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MORPHOLOGICAL CHARACTERIZATION OF *NICOTIANA TABACUM* L. PLANTS REGENERATED FROM TISSUE CULTURES.

CARACTERIZAÇÃO MORFOLÓGICA DE PLANTAS DE *NICOTIANA TABACUM* L. REGENERADAS A PARTIR DE CULTURAS DE TECIDOS.

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SUMMARY – A study of the relationship between the ploidy and some morphological features of *Nicotiana tabacum* L. plants, regenerated from tissue cultures, made it possible to establish four groups of plants (H, D, P and C). The H-plants were haploid, with 24 chromosomes. Their leaves were lanceolate and membranous, with about 11 chloroplasts per stoma. The corolla lobes were pointed and the stigma protruded beyond the corolla tube. Fruits were absent. The D-plants were diploid or aneuploid with chromosome number equal or close to 48. Their leaves were elliptical, leathery, bearing more or less 20 chloroplasts per stoma. The corolla lobes were triangular and the stigma lay in the same plane as the lobes of the tube. Plants almost always bore fruits. The P-plants were polyploid or aneuploid with chromosome number equal or close to 60, 72, 96 or 120. Their leaves were elliptical, ovate or lacerate and always more or less fleshy. Stomata with 30, 40 or more chloroplasts. Flowers were absent or presented blunt corolla lobes. The stigma was hidden in the corolla tube. Plants frequently were sterile or with few fruits. The C-plants showed features that were similar to those of D or P-plants, being characterized by leaves with more or less pronounced spots and mosaics or by their tendency to bear lateral branches showing developmental patterns clearly distinct from those of the main shoot.

RESUMO – O estudo das correlações entre a ploidia de plantas de *Nicotiana tabacum* L. regeneradas a partir de culturas de tecidos e algumas de suas características morfológicas permitiu o reconhecimento de 4 categorias distintas de plantas: H, D, P e C. Na classe H agrupamos as plantas haplóides com 24 cromossomos. Estas plantas apresentavam folhas lanceoladas, membranosas e com aproximadamente 11 cloroplastos por estômato. Os lobos da corola eram afilados e o estigma saliente. Nunca chegavam a formar fruto. Na classe D englobamos as plantas diplóides e aneuplóides com valores iguais ou próximos a 48 cromossomos. As folhas eram elípticas, coriáceas, com aproximadamente 20 cloroplastos por estômato. Os lobos da corola eram triangulares e o estigma rente aos lobos do tubo da corola. Quase invariavelmente apresentavam frutos. Na classe P englobamos as plantas poliplóides e aneuplóides com número de cromossomos variando ao redor de 60, 72, 96 e 120. As folhas eram elípticas, ovaladas ou laceradas porém sempre mais ou menos carnosas. O número de cloroplastos dos estômatos variava ao redor de 30, 40 ou mais. Não apresentavam flores ou quando estas estavam presentes, tinham os lobos da corola arredondados e estigma mergulhado no tubo da corola. Frequentemente eram estéreis ou formavam poucos frutos. As plantas da classe C tinham características similares às das classes D ou P, distinguindo-se destas pela presença de mosaicos mais ou menos extensos nas folhas ou pela tendência de formarem ramos laterais com aspecto e padrão de desenvolvimento diferente daquele do eixo principal.

INTRODUCTION

Plants in which the number of chromosomes was different from that of the original plant have been regenerated from tissue culture (Sacristan & Melchers 1969). The occurrence of polyploid forms in a batch of regenerated plants might be expected considering the polysomatic nature of most plants (D'Amato 1952). Tissue cultures originated from explants comprising both meristemátic and differentiated cells with all probability will contain some polyploid cells and some of the plants regenerated from

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these tissues may be polyploid (Murashige 1974). The determination of the chromosome number of plants regenerated in large batches may be a difficult task considering the available techniques of chromosome counting. In order to overcome this difficulty we tried to correlate the chromosome number with some morphological characteristics of the regenerated plants. These morphological features could be useful to infer the ploidy level of tobacco plants regenerated by anther culture and by other tissue culture techniques.

MATERIAL AND METHODS

Haploid and diploid plants of *Nicotiana tabacum* cv Wisconsin-38 were used as the source of pith tissues. The haploid plants were obtained by anther cultures as described by Nitsch (1969). Diploid plants were grown from seeds.

Explants were taken from ca. 100cm tall plants at the vegetative growth stage. Pith tissue cylinders of 5mm dia. were removed from three regions of the stem, at 5, 20 and 40 cm from the shoot apex. The tissues were cultured on 20ml of RM-65 medium (Linsmaier & Skoog 1965) containing 2.0mg/l indoleacetic acid and o.8mg/l kinetin. The cultures were incubated with a photoperiod of 16hr light (about 3.000 lux) at temperatures of 29°C (day) and 24°C (night). All buds that were present on the callus after 42 days of culture were transferred to rooting medium (RM-65 medium without growth substances). Rooted plantlets were transferred to potting compost and grown in the greenhouse.

Chloroplasts of both guard-cells of the stomata were counted on leaf peelings taken from mature leaves. The chloroplast number was recorded during the morning after their reaction with silver nitrate at 1% solution (Molish 1918 apud Butterfass 1959). At least 10 stomata per plant were recorded. The chromosome number was determined on aceto-·carmine root-squash preparations (Sass 1951).

RESULTS

The number of chloroplasts in the stomata of mature leaves was determined in 150 plants selected at random among haploid, diploid and regenerated plants. It was observed that the chloroplast number oscillated around median values that were characteristic of each plant. Considering the sample as a whole it was noticed that there were at leat four of these median values that proved to be most frequent v.g. 10, 20, 30 and 40 chloroplasts per stoma. This allowed us to arrange the plants into four groups called, respectively, H, D, P_1 and P_2. Table 1 shows the range of the chloroplast number, the sample mean and its standard error in each of these classes.

TABLE 1. Chloroplast number in the stomata of leaves of *Nicotiana tabacum* L.

Chloroplast number	Class H	D	P_1	P_2
mininum	8	14	22	24
maximum	18	30	52	63
mean	11	20	34	41
standard error	1.97	2.70	4.85	7.06

The number of chloroplasts in the stomata of the leaves was correlated with the number of chromossomes in the cells of the root tips. The results of a survey comprising 48 plants are presented in table 2. The mean chloroplast number of 12 plants characterized

them as being H-plants; their chromosome number was 24 which is the characteristic number of haploid *N. tabacum* plants. The D-plants, characterized by presenting mean values equal or close to 20 chloroplasts, proved to include diploid and aneuploid plants with chromosome numbers equal or close to 48. P_1-Plants included triploids and aneuploids with chromosome numbers equal or close to 72 and chloroplast values oscillating around 30. The P_2-class comprised tetraploids and aneuploids with chromosome number equal or close to 96 and more or less 40 chloroplasts in the stomata. Table 2 shows that chromosome counting disclosed the presence of at least two other classes, P_3 and P_4, which could not be separated either from D or from P-plants, based solely on their chloroplast number. The P_3-plants presented approximately 60 chromosomes and the P_4-plants presented 120 chromosomes.

TABLE 2. Correlation between the number of chromosomes in root tips and the number of chloroplasts in the stomata of mature leaves of *Nicotiana tabacum* L.

Class	Chromosomes (recorded values)	Chloroplasts (mean value)	number of plants
H	24	11	12
D	46	21	1
	48	20	12
	47, 48	20	1
	48, 49	19	1
P_1	70	34	1
	70, 72	32	3
	72	31	3
	72, 75	30	1
	75	37	1
P_2	92, 93, 104	42	1
	95, 96	41	1
	96	40	6
P_3	58, 60	42	1
	60	27	1
	58, 60, 70	51	1
P_4	120	45	1

TABLE 3. Morphological features of *Nicotiana tabacum* L. plants regenerated from tissue cultures.

	H	Class D	P
LEAVES:			
Shape	lanceolate	elliptic	ovate or lacerate
Consistency	membranous	leathery	fleshy
FLOWERING:			
Flowers	large number, sterile	smaller number than in H, mostly fertile	absent or in small number frequently sterile or with few seeds
Color of corolla-tube	pink	pink or red	red
Shape of the lobes of corolla-tube	pointed	triangular	rounded, blunt
Stigma	protruding beyond the corolla-tube	in the same plane as the lobes of the corolla-tube	sunken in the corolla-tube

Other morphological characters were also recorded besides chloroplast and chromosome numbers. In table 3 some features of H, D, and P-plants are shown. In the material under observation, the morphological features of H-plants were shared by all of them, making the group very homogenous and clearly distinct from all other plants. On the other hand, the D and the P-plants showed a gradation in their morphology ranging from plants similar to those of the Wisconsin-38 tobacco obtained from seeds to dwarfed polyploids with lacerate and fleshy leaves. Another feature that could be observed was the presence of D and P-plants with leaves showing white, yellow or light-green spots and mosaics. Some of these mosaics were observed only on the leaves produced on one side of the plant. Some dwarfed P-plants, after pruning of the main shoot produced lateral branches which after rooting, originated plants that showed less aberrant features than those presented by the mother plant, with a tendency to grow tall and to produce whole leaves. The most striking changes in morphological features could be observed in some cases in which the main shoot did not produce any flowers but, after pruning of the shoot apex, the lateral branches flowered.

DISCUSSION

In several species the number of chloroplasts of the stomata has been related to the number of chromosomes. It has been shown that in high ploidies this relationship was not linear and in aneuploids it was less clearly defined (Butterfass 1959, 1973). The results of our experiments showed that the number of chloroplasts in the stomata of the Wisconsin-38 tobacco plants, regenerated from tissue cultures, may be considered as a morphological feature which, taken together with other morphological features, might be useful to classify the regenerated plants as belonging to the H, D or P-class of plants. For practical purposes, the inference of the ploidy level on the basis of chloroplast numbers of the stomata alone has to be taken with great caution because the euploid forms cannot be distinguished from the aneuploid forms. The occurrence of mosaicism, the changes in growth pattern after pruning and the absence of a clear correlation between the chloroplast number and the chromosome number detected in plants with 60 and 120 chromosomes, led us to sugest the presence of chimaeric froms, or C-plants, composed of sectors with different meristematic chromosome numbers in the same shoot.

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