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SHORT REPORTS

CATALASE ACTIVITY AND LIPID CONTENT OF LEAVES FROM NORMAL AND STERILE-FLOWERED TOBACCO PLANTS*

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INTRODUCTION

During the breeding and hybridization of tobacco plants for the purpose of developing certain disease resistance, Burk [1] employed an interspecific bridge-cross, *Nicotiana repanda* through *Nicotiana sylvestris* to *Nicotiana tabacum*. Distorted, non-functional anthers occurred in some plants. The introgression of chromosomes from *N. tabacum* into the cytoplasm of *N. repanda* plus the concomittant loss of a specific *N. repanda* chromosome have been shown to be the cause of this anther distortion and consequent sterility [1, 2]. On the other hand, retention of a specific chromosome or segment results in the development of normal anthers. Pollination of a sterile-flowered plant by a normal-flowered plant results in restoration of normal anthers in some plants [3]. Cytological studies of the male-fertile plants showed the presence of the extra chromosome and determined that those plants possessed 48 chromosomes from *N. tabacum* plus one fragment chromosome from *N. repanda*, or a total of 49 chromosomes [2]. Thus, in the normal plant there are 49 chromosomes, while in the sterile-flowered plant there are 48.

RESULTS AND DISCUSSION

To determine what effect the deficiency of one chromosome might have on the overall oxidative metabolism of the tobacco leaf, we measured catalase (EC 1.11.1.6) activity in leaf samples of both sterile and normal-flowered plants derived from a bridge-crossing (N-2) and from a bridge-crossing followed by an extra back-crossing with *N. tabacum* (N-2 × NC95). As in our previous studies on tobacco leaf [4], we used catalase activity as an index of oxidative metabolism, because of its involvement in the recycling of O_2 for redox reactions. Using an oxygen electrode system for these measurements, we obtained the results shown in Table 1. In the bridge-cross the activity was identical for both samples. Apparently, at that point in the hybridization the oxidative metabolism of the leaf was not affected by the chromosome in question. In the case of the bridge-cross followed by an extra back-cross with NC95, the overall metabolism was decreased in the normal plants and even more so in the sterile plants. This finding illustrates, principally, the effect of the introduction of additional NC95 chromosomal material, but it may also be an indication that the additional back-crossing enhanced the one-chromosome difference between sterile and normal plants.

Since previous work in our laboratories [5] and elsewhere [6] has shown that neutral leaf lipids are major precursors of the polynuclear aromatic hydrocarbons (PAH) found in smoke, we have been interested in developing low lipid tobaccos. Therefore, the above leaf samples were also analysed for neutral lipids. The results are given in Tables 1–3.

As indicated in Table 1, the amount of hexane-extractable material, which may be used as a rough measure of the total amount of neutral lipids present, was increased considerably by the additional back-cross with NC95, but did not differ between normal and sterile-flowered plants of the same cross. Solanesol, which is a C_{45} isoprenol and the major component of this fraction, was also increased by the back-crossing. More noteworthy, however, was the fact that solanesol showed a large decrease in the sterile-flowered plants as compared with the normal in the back-crossed samples. Neophytadiene showed a considerable difference between normal and sterile-flowered plants of the bridge-cross, but not of the back-cross. The hydrocarbon waxes did not change.

Among the sterols (Table 2), only stigmasterol and sitosterol showed any real differences between normal and sterile-flowered plants: stigmasterol in the bridge-cross set and sitosterol in both sets. As noted in Table 3, levels of the major fatty acids were almost identical for normal and sterile plants of the bridge-cross; however, after the extra back-cross all acid levels were higher in the sterile-flowered plants as compared with the normals.

These findings point out that the loss of one chromosome in certain tobacco plants not only causes male sterility (as previously shown), but also results in some biochemical differences between normal and sterile-flowered plants, particularly in catalase activity, solanesol and fatty acid content of the leaves.

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Table 1. Catalase activity and analysis of certain lipid components in leaves of normal and sterile-flowered tobacco plants.

	Bridge-cross (N-2)		Bridge- and back-crosses (N-2 × NC95)	
	Normal	Sterile	Normal	Sterile
Catalase*	69.7	69.7	33.5	25.8
	% dry wt			
Hexane extractable	11.11	10.67	15.46	15.72
Solanesol (free)	4.14	4.06	7.52	5.75
Neophytadiene	0.11	0.07	0.13	0.13
Hydrocarbon waxes†	0.20	0.19	0.25	0.27

* Catalase activity in nkat H_2O_2/mg tobacco. † Hydrocarbon waxes were C_{27}–C_{34} aliphatic hydrocarbons.

Table 2. Total sterols* in leaves of normal and sterile-flowered tobacco plants (% dry wt)

	Bridge-cross (N-2)		Bridge- and back-crosses (N-2 × NC95)	
	Normal	Sterile	Normal	Sterile
Cholesterol	0.017	0.015	0.021	0.018
Campesterol	0.042	0.032	0.049	0.047
Stigmasterol	0.063	0.044	0.067	0.068
Sitosterol	0.064	0.044	0.079	0.064

* Total sterols = free + bound sterols. These were the major sterols found.

Table 3. Total fatty acids* in leaves of normal and sterile-flowered tobacco plants (% dry wt)

	Bridge-cross (N-2)		Bridge- and back-crosses (N-2 × NC95)	
	Normal	Sterile	Normal	Sterile
C_{14}	0.05	0.05	0.05	0.11
C_{16}	0.50	0.50	0.50	0.62
C_{18} unsat.†	2.02	1.98	2.03	2.56
C_{18}	0.32	0.37	0.35	0.45

*Total fatty acids = free + bound fatty acids. These were the major fatty acids found. † C_{18} unsat. = $C_{18:1}$ + $C_{18:2}$ + $C_{18:3}$

EXPERIMENTAL

Two accessions of tobacco, that were segregating for normal flowers and flowers with distorted anthers (male-sterile), were grown in field plots at the Tobacco Research Laboratory, Oxford, N.C. The first accession was obtained by crossing the line N-2 (2n = 48 + 2 chromosome fragments) with *N. tabacum*, cv NC95 (2n = 48). Normal-flowered plants of this accession (2n = 48 + 1 fragment) were crossed with NC95 to obtain a second back-cross generation. Each accession was grown in 2 plots of 20 plants each and consisted of normal and sterile-flowered plants. The plots were planted, harvested and fertilized conventionally. The only departure from standard flue-cured practices was that the plants were not topped and no suckering chemicals were applied.

Leaf samples were obtained from 3 normal and 3 male-sterile plants of each accession. Three intact leaves were obtained from the middle third of each plant. Thus, the total number of samples was 4:9 leaves from normal-flowered plants of accession #1, 9 leaves from male-sterile plants of accession #1, 9 leaves from normal-flowered plants of accession #2, and 9 leaves from male-sterile plants of accession #2. The midribs were removed and the laminae cut into small pieces and lyophylized.

Samples for catalase activity were prepared and assayed by the oxygen electrode method of Schepartz [7]. The enzyme units (K_f) determined in that method were converted to nkat H_2O_2/mg tobacco (1 K_f unit = 0.00129 nkat H_2O_2/mg tobacco). Lipids were analysed according to Ellington *et al.* [8, 9] and Severson *et al.* [10]. All analyses were performed in replication of at least 2 samples. All results are expressed on a dry-wt basis [8].

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