical induction of tetraploidy.

A definite relationship between pollen size and chromosome number of its gametes indicates that mechanical screening of pollen produced by triploids can be used to direct triploid maize progeny into the tetraploid chromosome number range (MNL 38: 20-21). Eight additional experiments were completely successful in inducing tetraploidy. Triploid embryos, produced upon a 4n female parent, grow very readily if the collapsed pericarp is removed. Sterile techniques are not needed. Fungicide-treated abscised embryos germinate vigorously on moist filter paper in petri dishes, and if transplanted promptly to small soil pots for further establishment and hardening-off before taking to the field, produce strong mature plants with ample pollen and vigorous ear production. Sib pollination of triploid plants with the 125 micron pollen fraction obtained by screening results in many viable, plump kernels per ear. These can be grown out in the normal manner, and again sib-pollinated. While this generation is almost entirely composed of hypo-tetraploid plants, gametophyte embryo selectivity shifts the population composition toward eutetraploidy (Can. J. Genet. Cytol. 4: 226-233). If desired, a second sib generation will result in a 4n population with normally distributed chromosome numbers from which genes or linkages introduced by the original diploid can be recovered, or from which desirable 4n plants can be selected for a second cycle of backcrossing to the original diploid, if more complete recovery of the diploid genome as a tetraploid is sought.

Actual counting of chromosomes is not necessary. 4n plants can readily be identified by visual examination, by test screening of pollen for the presence of 125 micron grains or by test crossing to an established tetraploid. The technique is therefore readily adaptable to ordinary maize breeding techniques since it requires a minimum of special equipment and skill. The technique appears to be by far the most rapid and easily applied method of inducing tetraploidy thus far proposed for maize. A natural application of the method would be to solve the difficult problem of establishing aleurone and plant color genetic 4n testers. For example, if a 4n stock "pure" for the $\underline{A_1}$ $\underline{A_2}$ $\underline{C}$ $\underline{R}$ $\underline{Pr}$ series were crossed to a 2n $\underline{r}$ tester, and the resulting triploids handled as described, any post-triploid 4n recovery having a colorless aleurone would be a newly-derived 4n $\underline{r}$ tester stock.

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