IDENTIFICATION OF THE CHROMOSOME CARRYING THE GENE FOR *CIS*-ABIENOL PRODUCTION BY THE USE OF MONOSOMICS IN *NICOTIANA TABACUM* L.

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The production of *cis*-abienol, a leaf surface diterpene of tobacco (*Nicotiana tabacum* L.), was confirmed to be genetically controlled by a single dominant gene in the cross between *cis*-abienol producing Red Russian and non-producing HM 195. To identify the chromosome carrying the gene designated as *Abl*, crosses were made between each of seven monosomics of Red Russian, Haplo-A, -B, -C, -E, -F, -H, and -K, and HM 195 or TI 245 as the pollen parent. An examination for *cis*-abienol production in the seven F_1 populations showed that only the F_1 of Haplo-A x HM 195 included plants not producing *cis*-abienol. The percentage of such plants was approximately equal to the ovular transmission rate of Haplo-A. Cytological examination of plants from the cross of Haplo-A x HM 195 revealed the association of the monosomic state with the deficiency of *cis*-abienol production. These results demonstrated that the gene for *cis*-abienol production in tobacco is located on chromosome A.

Key words: *Cis*-Abienol, Diterpene, Monosomics, *Nicotiana tabacum*

INTRODUCTION

Leaf surface lipids of tobacco (*Nicotiana tabacum* L.) have an important role in determining smoke qualities (5). The lipids are largely composed of diterpenes such as duvatrienediol (α- and β-isomers of 4, 8, 13-duvatriene-1, 3-diol), a thunberganoid, and *cis*-abienol ((12, Z)-labda-12, 14-diene-8α-o1), a labdanoid (3). According to a study on the components of the diterpenes of 188 tobacco varieties, duvatrienediol is produced by all the varieties examined whereas *cis*-abienol is produced only by a quarter of the varieties (8). A genetical study revealed that the ability for *cis*-abienol production is controlled by a single dominant gene (12).

Tobacco, which is of amphidiploid origin, has been considered to arise from chromosome doubling following hybridization between a progenitor of *N. sylvestris* Spegazzini and Comes and an ancestral type similar to *N. tomentosiformis* Goodspeed (10). The present form of the latter species produces *cis*-abienol whereas that of the former does not (6). It seems therefore to be highly probable that the gene for *cis*-abienol production was derived from the progenitor of *N. tomentosiformis* and is located on one of the chromosomes of the T genome.

The complete monosomic series of a tobacco variety, Red Russian, has been established (2), and has provided material for locating a number of genes on specific chromosomes (11). The purpose of the present investigation was to identify the chromosome carrying the gene for *cis*-abienol production by the use of the monosomic series.

MATERIALS AND METHODS

In a preliminary examination we found that Red Russian produces *cis*-abienol and HM 195 does not produce it. To examine the mode of inheritance of *cis*-abienol production, a cross was made between these two genotypes and the F_1, F_2, and BC_1 (F_1 x HM 195) generations were obtained. Fifteen plants of

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each parent and the F_1, 149 F_2 plants, and 49 BC_1 plants were grown in separate 12-cm pots containing soil and standard fertilizer in a greenhouse at around 25 °C for two months. An upper leaf about 15 cm long was taken from each plant and air-dried at a room temperature for 1 h. The fresh leaf was immersed in 10 ml of chloroform in a 15 cm petri dish for about 20 s with gentle shaking. The extract was filtered through TOYO No. 6 filter paper and evaporated to dryness in vacuum at 40 °C. The residue was taken up in 0.2 ml of chloroform, and a 4 μl sample was applied with a micro-pipette to a pre-coated glass thin layer chromatography plate (Merk Silica Gel 60). The plate was developed 5 cm high with a mixture of chloroform and methanol (100:2, v/v), and dried in air. The plate was then sprayed with a 5 % solution of *p*-anisaldehyde in 5 % ethanolic sulfuric acid, and heated with a handy hair dryer to visualize *cis*-abienol (Rf: 0.8 ca). *Cis*-Abienol from Red Russian was identified by gas chromatography and mass spectrometry.

Among the twelve types of Red Russian monosomics involving chromosomes of the T genome, Haplo-A, -B, -C, -E, -F, -H, and -K were chosen for convenience because of their high ovular transmission rates (2). Crosses were made between each of the seven monosomics and HM 195, and F_1 seeds were obtained in all crosses except Haplo-H x HM 195. Since the cross of Haplo-H x HM 195 was unsuccessful by accident, the F_1 of Haplo-H x TI 245 (Tobacco Introduction 245) was substituted. TI 245 does not produce *cis*-abienol. Twenty seedlings of each F_1 and six seedlings of TI 245 were examined for *cis*-abienol production by the same method described above. Since only the F_1 of Haplo-A x HM 195 included plants which did not produce *cis*-abienol, pollen mother cells from each plant of this cross were cytologically examined by the acetocarmine smear method.

The monosomic plants used in these crosses were those which had been identified according to the morphological and cytological characteristics associated with the deficiency for each of the chromosomes (1, 2). The original seed stocks containing monosomics were kindly supplied by Dr. D. U. Gerstel, Professor of Genetics, North Carolina State University at Raleigh, N.C., U.S.A. HM 195 is a chlorophyll mutant induced by X-rays (7); its $X_{6/7}$ was used here. TI 245 was obtained from the official seed stock of the Iwata Tobacco Experiment Station.

RESULTS AND DISCUSSION

The segregation ratios for *cis*-abienol production in the F_2 and BC_1 generations of the cross of Red Russian x HM 195 (**Table 1**) indicate that the *cis*-abienol production in this cross is controlled by a single dominant gene as in a previous report on the cross of Coker 139 x Galpão No. 1 (12). The gene of Red Russian is hereby designated as *Abl*.

The result of the examinaton for *cis*-abienol production in the F_1 generations from the crosses of the seven monosomics with HM 195 or TI 245 is presented in **Table 2**. Plants showing the phenotype of the pollen parent, namely the ones not producing *cis*-abienol, appeared only in the F_1 of Haplo-A x HM 195, and the percentage of such plants, 14 / 20 = 70 %, approximates the ovular transmission rate of Haplo-A, 78.7 % (2). A part of the chromatograms of the F_1 of Haplo-A x HM 195 is presented in **Fig. 1.** In the rest of the F_1 generations all plants produced *cis*-abienol.

Cytological examination of pollen mother cells from the F_1 plants of Haplo-A x HM 195 indicated that the plants not producing *cis*-abienol were all monosomics whereas the ones producing it were normal diploids. From these results it was concluded that the gene of tobacco for *cis*-abienol production is located on chromosome A.

In tobacco most of the genes whose chromosomal locations have previously been determined are associated with either morphological traits, plant color, or disease resistance (4,9,11), and no report on the location of a gene responsible for the production of a single chemical substance has appeared. The present investigation not only adds a new marker gene on chromosome A, but also shows the possibility of utilizing a gene for production of a chemical substance as the marker of a specific chromosome in tobacco.

By thin layer chromatography as used here duvatrienediol extracted from fresh leaf was also detected. Every sample from Red Russian showed a small spot of duvatrienediol as compared with that from HM 195 **(Fig. 1).** This suggests that Red Russian produces only a small amount of the component. Although the exact amount of duvatrienediol should be determined by other methods, it is noteworthy that Red Russian may have peculiarity in the production of duvatrienediol.

Table 1. Segregation for *cis*-abienol production in a cross between Red Russian and HM 195

	No. of plants[a]		χ^2(p)
	+	-	
Red Russian	15	0	
HM 195	0	15	
F_1	15	0	
F_2	117	32	0.99[b] (0.50-0.25)
F_1 x HM 195	21	28	1.00[c] (0.50-0.25)

[a] + With *cis*-abienol; - Without *cis*-abienol
[b] χ^2 value for a 3:1 ratio
[c] χ^2 value for a 1:1 ratio

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Table 2. *Cis*-Abienol production of the progenies in the cross of Red Russian monosomics with HM 195 or TI 245

	No. of plants[a]		χ^2(p)
	+	-	
Haplo-A x HM 195	6	14	0.90[b] (0.50-0.25)
Haplo-B x HM 195	20	0	
Haplo-C x HM 195	20	0	
Haplo-E x HM 195	20	0	
Haplo-F x HM 195	20	0	
Haplo-H x TI 245	20	0	
Haplo-K x HM 195	20	0	
TI 245	0	6	

[a] + With *cis*-abienol; - Without *cis*-abienol
[b] χ^2 value for the ovular transmission rate of Haplo-A, 78.7 %

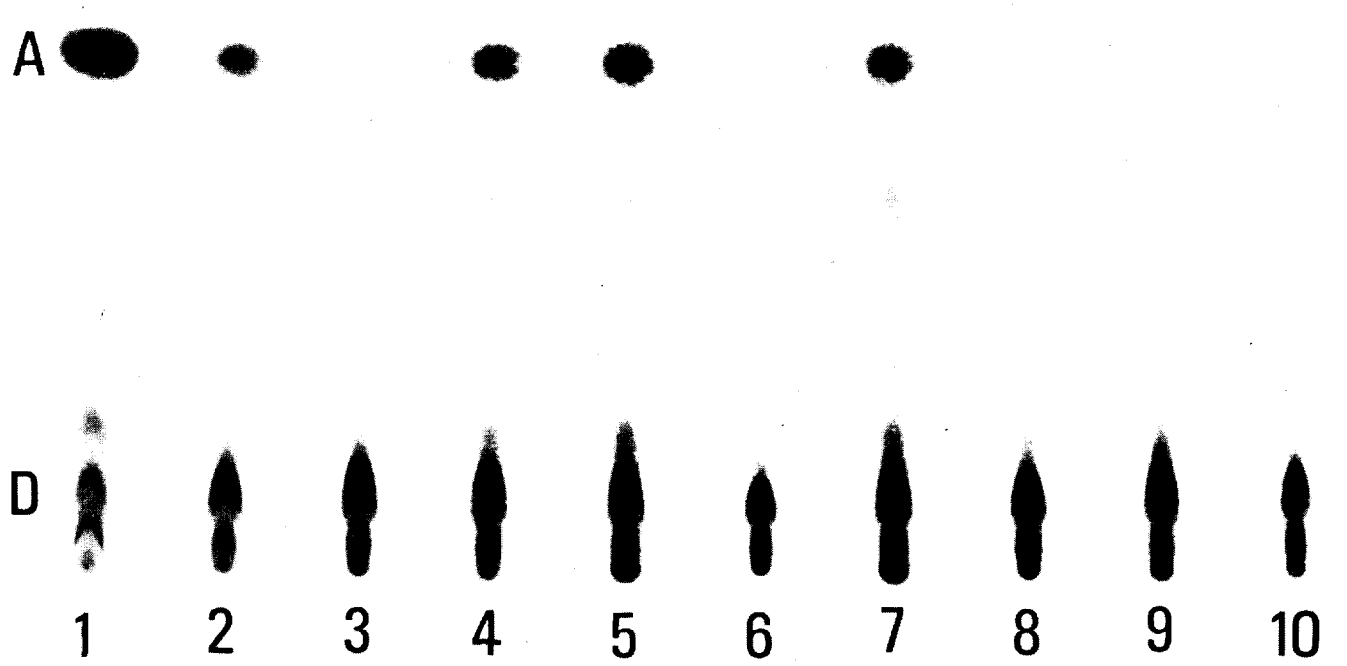

Fig. 1. Thin layer chromatograms of diterpenes from fresh tobacco leaves. A: *cis*-abienol (Rf ≈ 0.8), D:duvatrienediol (RF ≈ 0.1), 1: Red Russian, 2: Red Russian x HM 195 F_1, 3:HM 195, 4-10: Red Russian Haplo-A x HM 195 F_1

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