

3. Chemical treatment of pollen in vivo by the plastic bag method.

In the course of recent chemical mutagenic investigations (G. Ficsor, Ph.D. thesis, University of Missouri, 1965) indicator dyes were used to compare various methods of chemical treatment of pollen in vivo. The routine was to treat pollen by the various methods with a 0.1 per cent solution of methylene blue (MB) followed by visual examination of the plant and by microscopic examination of some anthers in quest of colored pollen grains. Five methods of pollen treatment were compared: treatment through leaf uptake, by stem or tassel injection, by the wick method, by the cut tassel method and by the cotton packing injection method. The result was that all methods stained the vascular systems to some degree, but only with the cotton packing injection method were a few colored pollen grains observed. Four of the five methods were also tested genetically, using the mutagen ethyl methanesulfonate (EMS) in place of MB to determine if the method which produced colored pollen grains would also produce increased mutation rates. The result was, that only with the cotton packing injection method were mutation rates increased significantly (Science 139, p. 1296, 1963). Thus one finds a close agreement between the penetration of methylene blue in the preliminary trials and the mutagenic efficiency of EMS with the various methods of treatment. But even with the cotton packing injection method, mutation rates were much lower than obtainable with optimum doses of x-rays in spite of the fact that in seeds EMS was capable of inducing mutation rates several times higher than x-rays (MNL 37, p. 104, 1963). In search for clues to explain the chemical's low mutagenic efficiency in the pollen it was noted that with the cotton packing injection method the tassel received uneven treatment due to the flow of MB or EMS solutions to the bottom of the pack. Moreover, with MB one could detect differences between florets in degree of pigmentation, in that younger florets were penetrated better than older ones which were protected by well sealed waxy glumes. Also, the leaking of the pack made the method risky to use with toxic chemicals.

To overcome these difficulties a new method of treatment was developed using 0.1 per cent MB as indicator with a few drops of Tween 20 as a wetting agent to break down the waxy protection of the glumes. As a first step the tassel to be treated was stripped of leaves exposing the upper part of the last internode. Then a long, narrow, seamless plastic bag was pulled down over the tassel (just like a tassel bag) and its mouth was firmly tied around the stem with a strong string so that all possibility of leakage down the stem was excluded. The dye was then poured in from the top of the bagged tassel through a 1" to 2" cut in the bag. The volume can be adjusted on the tassel by wrapping it with adhesive tape until the desired volume is obtained. In our experience 50-150 ml of solution was needed to submerge the tassel completely. Before pouring in the chemical the tassel should be supported with a stick to prevent it from breaking. Within 3 hours of treatment all the pollen grains examined from any part of the tassel and from any floret were stained, indicating that the difficulties of the cotton packing injection method were overcome.

Mutagenic tests with the plastic bag method have not yet been completed but it is expected that the correspondence found previously between indicator dye tests and actual mutagenic experiments will hold for this method as well.

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1. Classification of maize lines and selection of breeding materials by the application of multivariate statistical analysis.

Classification of local maize lines and selection of breeding materials were successfully carried out by the application of principal component analysis. Biological meanings of the extracted principal components in this study and the classification of the lines on four principal component axes were discussed in relation to plant breeding.

Materials used in this study were the data reported on the characteristics of 57 local Caribbean flint lines collected from Shikoku, Japan (Suto et al, unpublished). A part of the data were preliminarily reported in M.G.C.N.L. 33:84-88. Out of 65 botanical and agronomical characters observed, we selected ten characters which were of significance in plant breeding, and among which correlation coefficients were not so high. They were silking date, stalk length, leaf length, number of leaves, tassel length, ear length, ear diameter, ear weight, number of husks and 100 grains weight.

The correlation matrix of these ten characters was calculated, following principal component analysis. Twenty-eight, twenty-two, nineteen and thirteen per cent of the total variation of ten characters were accounted for by the first four principal components respectively, and hence more than 80 per cent could be explained in total (Table 1).