Identification of DNA from *Antirrhinum linkianum introgressed in A. majus with common molecular markers*

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Resumen

El género *Antirrhinum* se ha usado ampliamente en genómica comparativa. Actualmente, trabajamos en una Línea Recombinante Consanguínea (LRC) entre la conocida *A. majus* y *A. linkianum*. Por otra parte, contamos con una serie de marcadores que fueron desarrollados para identificar distintos *loci* de ambos parentales. Aquí mostramos una aplicación de éste conocimiento en nuestro trabajo diario.

Palabras clave: Indique 3 a 5 palabras clave separadas por punto y coma relacionadas con su trabajo (ninguna deberá estar en el título)

Abstract

The genre *Antirrhinum* has been widely used for comparative genomics. Currently, we are working on a Recombinant Inbred Line (RIL) between the well known *A. majus* and *A. linkianum*. On the other hand we count with a series of markers that were developed for identifying different *loci* from both parents. Here we show an application of this knowledge in our daily work.

Keywords: PCR, markers, *A. majus*

1. Introduction

Antirrhinum majus, popularly known as snapdragon, has been used as a model plant in genetics since the beginning of the 20th Century. The *Antirrhinum* species group also has been used in studies of natural variation. There are of approximately 20 species that are close relatives of *A. majus* and form a monophyletic group. This group is native to the Mediterranean region, mainly from southwestern Europe and northern Africa. The species vary widely in ecology and morphology [1].

All the species of *Antirrhinum* that have been studied can be cross-pollinated with one another and are able to form fertile hybrids, this facilitates the identification of genes that produce their differences [2]. Population genetic studies of *Antirrhinum* species have shown different geographic distributions, population sizes, and breeding systems, from self-fertility to obligate out-crossing [3]. This group share the same chromosome number (2n= 16) and the majority of the species are allogamous, though commercial *A. majus* lines and a few other wild species can be self-fertilized [4].

From cultivars of *A. majus* some laboratory lines were produced and also exists a large collection of mutants [4]. A collection of *A .majus* mutants has been produced from some laboratory lines of *A. majus* which have been selected for high transposon activity [5]. In some cases, these selected lines have been used to isolate genes by transposon tagging [6]. In addition, there is a collection of roughly four hundred classical mutants, mostly in an isogenic background (Sippe 50) [7].

Genetic recombination maps are an important source for comparative genomics because they allow identifying gene functions, assembling genome sequences and are useful also for breeding. Up to date, there are two molecular recombination maps published for *A. majus* (line 165E), both were made out of a F_2 cross line. The first one was made out of a cross between *A. majus* with the wild species *A. molle* [8]. The second one was published in 2010 [9] and it was carried out as result of a cross between *A. majus* (165E) x *A. majus* (Sippe 50). For allowing identification and alignment of linkage groups in the two populations, the genotypes from the first mapping population were used to construct the second map. Markers common to both maps permitted the identification of corresponding linkage groups and their orientations.

MITEs (miniature inverted-repeat transposable element) are present in relatively low copy-number in all *Antirrhinum* species. Due to the apparent inactivity of this transposon family, they have been called IDLE [10]. MITE transposons have commonly been found in gene-

rich regions [11], even though many transposon families are the main components of heterochromatin. This is consistent with the distribution observed by [9] of IDLE insertions in *Antirrhinum*, which are distributed among protein-coding genes and do not appear to be clustered in centromeric nor telomeric regions.

We have developed a Recombinant Imbred Line (RIL), between *A. majus* and its wild relative *A. linkianum*. Currently, we are working with the F_4 population and we are focused on a specific *locus* of the genome of the parents and their siblings. The aim of this work was to use different molecular sources to test the origin of our DNAs.

2. Materials and Methods

We used an inbred line from *A. majus*: 165E; and two different plants from *A. linkianum*(1 and 2).

2.1 DNA extraction

DNA was extracted from young leaves, using the Macherey-Nagel kit "NucleoSpin® Plant II", following the manufacturer protocol. We used 100mg of leaves, grinded with liquid nitrogen.

2.2 PCR

We used the high-fidelity polymerase: PrimeSTAR GXL DNA Polymerase. PCR conditions are specified in Table 1 and were used according to the PrimeSTAR GXL protocol.

2.3 Gel analysis

Electrophoresis for isolating DNA fragments has been done using an agarose gel with different percentages of agarose, depending on the expected size of the PCR fragments. Ethidium Bromide was used to stain the fragments. This agent has fluorescence when it is exposed to UV light (312nm), enabling the display of the DNA molecules in the gel. 1x TAE (Tris-acetate-EDTA) buffer was used, prepared from a stock solution of 50x TAE, according to [12]. For PM fragments we used a 1% agarose gel, whereas for ZS167 we used a 1.5% agarose gel.

3. Results and Discussion

While working with DNA from the parents of our RIL, we found heterozygosity (Fig.1) for the PM *locus* in *A. linkianum*(1) (two bands). The size of *A. linkianum*(1) bands were 1500bp and 2000bp, while the weight of the same locus in *A. majus* was 1500bp. Due to this double signal in *A. linkianum*(1), we thought of the risk of being working with a hybrid of *A. majus* and *A. linkianum,* because in the gel we could detect PCR products of different sizes with the same pair of primers.

So for testing this DNA from *A. linkianum*(1) was from a pure line, not from a hybrid; we used a couple of markers developed by [9] and used by [13] for the low resolution map of this RIL: ZS167 (Fig.2). This way, we found the results were consistent and *A. linkianum*(1) was effectively DNA from *A. linkianum.*

Moreover, we sowed seeds of *A. linkianum* from a secure stock. We extracted DNA from one of these plants (*A. linkianum*(2)) and probed again with the primers of interest (PM). As a result, two bands appeared with the same size than for *A. linkianum*(1) (Fig.1).

This is a preliminary result that may be explained by an insertion of 500bp in one of the alleles of this *locus* (PM). This insertion may be caused by a transposon [14]. Following studies will be focused in sequencing the different PCR products obtained by using PM primers. As far as a transposable element is the responsible of the insertion, further related studies could be: to identify the phenotype result of this insertion, to classify the type of transposon (MITE, a long terminal repeat LTR...) and its activeness[15].

4. Conclusions

The combination of molecular sources used for identifying the origin of our samples is a good approach for reaching our objective. Nevertheless, for ensuring our results, sequencing of the amplified fragments needs to be done.

5. Aknowledgements

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6. Bibliography

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Tables and Figures

Tabla 1. PCR conditions

Primers name	Annealing Tª	Elongation time (min)	Digestion
PM	60ºC	2	-
ZS167	55ºC	0.5	Hinf I

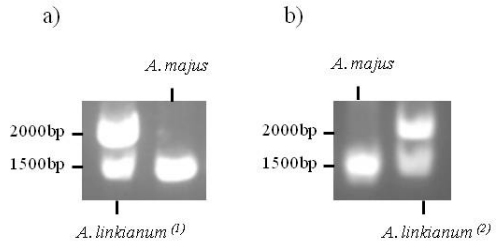

Figure 1. *PCR products from PM primers. a) Double band found for A. linkianum(1) and simple band in A. majus. b) Double band found for A. linkianum(2) and simple band in A. majus*

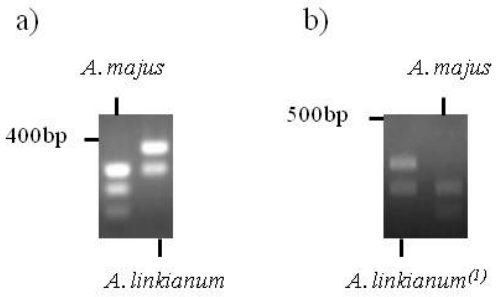

Figure 2. *PCR products from ZS167 primers. a) Results found in the low resolution map of [13]. b) Results found using A. linkianum(1) DNA*