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the data reported on the characteristics of 57 local Caribbean flint lines collected from Shikoku, Japan (Suto et al, unpublished).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).

Table 1

Eigen values (λ_j) and associated eigen vectors (l_jk) obtained from principal component analysis

Principal component	1	2	3	4	5	6	7	8	9	10
Eigen value (λj)	2.825	2.139	1.819	1.248	0.634	0.530	0.340	0.224	0.179	0.062
$\Sigma \lambda j$	2.825	4.964	6.783	8.031	8.665	9.195	9.535	9.759	9.938	10.000
Eigen vector	l_{1k}	l_{2k}	l_{3k}	l_{4k}	l_{5k}	l_{6k}	l_{7k}	l_{8k}	l_{9k}	l_{10k}
Silking date	.457	-.286	-.034	.199	.026	-.178	.558	.198	.525	.101
Stalk length	.497	-.083	-.102	.189	.273	.302	-.453	-.384	.349	-.244
Leaf length	.460	.155	.253	-.206	-.096	-.151	-.475	.613	-.155	.020
Number of leaves	.412	-.253	-.334	.162	.254	.030	.202	-.031	-.698	.187
Tassel length	.241	.046	.447	-.530	-.212	-.046	.087	-.591	-.235	.014
Ear length	.213	.269	.374	.462	-.261	.408	.400	.128	-.098	-.329
Ear diameter	.134	.423	-.478	-.084	-.142	-.449	.099	-.090	-.056	-.570
Ear weight	.193	.580	-.171	.215	-.185	-.043	-.005	-.175	.155	.679
Number of husks	-.037	.234	-.361	-.551	.027	.685	.093	.176	-.005	-.008
100 grains weight	-.055	.422	.291	-.068	.827	-.113	.177	.030	-.002	-.023

Table 2

Average squared distance between varieties and within a variety classified by using principal component analysis and squared distance between lines[1] in the four-dimensional space

Variety	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14
Number of lines	4	1	5	6	15	3	3	3	1	5	5	3	2	1
V1	3.5[2]	9.8	12.5	16.8	14.6	35.8	30.8	33.5	51.6	33.2	28.8	38.4	44.0	26.8
V2		0	22.0	16.2	15.3	29.5	25.9	41.5	46.6	35.0	45.6	54.1	46.2	50.6
V3			4.2	12.7	8.9	21.5	23.5	23.0	27.3	12.2	11.2	13.2	16.9	34.1
V4				4.6	9.3	11.7	19.0	18.2	14.9	20.8	20.3	21.0	20.6	32.2
V5					4.3	10.3	8.6	13.4	22.0	10.1	14.0	22.5	24.6	32.2
V6						4.0	8.1	11.3	8.2	12.5	19.3	24.4	22.8	45.9
V7							4.0	10.1	25.3	13.4	21.3	35.8	40.1	40.1
V8								2.3	22.4	18.1	11.1	24.2	39.1	19.4
V9									0	19.8	24.1	17.2	11.9	61.5
V10										4.2	11.5	16.9	17.4	52.2
V11											4.2	8.8	22.6	26.5
V12												3.9	10.0	43.0
V13													4.7	71.9
V14														0

1) Squared distance between lines was calculated from the scores of lines for the first four principal components
2) Figures in the diagonal indicate average squared distances within a variety

So as to classify the lines into line groups or varieties having similar characteristics, squared distances between lines in the four-dimensional space were calculated from scores of lines for the first four principal components. The smaller the squared distance between lines was, the more similar the characteristics of lines were expected to be. So the lines among which squared distances were very small were grouped as a variety. The criterion of grouping lines was that the average squared distances within a variety were always smaller than ones between varieties. In consequence, 57 lines were classified into 14 varieties (Table 2).

The classification based on the principal component analysis and distance method agreed generally with the previous one based on the conventional method. Furthermore, these varieties were classified into four major varietal groups by using the same procedure mentioned above (Table 3).

Table 3

Average squared distance between varietal groups and within one

Varietal group	A	B	C	D
A	6.0	22.2	37.4	31.5
B		11.4	18.9	38.1
C			10.6	40.5
D				0

Note: Varieties belonging to the respective varietal groups are as follows: A (V1-V2), B(V3-V10), C(V11-V13), D(V14)

These calculations were made on electronic computer OKITAC 5090C.

For the purpose of understanding the relation between characters and principal components, the characters were assorted into three classes, plus, minus and zero (in Table 4, class zero was omitted).

Table 4

Identification of characters by the degree of contribution to the first four principal components

Principal component	Class[1)]	Corresponding character
Y1	+	Stalk length, leaf length, silking date, number of leaves
	-	None
Y2	+	Ear weight, ear diameter, 100 grains weight, ear length
	-	Silking date, number of leaves
Y3	+	Tassel length, ear length, 100 grains weight, leaf length
	-	Ear diameter, number of husks, number of leaves
Y4	+	Ear length
	-	Number of husks, tassel length

Note: 1) l_{jk} in Table 3. + ; $l_{jk} \geq 0.250$, - ; $l_{jk} \leq -0.250$

This assortment was based on the value of eigen vectors corresponding to the respective characters in Table 1. In the case of the first principal component, characters concerned with the size of vegetative parts of a plant and earliness, such as stalk length, leaf length, number of leaves and silking date, contributed greatly to the principal component. So the biological meaning of the first principal component appeared to correspond to the general size of vegetative characters of a plant in relation to the duration of the growing period. In the case of the second principal component, the characters concerned with grain yield and earliness, such as ear weight, ear diameter, ear length, 100 grains weight, silking date and number of leaves, contributed greatly to the principal component. So the biological meaning of the second principal component appeared to correspond to the yielding ability, especially to the efficiencies of photosynthesis and translocation in the plant. By a similar consideration of biological meaning, the third principal component appeared to correspond to the degree of differentiation in organs.

Corresponding to the respective principal components, several plant types, i.e. forms of variation represented by the compound characters, were made clear. The first principal component corresponded to an early and small plant type vs. a late and large, the second an early and high yielding plant vs. a late and low yielding one, and the third a plant with short conical ear and short tassel vs. one with long cylindrical ear and long tassel.

Varieties or lines which seemed to be most suitable for breeding materials could be selected by choosing the principal components which were important in connection with the breeding objectives. In this study the first and the second principal components were most important in connection with the objectives of breeding early and high yielding hybrids.

Thus, classification of maize lines and selection of breeding materials were achieved by the application of principal component analysis and distance method.

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1. X-ray induced mutations during pollen tube growth in maize.

X-ray induced mutations in maize pollen were first discovered by Stadler (1928). Recently, the 24-28 hours old developing embryos in maize have proved to be a suitable system for a study of radiation- and chemical-induced mutagenesis (Singleton, 1961; Verma et. al., 1962; Caspar, 1965; Chatterjee et. al., 1965). However, very little is known about the frequency of X-ray induced mutation during pollen tube growth or early stages of fertilization. In order to obtain some information in this respect, ears having the recessive gene (su) were pollinated with Su pollen and were irradiated by X-rays at different times after pollination ranging from 0 to 30 hours. Total dose was 1210r ± 110r, with a dose rate of 96.1 r per min. (173 kVp, 25mA, 0.5mmCu + 0.5mmAl filter, 45-50cm distance). Whole and chimeral endosperm mutations at the Su locus were scored in the kernels resulting from these pollinations and data thus obtained are shown in Figure 1.

The frequency of whole mutations increased rapidly (from 2.91% to 5.81%) when ears were irradiated 0 to 12 hours after pollination; the frequency decreased slightly (from 5.81% to 5.60%) when X-irradiation was given 12 to 18 hours after pollination; the frequency declined rapidly (from 5.60% to 3.41%) from 18 to 30 hours after pollination. On the other hand, the frequency of chimeral mutations increased gradually (from 0.83% to 1.39%) when treatment was in the interval from 0 to 12 hours after pollination; the frequency increased rapidly (from 1.39% to 3.94%) in the interval from 12 to 30 hours after pollination. The data for the per cent of chimeral mutations are summarized in the following table:

	Hours after pollination					
	0	6	12	18	24	30
% chimeral endosperm mutations	21.6	18.3	19.5	28.1	41.0	50.4