Understanding genetic mechanisms underpinning volatile emission
Entendiendo los mecanismos genéticos que sustentan las emisiones de volátiles

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Abstract

From a cross performed between *A. majus* and *A. linkianum*, we obtained a F2 segregating population of 110 plants. We analyzed the scent emission of this population. We focused on four of the main volatiles present in the population: acetophenone, methyl benzoate, methyl cinnamate and β-ocimene. We analysed the possible mendelian segregations of these volatiles within the population, finding that all of these volatiles might be result of the segregation of one or two genes. Despite of being products of very complex metabolic pathways, the emission of these volatiles is result of simple or epistatic interactions. This basic approach is a useful tool for further molecular studies.

Keywords: Mendelian; RIL; gene.

Resumen

A partir de un cruce realizado entre *A. majus* y *A. linkianum*, obtuvimos una población F2 segregante de 110 plantas. Analizamos la emisión de aromas en esta población. Nos centramos en cuatro de los principales volátiles presentes en la población: acetofenona, metil benzoato, metil cinamato y ocimeno. Analizamos las posibles segregaciones mendelianas de estos volátiles en la población, encontrando que todos ellos pueden ser el resultado de la segregación de uno o dos genes. A pesar de ser el producto de rutas metabólicas muy complejas, la emisión de estos volátiles es el resultado de interacciones simples o epistáticas. Este enfoque básico es una herramienta útil para futuros estudios moleculares.

Palabras clave: Mendeliano; RIL; gen.

1. INTRODUCTION

Floral scent is one of the most important traits involved in pollination, yet it is a very complex trait due to the great amount of compounds found in plants [1]. Populations of Recombinant Inbred Lines (RILs) can be used for studying the genetic structure of complex traits [2]. Changes on the scent profiles can lead to major ecological changes, including speciation [3].

The aim of this study is to assess the possible mendelian segregations explaining the emission of acetophenone, methyl benzoate, methyl cinnamate and β-ocimene on a RILs (F2) population from a cross between *A. majus* and *A. linkianum*.

2. MATERIALS AND METHODS

The headspace volatile analysis was performed and quantified according to Ruiz-Hernández et al. (2017) [2]. Where flowers where isolated into desiccators and volatile compounds where adsorbed by magnetic stir bars (Twisters™) during 24 hours.

The statistical analysis of the segregation models have been done by using X^2 test (chi-squared). P-values under 0.05 rejected the null hypothesis.

3. RESULTS Y DISCUSSION

A. majus and *A. linkianum* are known for having contrasting scent profiles [2, 3] regarding the volatiles aim of study on this work. Whereas *A. majus* is a high producer of methyl benzoate and acetophenone, *A. linkianum* is a low producer of these compounds. On the other hand, *A. linkianum* is a high producer of methyl β-ocimene and a relatively high producer of β-ocimene, however *A. majus* produces relatively much less quantities or any emission at all of these compounds [3]. Because of this disparity in volatiles emission, these two species are a perfect model system for studying the genetic reasons underlying the emission of these important volatiles.

From the 110 different plants analysed we found that they could be grouped as high or low-absent emitters of acetophenone, methyl benzoate, methyl cinnamate and β-ocimene. The different groups are represented in Fig. 1. We performed several statistical analysis based on the different groups stablished on Fig1, to determine which mendelian segregations suit the data (Table 1).

To represent the emission of acetophenone, methyl benzoate, methyl cinnamate and β-β-ocimene we elaborated Fig. 2 where we display the mendelian segregations that based on the bibliography would probably be one of the most suitable to explain the emission of these compounds on the population.

In the case of methyl benzoate, it is known that the major enzyme responsible its emission in *Antirrhinum* is benzoic acid carboxymethyltransferase (BAMT) [4–6]. Our group has recently published the *A. linkianum BAMT locus* and unearthed that major rearrangements on the promoter region are responsible of the lack of emission of methyl benzoate in *A. linkianum* [2]. These evidences suport the mendelian segregation found for methyl benzoate in the *A. majus* x *A. linkianum* F2 segregation, where 3:1 segregation explains its emission with a p-value of 0.582.

β-ocimene synthase is the known enzyme responsible of the synthesis of β-ocimene in *A. majus*, being coded by the gene *ama0a23* [7]. We found two suitable mendelian segregations for the emission of β-ocimene in the population (3:1 and 13:3), meaning that its emission could be explained by the simple segregation of a gene or a epistatic segregation of two genes. It is yet to be stablished if β-ocimene synthase is the only enzyme synthesizing β-ocimene on *A. majus*.

These results also validate to some extent the mendelian segregation approach proposed in this work. Due to the lack of light over the genes implied on the emission of methyl cinnamate and acetophenone, these work is a guide for future research on the genetic basis of their emission. As our results indicate, acetophenone could be explained by the epistatic segregation of two genes, whereas methyl cinnamate emission is expected to be result of the segregation of a single gene where the dominant allele is non-functional.

4. CONCLUSIONS

Our results indicate that the usage of F2 populations to guide genetic molecular work are still a useful tool. Due to the lack of knowledge regarding acetophenone and methyl cinnamate

emission, our results have provided a helpful guide in our attempts to clone the genes underlying these compounds.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

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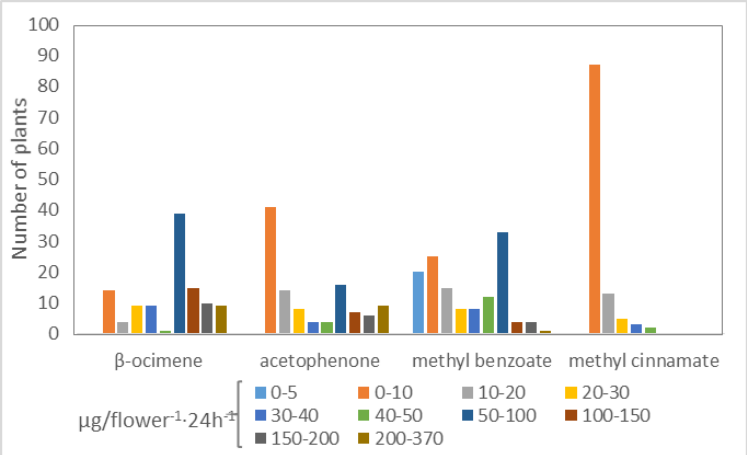

Figure 1. Number of plants emitting different ranges of quantities of volatiles

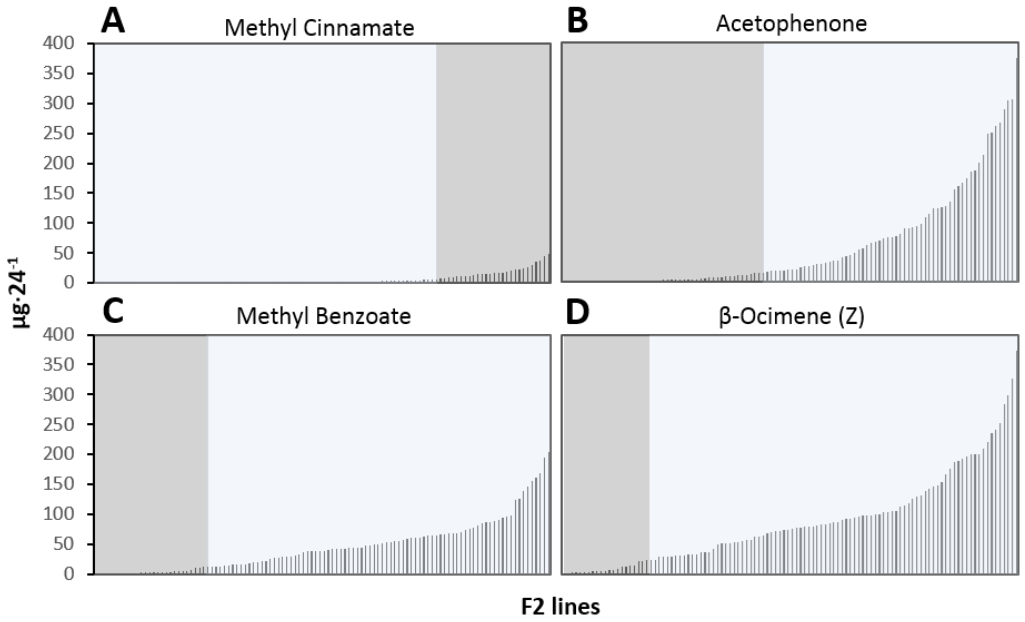

Figure 2. Individual emission of the interest volatiles by the 110 F2 lines of the population. Dark shaded areas indicate the proportion of lines considered as recessive. Light shaded areas indicate the dominant proportion of segregations: methyl cinnamate (3:1), acetophenone (9:7), methyl benzoate (3:1) and β-ocimene (13:1). See table 1.

Table 1. Possible segregations for the volatiles of interest

Compound	Segregation model	χ^2 (p-value)
Methyl benzoate	3:1 (85:25)	0.582*
β-Ocimene	3:1 (96:14)	0.002953
	3:1 (92:18)	0.03645
	3:1 (83: 27)	0.9123*
	13:3 (96:14)	0.1056*
Methyl cinnamate	3:1 (87:23)	0.3218*
Acetophenone	3:1 (68:41)	0.002354
	1:2:1 (41:46:22)	0.0001789
	9:7 (68:41)	0.1966*