NOTES AND COMMENTS

CYTOLOGICAL ANALYSIS OF MUTANT ALBINO SEEDLINGS IN *NICOTIANA TABACUM*

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Received 14.i.67

1. INTRODUCTION

MOAV (1957) showed that *Nicotiana tabacum* (*tbc*) plants, heterozygous for the recessive lethal mutant *ws* (*White-seedlings*) which, in the homozygous condition inhibits the production of chlorophyll, usually show a few albino (chlorophyllless) spots on their leaves. These spots are absent in homozygous dominant plants. Similarly, when a large number of *tbc* seeds, all heterozygotes *Ws*, *ws* are germinated, one seedling in several thousands is an albino mutant. Are the albino mutant seedlings and albino leaf spots the result of *point mutations*, gross chromosomal aberrations, or extrachromosomal aberrations? Chromosome counts in root tips of mutant albino seedlings revealed that about one third of the mutants were haploids, and that in most of the remainder one to six chromosomes were missing.

2. MATERIALS AND METHODS

The two species used were *Nicotiana tabacum* L. ($n = 24$), variety Red Russian, and *Nicotiana glauca* (L.) Link. ($n = 10$) (Goodspeed, 1954).

The cytological preparations of the root tips were made, after slight modifications, by the method described by J. Moav (1960).

The seedlings were (i) immersed in a para-dichloro-benzen solution for 90 minutes for condensation and spreading; (ii) transferred to a 7:1 solution of 2 per cent. aceto-orcein and a 0.1 N chloric acid for four hours; (iii) placed for one hour in three drops of 1 N chloric acid to dissolve the cell walls; (iv) macerated, lightly squashed, warmed and covered.

The optimal age for preparation of green seedlings was found to be three to four days after the emergence of the cotyledons. However, counts could be made 2-3 days earlier or later. The optimum age for the albino seedlings was only 6-12 hours after germination, and even slight deviation from this range reduced considerably the number of dividing cells.

3. THE COURSE OF THE EXPERIMENT AND ITS RESULTS

The production of a population, where all individuals (except rare albino mutants) are heterozygotes *Ws*, *ws*, requires crossing of homozygous dominant green plants with homozygous recessive albino plants. Ordinarily, the latter are lethal but they can develop almost normally through the addition of an alien chromosome carrying the dominant *Ws* marker. The male and female transmissions of an alien univalent, extracted from *N. glauca*, and carrying the *Ws* marker, were 3 per cent. and 20 per cent. respectively (Moav, 1961).

These alien addition plants (*pWs*, *ws*, *ws*) were used as the male parent in crosses with normal *tbc* plants, and with the four *tbc* monosomes Haplo-M,

Haplo-K, Haplo-E and Haplo-B. They were used as the female parent in crosses with normal *tbc* plants and with an alien addition *tbc* plant possessing *pbg* univalent marked by the dominant gene *Pollen-Killer* (*KI*) (Cameron and Moav, 1957).

Confidant chromosome counts were made in 32 albino seedlings, plus a large number of green seedlings. The majority of the albino seedlings revealed many chromosomal aberrations (table 1) but in green seedlings

TABLE 1

Chromosome numbers in root-tips of mutant albino seedlings of N. tabacum

Parents		No. of tested seedlings	Observed no. of chromosomes*				Expected no. of chromosomes
Female	Male		Haploids	48+F† or 49	47	Less than 47	
Monosomes (M, K, E, and B)	<i>pWs, ws, ws</i>	14	4	1(48+f)	5	2(44) 2(42)	47, 48 rare 49
Normal	<i>pWs, ws, ws</i>	3	...	...	3	...	48 rare 49
<i>pWs, ws, ws</i>	Normal	5	2	...	2	1(44)	48, rare 49
<i>pWs, ws, ws</i>	KI	10	4	1(49) 1(48+F)	1	1(45) 1(44) 1(42)	49 rare 48 or 50
Sums		32	10	3	11	8	

* Due to the early mortality and the scarcity of the albino mutants only a small number of cells, sometimes only one, could be confidently analysed in each seedling.

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not a single aberration was found. The two striking results are the high proportion of haploids and of seedlings with more than one chromosome missing among the albino mutants.

In the two crosses where the female parent was *pWs, ws, ws* the ovules transmitted the recessive *ws* allele of *tbc*, and the pollen-grains transmitted its dominant allele. Thus the haploid albino offspring of this crosses were most likely parthenogenetic. The reverse existed in the five crosses where the female parent transmitted the dominant *Ws* of *tbc* and the male parent its recessive allele. Here, the haploid albino offspring probably had an androgenic origin.

4. DISCUSSION

Only 6 of the 32 albino mutant seedlings tested possessed the expected chromosome numbers. The remaining 26 fell into three classes, 10 were haploids, 2 contained fragments—not present in their parents, and in 14 from 1 to 6 chromosomes were missing (table 1).

Thus, the great majority of the albino mutants of *tbc* are a consequence

of gross chromosomal aberrations, covering the wide range, from loss of fragments to the loss of whole sets of chromosomes.

At least two mechanisms are needed to explain the origin of the haploid mutants. These are: ovum abortion following the penetration of a pollen-grain nucleus into the embryo-sac, and apomictic development of an unfertilized ovum or another haploid nucleus within the embryo-sac.

Loss of a fragment, or one to several chromosomes, rather than a whole set, may be due to meiotic or mitotic disorders. The latter could be responsible also for the albino spots found on the leaves of plants heterozygous for *ws*.

In the strongly mottled hybrid derivatives of *tbc* and *N. plumbaginifolia* it was shown that albino shoots are always deficient for a fragment or a whole chromosome (Moav, 1961; Moav, Moav and Zohary, unpublished). This, with the present observations indicates that the albino spots on leaves of unhybridised heterozygous *tbc* plants, are also due to gross chromosomal aberrations, rather than to point mutations.

5. SUMMARY

1. Of several thousand *N. tabacum* seedlings, all heterozygous for the recessive allele *ws* which in the homozygous condition inhibits chlorophyll production, one is an albino mutant.

2. Ten of 32 albino seedlings were found to be haploids of which 4 were androgenous and 6 had a parthenogenetic origin. Two contained fragments that were not present in the parents, in 14 mutants 1 to 6 chromosomes were missing, and only 6 mutants possessed an expected chromosome number.

3. The great majority of the albino mutants in *N. tabacum* are the consequences of gross chromosomal aberration, rather than point mutations.

Acknowledgment.—The writers express their gratitude to Professor D. R. Cameron for his generosity in supplying the seed needed for the present investigation.

6. REFERENCES

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