A LOCALISED CHLOROPHYLL DEFICIENCY ASSOCIATED WITH MALE STERILITY IN *NICOTIANA TABACUM* L.

SPONTANEOUS occurrence of male sterility controlled by single or duplicate factors is known only in a few cases in *Nicotiana tabacum* (Raeber and Bolton, 1955, and Bhat and Krishnamoorthi, 1956). Other teratological modifications of the stamens and consequent male sterility controlled by a single gene are also reported in X-ray populations of the same species (Goodspeed, 1930).

During the screening of the irradiated material of flue-cured varieties of *Nicotiana tabacum* for viable mutations after seed treatment with thermal neutrons. (flux 1×10^{12} to 5×10^{13} N.sec.cm.^{2}), male sterile plants appeared in the n_2 generation in five different lines.

Three varieties, *viz.*, Hicks, Delcrest and Chatham, were irradiated at the levels mentioned above. But male sterile plants appeared only in Hicks and at the dose 1×10^{12} N(cm.)^{2}. Several plants had been selfed in the n_1 generation both in the control and irradiated populations. Of them, 10 lines were raised in each category. Only 5 of the 10 lines in the irradiated population seggregated for male sterility.

Two categories of male steriles were found. In both cases the anthers were rudimentary.

In one there was development of appendages with no sporogenous tissue; in this case seed set could be obtained by pollinating with normals indicating that the female gametes were functioning normally.

In the second category, appendages were absent, the stigma was yellow and the style was very short, 5–6 mm., compared to over 40 mm. in the normal plants. These plants were also characterised by condensed panicles, clustering of flowers and cleistogamy (Figs. 1 and 2 *c*, *d*). The corolla was whitish and the younger leaves below the peduncle turned yellowish-green with the onset of reproduction, while the lower leaves remained normal green. The stamens were reduced in size and obliterated. Pollen was absent in all cases except in one of the flowers which had nearly 10% stainable pollen. Pollination with abundant pollen from the normal sibs failed to give any seed set indicating female sterility also.

FIG. 1. Panicle of the 'yellow stigma' mutant showing crowding and cleistogamy of flowers.

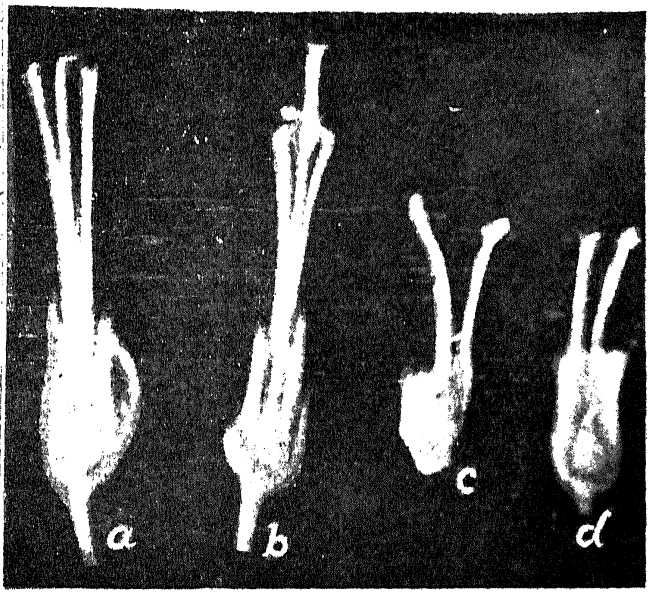

FIG. 2. Flowers of *a*, normal; *b*, males sterile; *c* and *d*, yellow stigma mutant. Note the dwarf style in *d*.

The n_1 plants from whose progenies these two types of male steriles were obtained were phenotypically normal and the F_1 between the first category of male sterile mutant and the normal was completely fertile. This character can therefore be considered to be recessive to normal. The distribution of the different categories of mutants in the n_1 and n_2 generations is given in Tables I and II. The two classes of mutants are called "male sterile" and "yellow stigma" respectively for brevity.

Among the five lines three seggregated only for male sterility, one for 'yellow stigma' only and one for both 'male sterility' and 'yellow stigma'. In the 'yellow stigma' seggregants none were present with appendages on the anthers similar to those found in the first category of male sterility. The data suggest that the first case of male sterility is due to duplicate recessive factors. Line-1 gave simplex seggregation suggesting that it is homozygous for one of the recessive genes. The 'yellow stigma' character also appears to be due to duplicate recessive factors. To ascertain whether the male sterility in 'yellow stigma' plants is due to pleiotropic effect of the genes for chlorophyll deficiency or due to close linkage with genes for male sterility, test for linkage was carried out by Fisher's scoring method. The test confirmed that linkage was not involved. Therefore the male sterility in the 'yellow stigma' is due to the pleiotropic effect of the genes for chlorophyll deficiency and appears to be distinctly different from the male sterility with the anther appendages encountered in the same population. These two mutants appeared only in Hicks but not in the other varieties (Table II). Such a specific response by a variety to a certain dose

TABLE I

Distribution of mutant categories in the n_1 and n_2 generations

Generation	Variety	Total plants studied	Chlorophyll mutants (seedlings)		Leaf modifications No. of plants different in leaf characters from normal	Floral teratology			Sterile mutants		
			Normal	Albina		Split flr.	Calycina	Cleistogamous	Pollen abortion over 30%	Male sterile anthers	Complete sterility
n_1	Hicks	480	480	..	14	1	1	1	136	1	..
	Delcrest	420	420	..	6	..	..	..	75	..	..
	Chatham	270	270	..	1	..	..	..	69	..	..
n_2	Hicks	800	540 (separate scoring)	168	2	..	..	..	20	185 (Normal) 28 Male sterile	101 (Normal) 8 Yellow stigma
	Delcrest	900	..	..	1	..	..	..	16	..	..
	Chatham	900	..	..	..	..	..	..	..	..	..

TABLE II

Distribution of 'male sterile' and 'yellow stigma' mutants in the n_2 generation

Sl. No.	Lines	Total No. of plants	Normal	'Male sterile'	'Yellow stigma'	X^2
1	7–44 (3 : 1)	55	40	15	..	0·1514
2	23–8 (15 : 1)	48	43	5	..	0·893
3	20–32 (15 : 1)	54	51	3	..	0·0743
4	23–16 (15 : 1)	56	50	..	6	1·167
5	23–42	58	51	5	2	2·157

indicates that the residual genotype has considerable influence on the mutability of the concerned loci. It is interesting that both these cases are due to duplicate factors while in X-rayed population studied by previous workers, male sterility was a simple recessive. The denser ionizing radiation of neutrons is responsible for simultaneously knocking out the two loci concerned and the higher viability of the neutron-treated material compared to X-rayed material has permitted the detection of the mutants which would have been lost due to the drastic side-effects of the X-rays.

Studies are under way to ascertain whether the male sterility genes are iso-allelic with other male sterile lines maintained at this Institute. The genes for yellow stigma were designated as ys_1 and ys_2. These are different from the stigmatoid gene isolated by Goodspeed (1930). The manifold effects associated with the dwarfing of stigma without any reduction in the vegetative plant organs is unique in this case.

Central Tobacco Research Institute, Rajahmundry, July 22, 1961.
G. S. MURTY.
K. V. KRISHNAMURTY.
B. R. MURTY.

1. Bhat, N. R. and Krishnamoorthi, T., *Curr. Sci.*, 1956, **25**, 297.
2. Goodspeed, T. H., *Univ. Calif. Pub. Bot.*, 1930, **11**, 285.
3. Raeber, J. G. and Bolton, A., *Nature*, 1955, **176**, 314.