Phytochemistry, Vol. 25, No. 9, pp. 2085–2087, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

GENETIC CONTROL OF PHENYLPROPANOIDS IN *PERILLA FRUTESCENS*

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(*Received* 6 *January* 1986)

Key Word Index—*Perilla frutescens*; Labiatae; volatile oil; chemotype; gene analysis; myristicin; elemicin; dillapiole.

Abstract—Genetic analysis of three chemotypes of *Perilla frutescens* which differ in the chemical composition of phenylpropanoids in volatile oils has shown that the biosynthesis of dillapiole and elemicin are controlled by two independent genes, *D* and *E*, respectively, whereas only myristicin is produced in recessive forms.

INTRODUCTION

Local varieties of *Perilla frutescens* cultivated in Japan [1, 2] can be classified into five chemotypes according to the main components of volatile oils: perillaldehyde (PA), perillaketone (PK), elsholtziaketone (EK), citral (C) and phenylpropanoid (PP) types [3]. Genetic analysis of these chemotypes has demonstrated that the chemical diversity is due to two pairs of independent genes, *G* and *H* [4]. We have also found that strains of the PP type (*ggHH* or *gghh*), which lack monoterpenoids in volatile oils, vary in the composition of specific phenylpropanoids (myristicin, dillapiole and elemicin) [1, 3, 5–7]. To investigate the genetic basis of chemical variation in the phenylpropanoids, we have undertaken hybridization using three different strains of the PP type. This paper reports that variation in the phenylpropanoid composition is controlled by two pairs of independent genes. Also, a hypothetical biosynthetic pathway of the three phenylpropanoids is discussed on the basis of genetic data.

RESULTS

Seven parental strains of *Perilla* used for hybridization were classified into three chemotypes according to the composition of phenylpropanoids in volatile oils: (1) the M type containing only myristicin; (2) the DM type containing dillapiole and myristicin; and (3) the EM type containing elemicin and myristicin (Table 1). Dillapiole and elemicin are the main constituents of volatile oils in the DM and EM types, respectively, although these chemotypes also contain a small amount of myristicin. Genetic data obtained from intercrosses made between three chemotypes are shown in Table 2.

M × *DM*

In the cross 30 (M) × 53 (DM), the phenotype of F_1 was DM, and the F_2 progeny segregated for DM and M in a 3:1 ratio. These data suggest that the difference between the DM type and the M type is due to a dominant allele *D*; the genotypes of 30 and 53 may be designated as *dd* and *DD*, respectively.

M × *EM*

In the cross 5 (M) × 70 (EM), the F_1 hybrid was of the EM type, and gave a 3:1 segregation ratio for EM and M in F_2. Thus, genotypes *ee* and *EE* may be assigned to strains 5 and 70, respectively.

EM × *DM*

In both crosses, 10 (EM) × 53 (DM) and 16 (DM) × 70 (EM), all the F_1 plants were of the 'DEM type' containing dillapiole, elemicin and myristicin. In F_2, four kinds of phenotypes, DEM, DM, EM and M, were observed in a

Table 1. Main phenylpropanoid components of volatile oils in parental strains of *Perilla frutescens*

		Phenylpropanoids of volatile oils (% of fresh weight of leaves)		
Strain No.	Chemotype	Myristicin	Dillapiole	Elemicin
5, 30	M	0.21–0.38	—	—
16, 53	DM	0.03–0.05	0.22–0.34	—
10, 70	EM	0.05–0.09	—	0.25–0.32

—Not detected.

Table 2. Segregation for phenylpropanoid components in F_2 progenies of intercrosses between different chemotypes of *Perilla frutescens*

Cross ($P_1 \times P_2$)*		Phenotype of F_1	Segregation ratio in F_2 progenies: Phenotype	Observed ratio	Expected ratio	P value of χ^2 test
30 × 53	M × DM	DM	DM:M	25:7	3:1	0.8
5 × 70	M × EM	EM	EM:M	20:5	3:1	0.5
10 × 53	EM × DM	DEM	DEM:DM:EM:M	31:11:11:5	9:3:3:1	0.8
16 × 70	DM × EM	DEM	DEM:DM:EM:M	23:12:9:4	9:3:3:1	0.6
10 × 115†	EM × DEM	DEM	DEM:EM	33:10	3:1	0.8

*P_1 and P_2 represent the female and the male parents, respectively.
†F_1 of 16 × 70.

ratio of *ca* 9:3:3:1 (Table 2). This ratio could be explained by assuming that the genotype of the parental strains were *ddEE* for the EM type (10 and 70) and *DDee* for the DM type (16 and 53), and that both dillapiole and elemicin are formed in addition to myristicin when a dominant allele *D* is present with another dominant allele *E*. On the basis of this assumption, the expected phenotypes in F_2 would be DEM (*D-E-*), DM (*D-ee*), EM (*ddE-*) and M (*ddee*) in a ratio of 9:3:3:1, where e.g. *D-* stands for *DD* and *Dd*.

EM × DEM

In order to confirm the validity of the above assumption, the F_1 hybrid (DEM type, *DdEe*) between strain 16 (DM type, *DDee*) and strain 70 (EM type, *ddEE*) was backcrossed to strain 10 (EM type, *ddEE*) to obtain a BC_1 plant of the DEM type. Since the genotype of this BC_1 plant should be either *DdEe* or *DdEE*, its self-pollinated progeny plants would be expected to segregate for either DEM, DM, EM and M types (9:3:3:1) or DEM and EM (3:1), respectively. The observed segregation ratio was found to be in accord with the latter ratio (Table 2), suggesting that the genotype of the BC_1 plant was *DdEE*.

All the results of breeding experiments (Table 2) can be fully explained by assigning the following genotypes to the parental strains; *ddee* to strains of the M type (5 and 30), *DDee* to strains of the DM type (16 and 53), *ddEE* to strains of the EM type (10 and 70). Furthermore, strains having the genotype *DDEE* (DEM type) were obtained in the F_3 generation of an F_1 hybrid (*DdEe*).

Table 3 shows the relationship between the genotype and the relative amount of myristicin in the volatile oil. It appears that the proportion of myristicin to total phenylpropanoids in volatile oils is dependent on the dosage of dominant alleles, *D* and *E*. Almost no myristicin but only dillapiole and elemicin were found in plants homozygous for both *D* and *E*. It is also interesting that *DDee* plants contain a smaller amount of myristicin than *ddEE* plants.

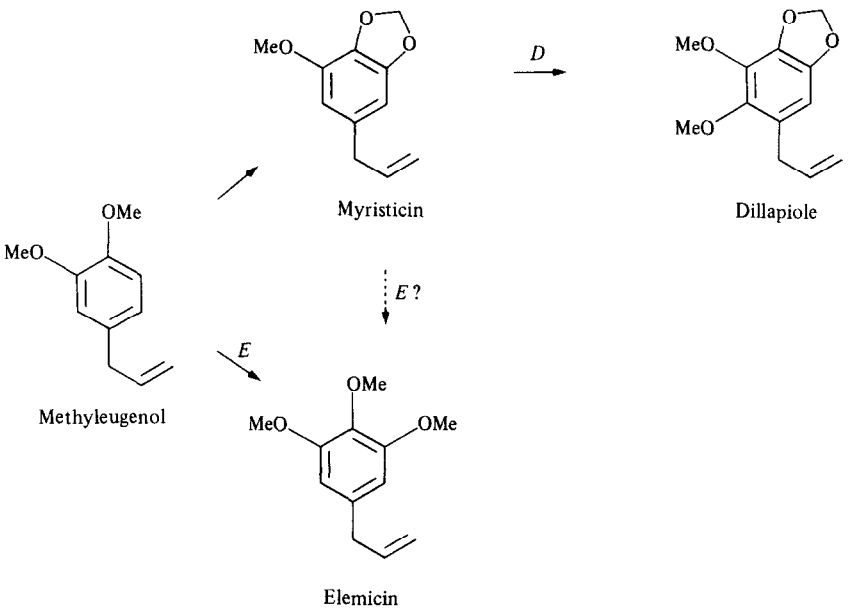
Table 3. Relationship between the genotype and the proportion of myristicin to total phenylpropanoids (D, E and M)*

Genotype	Proportion of myristicin†
DDEE	trace
DdEe	15.7–34.2
DDee	5.1–13.6
ddEE	18.7–21.5
ddee	100

*D = dillapiole, E = elemicin, M = myristicin.
†M/(D + E + M) × 100 (%).

DISCUSSION

The present experiments have shown that the production of dillapiole and elemicin in *Perilla* is controlled by dominant alleles, *D* and *E*, respectively, and that only myristicin is produced in the absence of both dominant alleles. It is known that myristicin, dillapiole and elemicin are also found in volatile oils of *Myristica fragrans* [8, 9], *Piper aduncum* [10], *Ligusticum scoticum* [11] and some umbellifers [12]. However, no investigation has been made on phenylpropanoid variations in these plants. As regards the biosynthetic relationship between these phenylpropanoids, Fujita [13] has proposed from a chemotaxonomic study on the volatile oils in *Mosla* that myristicin and elemicin would be derived independently from a common, presumptive precursor, methyleugenol and that dillapiole would be derived from myristicin. Our genetic data apply to his hypothetical scheme, if the two genes, *E* and *D*, control the conversions of methyleugenol → elemicin and myristicin → dillapiole, respectively (Scheme 1). However, no chemotype that contains methyleugenol has been reported in *Perilla* and we were unable to detect the presence of this compound in any of the *Perilla* strains tested. Furthermore, the possibility that elemicin might be derived from myristicin is not contradictory to the genetic data. Thus, the biosynthetic sequence of these phenylpropanoids still remains to be determined by biochemical studies on various genotypes.



Scheme 1. The biosynthetic pathway postulated by Fujita [13] and the possible reaction steps controlled by genes *D* and *E*.

EXPERIMENTAL

Plant materials. Three strains (5, 10 and 30) of the green-leafed variety 'Aojiso' (*Perilla frutescens* Britton var. *acuta* Kudo f. *viridis* Makino), two strains (53 and 70) of the red-leafed variety 'Akajiso' (*P. frutescens* Britton var. *acuta* Kudo), and one strain of the variety 'Katamenjiso' (*P. frutescens* Britton var. *acuta* Kudo f. *crispidiscolor* Makino) having leaves with the red reverse face were intercrossed for genetic analysis. All the strains used as parents had been self-pollinated for more than two generations to confirm that no genetic segregation for chemical components of volatile oils occurred in the progeny plants. Methods of artificial pollination and cultivation have been described elsewhere [14, 15].

GC analysis. Four to five fresh leaves (2–3 g, *ca* 4 × 2 cm) that were the youngest of fully expanded leaves, were sampled from individual plants in early July and extracted with Et_2O (1.25 ml/g fr. wt) overnight at 4°. Five μl of the Et_2O layer were subjected to GC. GC analysis was carried out using a stainless column (3 mm × 2 m) packed with PEG-6000 chromosorb W 17% (30–60 mesh) under the following conditions: injection port temp. 250°; column temp. 170°; carrier gas, N_2 at a flow rate of 30 ml/min; detector, FID [3–6]. Peaks of elemicin, myristicin and dillapiole were detected on the chromatogram at RR_t 3.8, 4.3 and 5.9, respectively, relative to the peak of perillaldehyde ($RR_t = 1$) [3–6] used as an internal standard. The lower limit of detection for any of the compounds by GC was approximately 0.12 ng, which corresponds to 0.003% of the fr. wt of leaves.

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