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A REPORT:

Identifying Genetic Markers and Their Role in Selecting Chemotypes in Perennial *Lamiaceae*

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This is a report of research progress in this project through early 1995. The results of these initial experiments have led us to propose hypotheses about the patterns of inheritance of aromatic compounds in *Agastache foeniculum*. We are now testing those hypotheses with larger plant populations. On request, we will be pleased to provide readers with more up-to-date information as we complete the analyses of our ongoing experiments. Until such results are subject to scientific peer review, the data presented in the following report must be considered strictly preliminary.

Agastache foeniculum is one of seven species of *Agastache* section *Agastache* (*Lamiaceae*) native to North America (Lint and Epling, 1945; Vogelmann, 1984; GPFA, 1986). It grows frequently along prairie sloughs in the Great Plains of the North Central U.S. and prairie provinces of Canada (Vogelmann, 1984; GPFA, 1986). Plants of *A. foeniculum* are perennial and can grow to about one meter tall; they have spike-like inflorescences generally with purple corollas and calyces. The fragrance of their crushed leaves usually has an anise-like aroma (Vogelmann, 1983; Wilson et al., 1992). Several studies have reported the composition and content of the essential oils of *A. foeniculum* (Charles et al., 1991; Mazza and Kiehn, 1992; Nykänen et al., 1989; Wilson et al., 1992). In those studies, methylchavicol and limonene have been reported to be the main components of its essential oil. To date there are no reports on the inheritance or genetic control of the components of the essential oil of *A. foeniculum*. This study has been conducted to determine the inheritance of some of the main components of the essential oil of *A. foeniculum* and to determine if there are isozyme markers that correlate with the inheritance of important aromatic volatiles.

Populations of *A. foeniculum* (Table 1), maintained at the North Central Regional Plant Introduction Station (NCRPIS), with specific isozyme banding patterns and aromatic content were used in this study. Parental populations were screened for isozyme variability. Eleven enzyme systems that included 19 putative loci were successfully stained and some degree of polymorphism was detected in seven of the 19 loci (Fuentes and Widrlechner, 1995). Parental plants, which differed in banding patterns at one or more

Table 1: Origin of the accessions of *A. foeniculum* used in the study

Species	Accession #	Origin
A. foeniculum	PI-561057	Wild: Barnes Co., ND
A. foeniculum	PI-561058	Wild: Cass Co., MN
A. foeniculum	PI-561059	Wild: Hennepin Co., MN
A. foeniculum	PI-561060	Wild: Manitoba
A. foeniculum	PI-561061	Wild: Las Animas Co., CO

loci, were selected for controlled crossing. The offspring from these crosses were analyzed and their hybridity verified by scoring these enzyme systems for which the parents differed. Observed banding patterns were compared to the expected patterns of hybrids according to their quaternary structure.

Table 2 summarizes the enzyme banding patterns of parents and hybrids and Table 3 summarizes the composition of the oils of the parents.

volatile content, containing methylchavicol. Chromatographic analyses of the parents confirmed the results of sensory analyses in populations PI-561057 and PI-561058 where both were low in methylchavicol. Population PI-561059, originally sensed as high in methylchavicol, had no detectable methylchavicol in its volatiles but instead was high in limonene. Both populations PI-561060 and PI-561061 had a moderate to high content of methylchavicol in their volatiles. Efforts to identify the unknown peak (RT= 6.38) has not yet been successful. None of the studies (Charles et al., 1991; Mazza and Kiehn, 1992; Nykänen et al., 1989; Wilson et al., 1992) reporting on the components of the essential oil of *A. foeniculum* indicates any compound with a retention time close to the unknown detected by our chromatographic analyses. This highly volatile compound may not be aromatic at all and that may be why previous studies (Charles et al., 1991; Mazza and Kiehn, 1992; Nykänen et al., 1989;

Table 2: Enzyme banding patterns of parents and their offspring at some polymorphic loci.

	Parent								
Locus	-057	-058	Offsp	-058	-059	Offsp	-060	-061	Offsp
AAT-1	22	22	22	22	22	22	22	22	22
AAT-2	22	22	22	22	11	12	22	22	22
CAT	11	11	11	11	11	11	11	12	11&12
PGM-2	33	22	23	22	22	22	22	22	22
PHI-2	22	22	22	22	22	22	22	22	22
TPI-1	11	22	12	22	23	22&23	12	33	13&23
TPI-2	22	22	22	22	12	12&22	22	22	22

Table 3: Composition of the headspace of the leaves of parental lines, given as percentage of the total peak area.

	Retention	Population				
Component	time (min)	PI-561057	PI-561058	PI-561059	PI-561060	PI-561061
Unknown	6.38	--	47%	--	--	--
∝-pinene	11.33	--	--	8%	--	--
myrcene	12.23	--	22%	9%	--	3%
R-+ limonene	13.16	--	16%	71%	62%	21%
methylchavicol	16.22	--	--	--	19%	76%
spathulenol	21.28	100%	15%	2%	19%	--

These populations were selected based on sensory analyses of their volatiles because at the time of making controlled crosses there was not enough leaf material to conduct chromatographic analyses. Populations PI-561057 and PI-561058 were sensed as being very low in methylchavicol. On the other hand, populations PI-561059, PI-561060, and PI-561061 were sensed as having a high

Wilson et al., 1992) have not detected and/or reported it. We have run a series of standard aromatic compounds and straight-chain hydrocarbons to calculate Kovats' indices to identify the unknown compound but the attempts have not yet been successful.

Equilibrium headspace gas chromatography was used to analyze and identify the volatiles released by the leaves

Results

Tables 4, 5, and 6 summarize the major essential oils from leaves of the offspring from controlled crosses.

Table 4: Major components of the volatiles of the offspring from the cross 561057 X 561058 (expressed as percent of total peak area).

		Plants									
Compound	RT	1	2	3	4	5	6	7	8	9	10
Unknown	6.38	34	29	76	94	87	65	60	100	--	--
∝-pinene	11.33	--	--	--	--	--	--	--	--	--	--
myrcene	12.23	35	48	10	6	13	35	15	--	--	--
R-+ limonene	13.16	11	23	--	--	--	--	--	--	--	--
methylchavicol	16.22	--	--	--	--	--	--	--	--	--	--
spathulenol	21.28	13	--	14	--	--	--	25	--	100	100

Table 5: Major components of the volatiles of the offspring from the cross 561058 X 561059 (expressed as percent of total peak area).

		Plants															
Compound	RT	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Unknown	6.38	17	11	--	37	8	9	17	100	61	100	100	100	100	--	--	100
∝-pinene	11.33	6	6	--	5	--	7	6	--	--	--	--	--	--	--	--	--
myrcene	12.23	25	60	71	39	34	60	24	--	--	--	--	--	--	--	38	--
R-+ limonene	13.16	35	12	14	--	49	13	36	--	39	--	--	--	--	--	--	--
methylchavicol	16.22	--	--	--	--	--	--	--	--	--	--	--	--	--	--	29	--
spathulenol	21.28	3	6	15	10	--	3	--	--	--	--	--	--	--	100	32	--

Table 6: Major components of the volatiles of the offspring from the cross 561060 X 561061 (expressed as percent of total peak area).

		Plants						
Compound	RT	1	2	3	4	5	6	7
Unknown	6.38	--	--	47	--	--	--	--
∝-pinene	11.33	--	--	--	--	--	--	--
myrcene	12.23	--	--	--	--	28	--	8
R-+limonene	13.16	--	45	32	45	19	10	64
methylchavicol	16.22	100	55	--	39	22	85	28
spathulenol	21.28	--	--	21	16	18	5	--

of the progeny of the crosses. Five to seven grams of leaves were placed in glass bottles and immediately sealed with headspace sampling caps containing Teflon-coated septa and aluminum seals (Ong, 1988). The samples were equilibrated for 4 to 7 hours at 20°C before the analyses were conducted.

The chromatographic analyses were conducted on a Varian 3700 gas chromatograph equipped with a FID detector and a Hewlett-Packard 3390A integrator. A DB-5 fused-silica capillary column (0.25 mm i.d. x 30 m) was used through the study. Flow rates of oxygen, hydrogen, and nitrogen (carrier gas) were 300ml/min, 30 ml/min, and 30 ml/min, respectively. Oven temperatures were programmed from 40°C to 220°C with an increase of 10°C/min. A 2-min hold was set at 40°C. The sample size was 2 ml. Following Wilson et al. (1992), the samples were cryofocussed before the start of the temperature program. Headspace samples of standard compounds were used to identify some of the volatiles present in the samples, except for spathulenol which was identified based on a study by Charles et al. (1991), and the unknown highly-volatile compound.

Analyses of the volatiles of 10 offspring from the cross PI-561057 X PI-561058, 16 from the cross PI-561058 X PI-561059 and 7 from the cross PI-561060 X PI-561061 revealed the presence of the unidentified peak (RT 6.38 min) detected in parental population PI-561058. Twenty-one of the 26 offspring from crosses which had PI-561058 as a parent (Tables 4 and 5) had that unidentified compound as component of their volatiles. In seven of those 26 individuals, the unidentified peak accounted for 100% of the volatiles given off by the leaves. On the other hand, in five of the 26 individuals this unidentified compound was not detected. As noted before, this peak was detected in the volatiles only of the parent PI-561058. A possible hypothesis of the genetic control of this compound may be as follows. Eight from 10 offspring from the cross PI-561057 X PI-561058 and 13 from 16 offspring from the cross PI-561058 X PI-561059 suggest that this is a dominant trait; however, the results do not fit any simple ratios. Furthermore, 1 from 7 offspring from the cross PI-561060 X PI-561061 suggests the need for 2 complementary-dominant genes U and A to observe the unknown compound in the progeny. The proposed genotypes of the parents would then be:

Parent	Genotype
PI-561057	uuAa
PI-561058	UUAa
PI-561059	uuAa
PI-561060	Uuaa
PI-561061	uuAa

∝-pinene (RT 11.33) was detected in relatively low concentrations (less than or equal to 7% of total peak area) in 5 of the 16 offspring from the cross PI-561058 X PI-561059. ∝-pinene was detected in the volatiles of the parent PI-561059 in relatively low concentration, 8% of total peak area. The synthesis of this compound may be controlled by a single dominant gene P that requires the effect or product of another gene, M, responsible for myrcene synthesis. ∝-pinene was only present in the volatiles of the plants that contain myrcene. Proposed genotypes: PI-561058 pp and PI-561059 Pp.

Myrcene (RT 12.23 min) was detected in seven of the ten offspring from the cross PI-561057 X PI-561058 and in eight of the 16 offspring from the cross PI-561058 X 561059. Myrcene was detected in the volatiles of the parent PI-561058 with 22% of the total peak area and with 9% of the volatiles of parent PI-561059. It should be noted, that the individual plants lacking myrcene were the ones in which 100% of the volatiles were made up of only one component, either the unidentified or spathulenol. We propose that myrcene's production is controlled by a single gene controlling presence or absence of this compound and a complementary factor controlling the quantity produced. The gene M (with at least three alleles, M, M*, and m) would control presence/absence of myrcene. M is a dominant allele with good penetrance, M* is a dominant allele with poor penetrance, and m is a recessive with no expression of myrcene. Locus Q would be responsible for quantity of myrcene produced. Allele Q would be dominant, allele Q* overdominant, and q recessive. The proposed genotypes of the parents would be:

Parent	Genotype
PI-561057	mmqq
PI-561058	MmQQ
PI-561059	M*mQ*Q*
PI-561060	mmQ*Q*
PI-561061	M*mQ*Q*

Limonene (RT 13.16) was detected in two of the ten offspring from the cross PI-561057 X PI-561058, in 7 of the 16 offspring from the cross PI-561058 X PI-561059, and in 6 of the 7 offspring from the cross PI-561060 X PI-561061. These results suggest that synthesis of limonene may be controlled by 2 factors with additive gene action. In the genus *Mentha*, Lincoln et al. (1971) reported limonene as being inherited as a single dominant gene. This compound has also been reported as being an immediate precursor for other aromatic compounds and our lack of detection may not necessarily mean that it was not synthesized earlier.

Methylchavicol was detected in all seven offspring from the cross PI-561060 X PI-561061. In addition, all of

the seven offspring of the cross PI-561059 X PI-561061 had methylchavicol as the major, and only detectable, component of their volatiles (data not shown). Probably the parent PI-561061 is homozygous dominant for this trait and methylchavicol may be inherited as a single dominant gene, C. Detection of methylchavicol in one of 16 offspring from cross PI-561057 X PI-561058 could have been due to a sampling error or perhaps the existence of a different pathway or genetic control for its synthesis.

Synthesis of spathulenol seems to be controlled by two loci, S and T. Five of ten offspring from the cross PI-561057 X PI-561058 had spathulenol in their aromatic oils. Seven of 16 offspring from the cross PI-561058 X PI-561059 had spathulenol in their oils. Four of 7 offspring from the cross PI-561060 X PI-560161 had spathulenol in their oils. Proposed genotypes of the parents would be:

Parent	Genotype
PI-561057	SsTt
PI-561058	SsTt
PI-561059	SsTt
PI-561060	SsTt
PI-561061	sstt

Isozyme banding patterns for individual plants were used to identify offspring from controlled crosses and to prove their hybridity. We could not correlate, however, any specific isozyme banding pattern with the production of a specific volatiles. It does not seem that isozymes alone can be used as genetic markers for selection for specific volatile production. We observed that inheritance of each essential oil is controlled by one or two major genes. The proposed hypothesis for the genetic control of essential oil production needs, however, to be tested to be proved. We have a relatively good supply of F_2 seeds from controlled crosses which we plan to grow in 1995 and evaluate both their isozymes and essential oils to confirm our hypothesis regarding genetic control. Given the availability of sufficient funds, we will proceed to screen certain F_2 populations for DNA-based markers to determine if we can link any of them to essential oil synthesis.

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