Scientia Horticulturae 144 (2012) 161–167

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Genotyping *Antirrhinum* commercial varieties using miniature inverted-repeat transposable elements (MITEs)

Julia Weiss[a,b], Izaskun Mallona[a,b], Perla Gomez-di-Marco[b], José María Fernández-Valera[c], Marcos Egea-Cortines[a,b,*]

[a] Genetics, Departamento de Ciencia y Tecnología Agraria, ETSIA, Universidad Politécnica de Cartagena, 30203 Cartagena, Spain
[b] Instituto de Biotecnologia Vegetal, Departamento de Ciencia y Tecnología Agraria, Universidad Politécnica de Cartagena, 30202 Cartagena, Spain
[c] Departamento de Ingeniería de los Alimentos y del Equipamiento Agrícola, Universidad Politécnica de Cartagena, 30203 Cartagena, Spain

ARTICLE INFO

Article history:
Received 24 February 2012
Received in revised form 16 May 2012
Accepted 27 June 2012

Keywords:
Antirrhinum majus
Floral size
Floral architecture
DNA fingerprint
MITEs transposons

ABSTRACT

A special challenge for variety fingerprinting arises in ornamentals sold as population mixture or F1 hybrids displaying colour combinations. We developed a fingerprint protocol for snapdragon (*Antirrhinum* spp.) commercial plant material based on mapped miniature inverted-repeat transposable elements. We used 15 MITEs-based markers to discriminate two laboratory inbred lines 165E and Sippe 50 and twelve commercial varieties including six sold as colour mixtures, out of which three were F1 hybrid varieties, three were population mixtures and three single coloured. As MITEs share a common sequence the number of primers was reduced over 33% compared to regular primer pairs. In spite of the obvious variability, we found single markers homozygote for a given variety, and as expected, several heterozygote markers. We developed a dichotomic amplification protocol that allows unambiguous identification of snapdragon varieties thus showing that all varieties have a unique pattern of MITEs marker amplification that allows the reliable genotyping of these ornamental cultivars. Distinctiveness, uniformity and stability of ornamental F1 hybrid varieties can be established using these molecular markers.

1. Introduction

Commercial snapdragon belong to the genus *Antirrhinum*, family Scrophulariaceae, consisting of more than 20 species, which are mostly found around the Mediterranean Sea and have their centre of origin and distribution in the Iberian peninsula (Wilson and Hudson, 2011). There are three subsections in the genus *Antirrhinum*: *Kicksiella*, *Streptosepallum* and *Antirrhinum* that differ in the size of both leaves and flowers (Feng et al., 2009). Most commercial varieties are thought to be *A. majus* but a recent market tendency to sell wild species has been detected with inclusion of other wild species like *A. barrelieri*, *A. siculum* o *A. braun-blanquetii*.

Successful release of new and better varieties of agricultural and horticultural crops requires their unambiguous identification, both for breeding and the maintenance of varietal purity, registration, trade and plant patent protection. Whilst most plant varieties sold are either as F1 hybrids and in some rare cases pure lines, in ornamental bedding plants like Petunia, Begonia, Pansy, Geranium, Viola, Abutilon, Gazania, Cyclamen, Mimulus or snapdragon, just to name a few species, varieties are often sold as F1 hybrids, or mixed populations that display colour combinations. These mixed populations with different colours increase the aesthetic impact of the plantation. This type of mixed populations are a challenge when trying to obtain a fingerprint for a variety as we expect to have markers that segregate and others that are fixed in homozygosis.

Molecular markers may serve as a cost- and time saving alternative to morphological markers for variety identification. In contrast to food crops or model organisms, molecular data on ornamentals is very scarce (De Riek and Debener, 2009). Possible reasons for this lack of molecular resources are the amount of species used in the ornamental industry, or the fact that ornamental breeding looks for beauty and it is not always well characterized at the genetic level (De Riek and Debener, 2009). Recent work has shown the feasibility of transposon and retrotransposon based molecular marker development (Casa et al., 2002; D'onofrio et al., 2010; Kalendar et al., 2011; Kwon et al., 2005). Miniature inverted-repeat transposable elements (MITEs) represent a new class of molecular markers. They belong to a group of small non-autonomous transposable elements with terminal inverted repeats. They are structurally reminiscent of class 2 non-autonomous elements with their small size (<600 bp), lack of coding capacity, and terminal inverted repeats (TIRs) (Feschotte et al., 2002). Most of the MITEs seem to be

* Corresponding author at: Genetics, Departamento de Ciencia y Tecnología Agraria, ETSIA, Universidad Politécnica de Cartagena, 30203 Cartagena, Spain. Tel.: +34 868071077; fax: +34 968325433.
E-mail address: marcos.egea@upct.es (M. Egea-Cortines).

0304-4238/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scienta.2012.06.040

inactive and the short terminal repeats (TIRs) are usually conserved in length between members of a family (Feschotte et al., 2003). Importantly MITEs tend to have homogeneous distribution in genomes as distant as Brachypodium (Vogel et al., 2010) or *A. majus* (Schwarz-Sommer et al., 2010). One MITEs family identified in *A. majus* was named *Idle* due to its apparent stability and was also found in the genera *Misopates* and *Linaria* belonging to the family of the *Scrophulariaceae* (Cartolano et al., 2007). They are characterized by a region of 215 bp flanked by TIRs. Altogether, 51 MITEs markers are mapped to the molecular linkage map derived from an intraspecific hybrid between the laboratory lines 165E and Sippe 50 (Schwarz-Sommer et al., 2010). Four to ten MITEs have been placed on each chromosome with the exception of chromosome 3, which only has two identified *Idle* MITEs.

The aim of this work was to analyse the suitability of MITEs positioned in the *A. majus* chromosomes as markers to unambiguously identify snapdragon commercial lines in spite of the challenge caused by colour mixtures.

2. Materials and methods

2.1. Plant material and growing conditions

The Antirrhinum laboratory line Sippe 50 was obtained from the germplasm bank in Gatersleben (Kulturpflanzenbank Gatersleben, Germany; www.ipk-gatersleben.de). The laboratory line 165E was donated by Zsuzsana Schwarz-Sommer from the Max-Planck Institute of plant breeding (MPI-Pflanzenzüchtungsforschung, Köln, Germany; www.mpiz-koeln.de). Both lines are inbred lines resulting from repeated self pollinations (Delgado-Benarroch et al., 2009). The commercial varieties Vilmorin Maximé, Vilmorin Muflier Grand Gueule De Loup Varie, Tall Mix, Vilmorin naín, *A. majus* Kim Bicolor Mixed F1 Hybrid, *A. majus nanum* Frosted Flames, *A. majus* Double Madame Butterfly Mixed F1 Hybrid, *A. majus nanum* Bronze Dragon, *Antirrhinum* Pendula Multiflora Chinese Lanterns, F1 Hybrid Royal Bride and Kousei-ichidai-kouhaikei Mix were obtained from different commercial providers (Table 1). Plants were germinated on vermiculite and transplanted to pots with a mixture of vermiculite–coconut fibre–floral substrate in a ratio of 0.2:1:2 and watered as required. Plants were grown in a Sanyo MRL 350 growth chamber under a regime of 16 h fluorescent light at a photosynthetically active photon flux density of 250 mE s^{-1} m^{-2} and 8 h darkness with day/night temperatures of 22/15 °C, and further transplanted to greenhouse conditions as described (Bayo-Canha et al., 2007).

2.2. DNA isolation

A total of six independent DNA extractions were performed from six independent plants for every line using a total plant DNA extraction kit which included treatment with RNase (NucleoSpin; Macherey-Nagel, http://www.mnnet.com). DNA concentration was determined spectrophotometrically at 260 nm and adjusted to 200 ng/μl with TE (10 mM Tris–HCl, pH 8. 0.; 1 mM EDTA).

2.3. MITEs amplification

Amplification of the Idle-MITEs was performed using an *Idle*-specific primer and a second primer specific for the flanking genomic region of insertion (Table 2). All oligonucleotides were synthesized by Invitrogen. PCR was performed in a final volume of 25 μl containing 2 μl of the extracted DNA, 0.08 mM each of dATP, dCTP, dGTP and dTTP, 1.5 mM $MgCl_2$, 1× PCR buffer and 1 U GoTaq Flexi DNA polymerase (Promega). The amplification was carried out as follows: initial denaturation at 95 °C for 2 min, 30 cycles of 95 °C for 30 s, 54–58 °C depending on the particular MITEs for 30 s and 72 °C for 30 s, and a final extension at 72 °C for 2 min. Amplification was performed in a GeneAmp PCR System 9700 Thermocycler from PE Applied Biosystems. Samples of 10 μl of PCR products were analysed on 1–2.5% agarose gels stained with ethidium bromide in TAE buffer together with a standard marker DNA ladder (Fermentas, Spain). Performing six independent DNA isolations per variety and two PCR reactions per DNA extraction and MITEs marker set assessed consistency of band profiles. Each PCR experiment included two independent amplifications from 165E and Sippe 50 as positive control reactions.

2.4. MITEs analysis

The MITEs sequences were retrieved from GenBank and annealed with CLUSTALX (Larkin et al., 2007). Sequences were manually trimmed to obtain MITEs sequences without *Antirrhinum* genomic flanking sequences. Analysis of Direct Repeats (DRs) and Terminal Inverted Repeats (TIRs) was performed using the MUST program (Chen et al., 2009).

2.5. Data analysis

In order to classify the segregating individuals according to the MITEs markers tested, we split each marker into two logical variables: Sippe 50- and 165-like amplification. Therefore we built a binary matrix assigning '1' when there was a band corresponding to such a marker and '0' when there was not. The dissimilarity indices used were computed between all pairs of varieties using three different methods, Euclidean, Manhattan and Gower. Hierarchical clustering was performed by the method described by Ward (Ward, 1963).

Regarding the classification by lines, data gathered from all the individuals of the same line were coded as follows: 'A', homozygous for the genetic background 165E; 'B', homozygous for the genetic background Sippe 50; 'C', heterogeneous population; 'D', no amplification and recursively partitioned. The decision tree built up after the partitioning had two choices in each node, resulting in a binary tree that could be read as a dichotomous key.

Both procedures of individual and genotype multivariate analysis were conducted with the R statistical environment with the packages "cluster" v1.14.0 and "rpart" v3.1-50.

3. Results

3.1. Phenotypic characters of commercial varieties

The snapdragon lines used to create the *A. majus* linkage map are two inbred lines that show complete apical dominance and differ from each other in their flower colour due to known mutations (Fig. 1a and b). Further differences include tendency of the 165E line to produce radially symmetrical flowers in winter (Fig. 1b). The different snapdragon commercial varieties could be readily sorted by their body size, with a group of varieties showing a high level of apical dominance and longer inflorescences (Fig 1c, Bronze Dragon) a second group with low apical dominance and shorter size (Fig 1d, Vilmorin naín). One of the varieties, Pendula Multiflora Chinese Lanterns F1 Hybrid, showed a lack of negative gravitropism (Fig. 1e), indicating that changes in plant structure used in breeding include stem size, apical dominance and shoot gravitropism. Size of the plants announced in the commercial bag of seeds was slightly larger than the ones we observed, probably due to local environmental conditions (Table 1). Visual inspection of the Pendula line showed a strong venation pattern in the petals, typical of *Venosa+* plants (Schwinn et al., 2006). Additional characters included completely red to purple colour in stems and leaves of Bronze Dragon

Table 1
List of Antirrhinum lines and commercial origin.

Origin	Name of line	Major characteristics
IPK Gatersleben	Sippe 50	*nivea*, 40 cm tall apical dominant
Max Planck Institute – MPIZ	165E	*Pallida rec*, *delila* 40 cm tall apical dominant
Vilmorin	*Muflier Grand Gueule De Loup Varie*	Colour mixture 80 cm tall apical dominant
	Maximé	Colour mixture 60 cm tall apical dominant
	naín	Semi dwarf, colour mixture Bushy, bushy 20 cm tall
	Tall Mix	Colour mixture 60 cm tall apical dominant
Thompson & Morgan	*Kim Bicolor Mixed F1 Hybrid'*	Semi dwarf, colour mixture Bushy 20 cm tall
	Pendula Multiflora 'Chinese Lanterns'Laterns'	Trailing, possible hybrid of *A. majus* × *A. molle*
	A. majus nanum Bronze Dragon	Dark purple leaves Bushy, 20 cm tall
	Madame Butterfly	Colour mixed, modified floral architecture (double petals), apical dominance 60 cm tall
Mr. Fogerhills direct	*A. majus nanum Frosted Flames*	Semi dwarf, variegated leaves 20 cm tall
	F1 Hybrid Royal Bride	Colour mixture 80 cm tall apical dominance
	Kousei-ichidai-kouhaikei Mix (Hybrid F1 Mix)	Colour mixture 60 cm tall, apical dominance

and variegated leaves in the case of Vilmorin Muflier Grand Gueule De Loup Varie, as announced in the advertisement.

Concerning organ-specific characters, different snapdragon lines could be distinguished by important differences in floral size (Fig. 1f). The Japanese line Double Madame Butterfly Mixed F1 Hybrid produced the largest flowers observed. Visual inspection showed that this line also has modified floral architecture. We investigated further organ number differences and found it displayed two whorls of petals, one whorl of petals, a fourth whorl of filamentous petals, stamens and carpels (Fig. 1g and h).

All the lines that were sold as mixtures displayed a combination of colours in the plants grown from a single batch of seeds

Table 2
Primer sequence, annealing temperature, PCR product size, genetic background and chromosome position of Idle MITE markers. The primers specific for a MITE are labelled as P1 and P2, for those cases that are common to several *Idle*-MITEs.

Chr. Nr.	Idle-MITE	Accession nr.	Primer sequences	Anneal. Temp.	Product size	Presence	Position in chromosome (cM)
1	01p23	FM992453	CCTTCGCATAACACATGGGGTAC **P1 CCATGCGTTTCCTCGAAAATCACC**	57.1	600	165E	29
1	88i24	FM992458	GTTTGTAGAGCTCGAAGGACTGC **P2 CGGTTTCGATATATGTTTCGGTCTGC**	57.1	350	165E	76
2	37	FM992446	GTGTGTGTACAAAACTCCCTTTATTTTCG **P1**	57.3	260	Sippe 50	36
2	31b13	FM992456	CAACCCTTCAATGCCTAAAGGCAAG **P2**	58.0	400	165E	52
3	67m10	FM992478	GGCGTTTGAGTGATTAAATAGGCACTTC **P2**	58.0	380	165E	49
4	67i01	FM992481	GGTTTAGGATCACATTTTCCCCTTGC GTGAAAATCTTGGCTTATGAAAACTCTGG	57.3	940/700	165E	62
4	2	FM992411	GTGTCCCACTTAGCCATTTGCTTGG **P1**	57.4	320	Sippe 50	40
5	06n14	FM992454	CGCATTTCATTGAATTACTCCACCAAGTC **P1**	57.4	1000	165E	45
5	81k24	FM992460	GCTAAACTAACTCCGATCTTGCTCTAGC **P1**	57.4	600	165E	52
6	34	FM992443	GCTTTCTGAAGACCAAAGGCTGTAGG **P1**	57.4	250	Sippe 50	14
6	83d24	FM992459	GAGGGTATAATAGGCATGGCTAAATTGG **P1**	57.4	400	165E	66
7	8	FM992417	GCTCGTATATGTCCCAACTCAGTATGG **P2**	58	570	Sippe 50	14
7	51g09	FM992487	GAGAAAGAGACTTACTTTAGGGTTGAAGC **P1**	57.4	440	165E	17
8	94b13	FM992499	CTAGCTCATTCGTCATACAAGTATCTCC **P1**	58.0	530	165E	17
8	12	FM992421	GCTCTATTGCGTAGGTTGGAGG CAACCGTATCAGGTAACGTAAAGG	55.7	320/520	Sippe 50	24

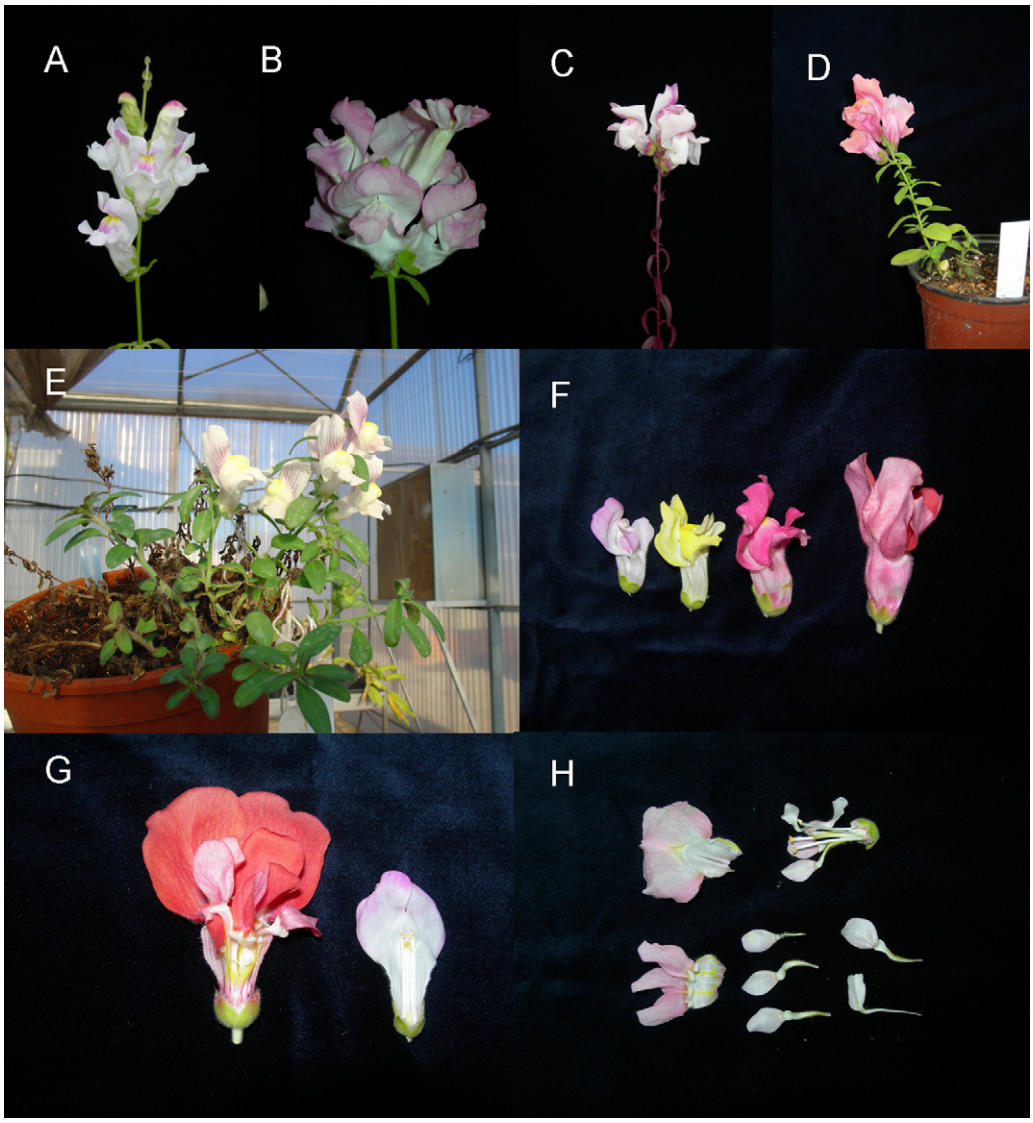

Fig. 1. Picture of several lines used in the study highlighting major phenotypic characteristics. (A) Sippe 50, (B) 165E, (C) Bronze Dragon, (D) Vilmorin naín, (E) Pendula, and (F) Floral size differences in commercial varieties. From left to right 165E, Vilmorin naín, Muflier and Madame Butterfly. (G) Cross section of Madame Butterfly and 165E and (H) Floral organs of Madame Butterfly.

confirming the complex mixture of at least three different colours and a maximum of five.

3.2. Characteristics of the selected MITEs markers

We used two MITEs per chromosome except for chromosome 3. Although the *Idle* MITEs family had been reported previously as part of a positional cloning experiment (Cartolano et al., 2007), there was no detailed information about this MITE family. We performed a multiple alignment of these 15 MITEs that show a high degree of sequence conservation (Fig. 2). We characterized the DRs and TIRs of the Idle clones under study and found that the DRs ranged between 2 and 5 base pairs while the TIRs varied between 8 and 13 base pairs (Supplementary Table S1). The primer combinations previously developed (Schwarz-Sommer et al., 2010) had a common primer that aligns to several MITEs and a second primer that is specific for the genomic region flanking the insertion (Table 2). This allowed the amplification of 15 MITEs with a total of 19 primers which is more than one third decrease in costs at this stage compared to 30 that would be required for EST-based markers.

Most MITEs behave as dominant markers that are inserted in a genomic region compared to a non-carrying allelic region. This can be regarded as an insertion/deletion situation. Among the 15 MITEs and under our experimental conditions, 8 MITEs amplified as dominant markers, amplifying as predicted either in 165E or Sippe 50, 4 were codominant markers whose amplicons differed in size as indicated between 165E and Sippe 50 (Supplemental Fig. S1) and three markers, which were originally designed as dominant (Table 2) (Schwarz-Sommer et al., 2010), did not show a polymorphism between 165E and Sippe 50 on agarose gels (Supplemental Fig. S1). This lack of polymorphism was tested and resulted stable even under stringent PCR conditions in a primer hybridization gradient assay. Size differences between the amplicons of the codominant markers were easy to score on agarose gels. PCR product size of the MITEs corresponded to those listed in Table 2 with the exception of *Idle*-6n14, which amplified a larger product in Sippe 50 of roughly 1.8 kb and *Idle*-83d24 that amplified a smaller product in 165E. The markers *Idle*-51g091, *Idle*-1p23 and *Idle*-94b13 amplified in some varieties additional PCR products differing in size from those described for Sippe 50 and 165E (Table 2). Differing products were excluded from further analysis.

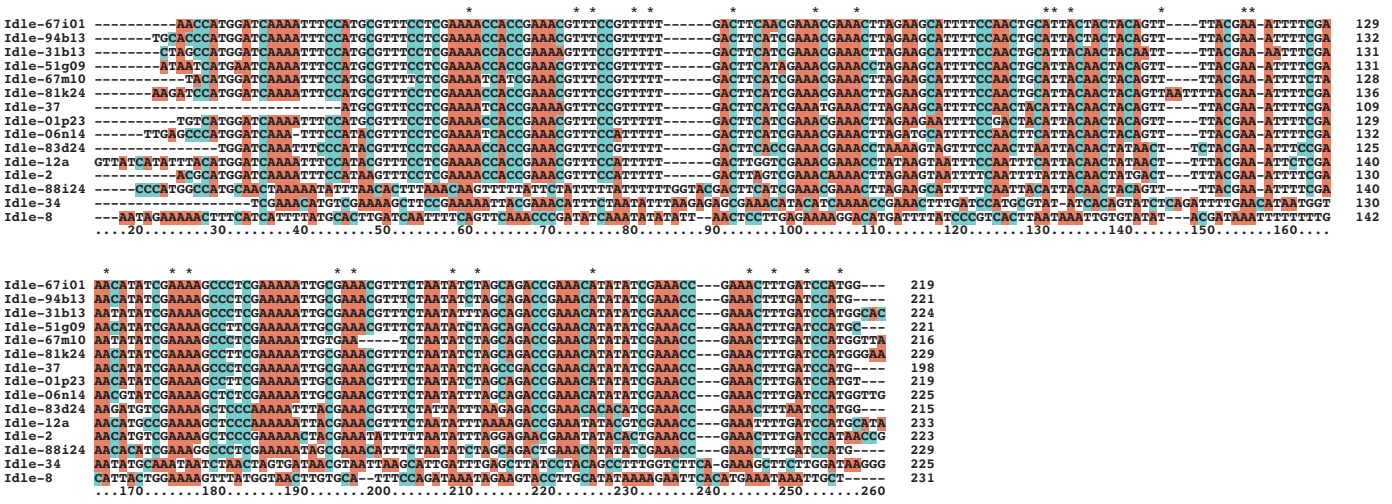

Fig. 2. Multiple alignment of the Idle-MITES used in this study. Alignment was performed with CLUSTALX using standard parameters. Coloured bases correspond to identical bases in the sequences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

3.3. Discrimination between varieties based on MITEs markers

Except for *Idle*-1p23 and *Idle*-8 on chromosome 1 and 7, we were able to obtain PCR products for all of the 15 selected *Idle*-MITEs markers using DNA preparations from our collection of 13 commercial *Antirrhinum* varieties, although not every marker amplified within each analysed variety population (Fig. 3). Applying the letter code described above, Table 3 presents the pattern of amplification for each variety. The results show that, with the exception of the varieties Kim Bicolor Mixed F1 Hybrid, whose analysed population was highly heterogeneous for all of the fifteen selected *Idle*-MITEs markers, all varieties showed amplicons coinciding with Sippe 50 or 165E for at least one (Vilmorin maximum, Tall mix) and a maximum of five MITEs markers (Madame Butterfly). The varieties showed a specific and unique profile concerning the combination of homozygous markers.

3.4. Marker distribution

We observed a preference of MITEs markers, appearing on certain chromosomes for the analysed varieties. Markers derived from 165E frequently appeared on chromosome 5, 6, and 8 whereas markers originating from Sippe 50 were often found on chromosome 6.

3.5. Amplification pattern profiling by clustering

The dendrogram shown in Fig. 4 is a clustering plot based on the amplification pattern of the dominant and co-dominant MITEs markers excluding *Idle*-01p23 and *Idle*-8 that did not amplify in any of the commercial varieties. Each of the six analysed individuals per variety was treated individually. The dendrogram shows that in case of some varieties, i.e. Royal bride, the individuals appear in many distant branches, indicating a very heterogeneous plant material, while individuals of other varieties like Vilmorin naín appeared in neighbour branches and therefore seem to be genetically more homogeneous.

3.6. Development of a dichotomic key for variety identification

In order to speed up the process of genotyping we developed a dichotomized amplification guide that allows the identification of a single variety in a set of four PCR reactions. This simple key (Fig. 4) shows that MITEs-based markers can be used to obtain DNA fingerprints for commercial varieties even in those cases where

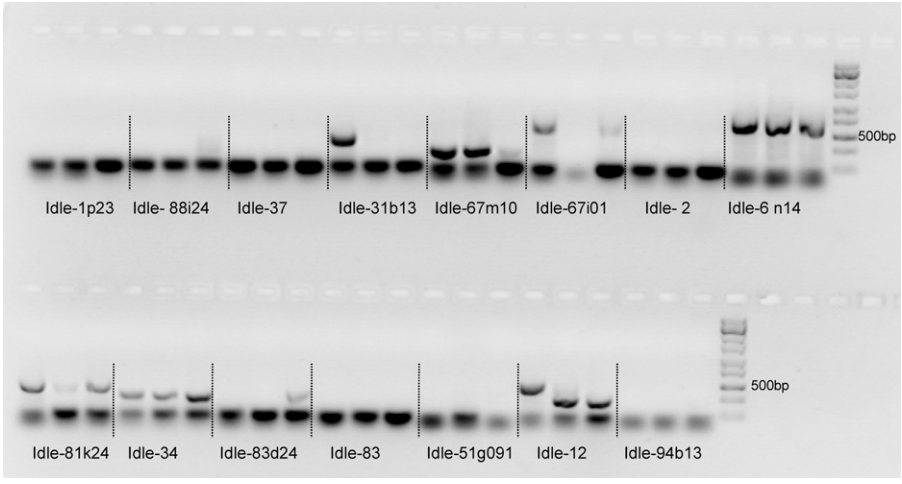

Fig. 3. An example of amplification of 15 *Idle*-MITEs markers in three individuals of the *A. majus* Muflier variety.

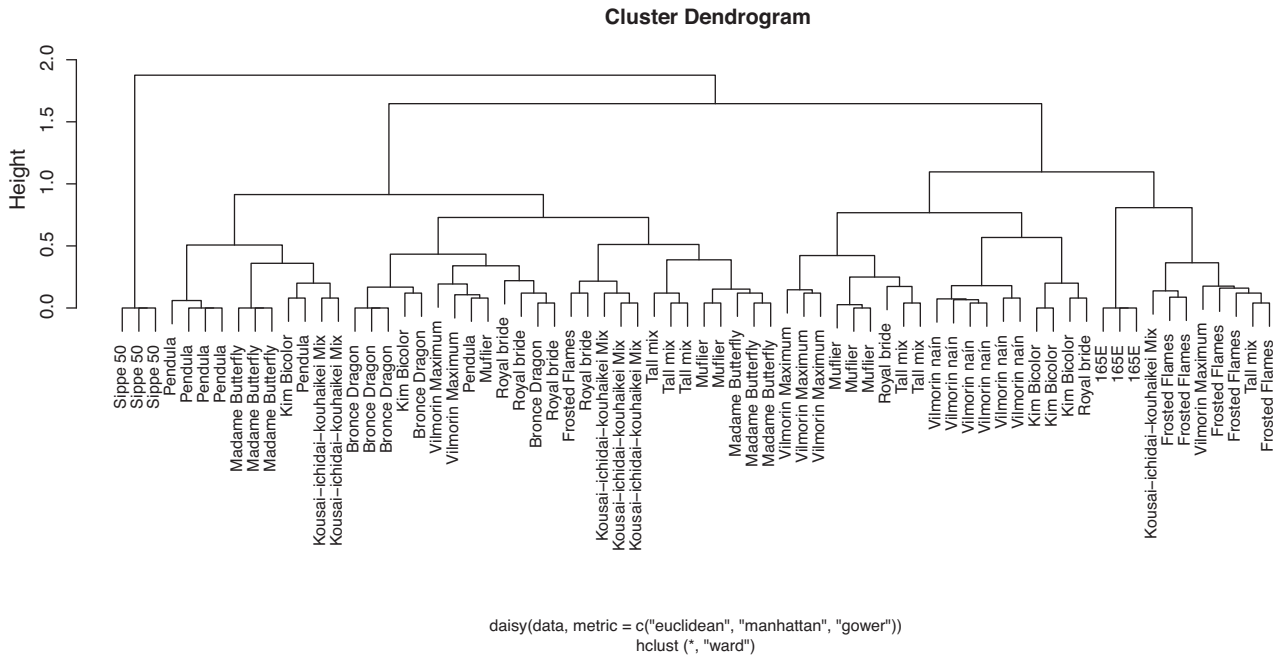

Fig. 4. Overview of the diversity of MITEs segregating patterns. Each leaf of the tree corresponds to a single individual and it is nominated according to its commercial name. Hierarchical clustering was performed taking into account the resemblance to the 165- or to the Sippe 50-lines.

some characters segregate. Inclusion of additional varieties would require model updating to construct a proper decision tree that might use other MITEs combinations described in this work, specific for a given variety.

4. Discussion

In this study we have successfully applied 15 mapped MITEs transposable elements to create a dichotomic key to identify snapdragon commercial varieties. A major advantage compared to other molecular markers is that once the sequence of a transposable element is known, obtaining flanking sequence is straightforward. This approach has been effectively used to create markers in maize, barley, rice or snapdragon (Casa et al., 2000; Monden et al., 2009; Schwarz-Sommer et al., 2010; Takahashi et al., 2006). Furthermore as the core sequence of MITEs is conserved, the number of primer combinations required is significantly lower than with other markers that require two new pairs of primers per locus. In our case we could cover 15 loci with 4 MITEs–based primers and one specific for the genomic sequence flanking a particular insertion. This approach allows over 30% cost reduction in primers that can be a considerable amount when multiple sets of primers have to be designed and synthesized.

Two types of molecular markers had been developed in the genus *Antirrhinum* to study wild populations. One is based on allozymes (Mateu-Andres, 1999) and a second development has been based on RAPD markers (Jimenez et al., 2002). Finally chloroplast based markers (ITS and *ndhF*) have been also develop to study species distances (Vargas et al., 2004). Although these markers were available between the publication of the first map of *A. majus* × *A. molle* (Schwarz-Sommer et al., 2003), and the second map based on the two *A. majus* lines used in this study (Schwarz-Sommer et al., 2010), they are less reliable than those based on specific amplification products. In this respect, MITEs-based markers are as robust as markers based on EST-based amplification and digestion, but are

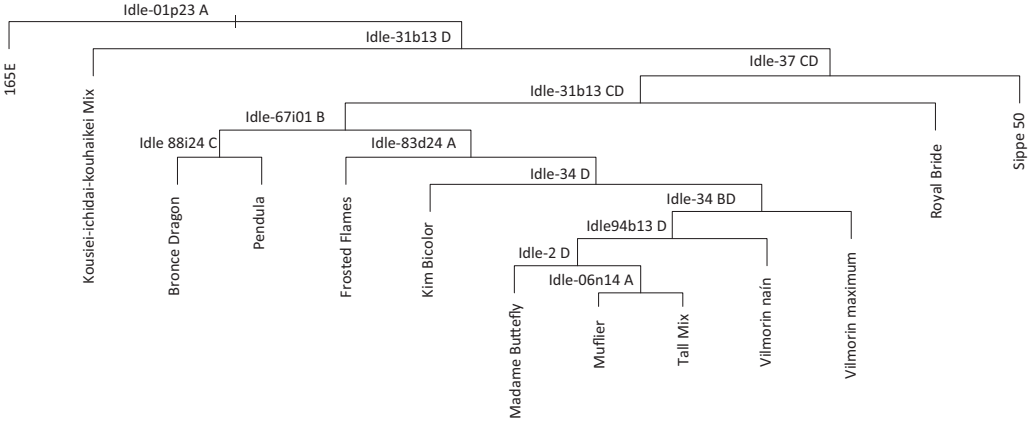

Fig. 5. Dichotomous key of variety identification by its amplification profile A: homozygous for the genetic background 165E; B: homozygous for the genetic background Sippe 50; C: heterogeneous population; D: no amplification. Tree node labels show the pattern leading to the left branch; i.e. *Idle*-01p23 A means that if the marker shows the A pattern the path goes to the left subtree and otherwise (B, C or D) to the right subtree.

easier to identify unless Next-Generation Sequencing and bioinformatic analysis is applied, followed by suitable SNP detection technology. This last approach requires larger economic inputs than in the case of MITEs identification.

The varieties could be characterized by a specific combination of 2–5 amplifying markers. Considering the existence of a total of 51 mapped MITEs, it should be possible to establish a unique MITEs-DNA profile for any cultivar. The present work also indicates that a specific MITEs pattern could be used as single variety marker. Apart from their capacity to discriminate among cultivars, MITEs markers have the advantage of an easy access, as they require conventional PCR and agarose gel electrophoresis. Automation might be possible using multiplex-PCR for simultaneous amplification of a set of selected MITEs. Even so not all of our MITEs markers resulted codominant as indicated in former experiments, MITEs-based analysis is highly reproducible and transferable (Fig. 5).

The absolute number of PCR reactions required to identify one variety from the rest ranged between two and nine, for those lines with a high level of similarity like Muflier, Tall mix and Madame Butterfly. New varieties would have to be analysed against others to determine this parameter that is obviously variable depending on the scheme of the comparison but is logical and straightforward. The number of samples per variety would have to be adjusted to the number of colour phenotypes present. We think this technological setup has great potential in any species where varieties are sold as colour combinations.

Acknowledgments

Work in the lab was supported by grants AGL2007-61384 and MICINN-FEDER BFU2010-15843 to MEC. The funding agencies had no role in the experimental design or publication of the present work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scienta.2012.06.040.

References

Bayo-Canha, A., Delgado-Benarroch, L., Weiss, J., Egea-Cortines, M., 2007. Artificial decrease of leaf area affects inflorescence quality but not floral size in *Antirrhinum majus*. Sci. Hortic. 113, 383–386.

Cartolano, M., Castillo, R., Efremova, N., Kuckenberg, M., Zethof, J., Gerats, T., Schwarz-Sommer, Z., Vandenbussche, M., 2007. A conserved microRNA module exerts homeotic control over *Petunia hybrida* and *Antirrhinum majus* floral organ identity. Nat. Genet. 39, 901–905.

Casa, A.M., Brouwer, C., Nagel, A., Wang, L.J., Zhang, Q., Kresovich, S., Wessler, S.R., 2000. The MITE family Heartbreaker (Hbr): molecular markers in maize. Proc. Natl. Acad. Sci. U.S.A. 97, 10083–10089.

Casa, A.M., Mitchell, S.E., Smith, O.S., Register, J.C., Wessler, S.R., Kresovich, S., 2002. Evaluation of Hbr (MITE) markers for assessment of genetic relationships among maize (*Zea mays* L.) inbred lines. Theor. Appl. Genet. 104, 104–110.

Chen, Y., Zhou, F.F., Li, G.J., Xu, Y., 2009. MUST: a system for identification of miniature inverted-repeat transposable elements and applications to *Anabaena variabilis* and *Haloquadratum walsbyi*. Gene 436, 1–7.

D'onofrio, C., De Lorenzis, G., Giordani, T., Natali, L., Cavallini, A., Scalabrelli, G., 2010. Retrotransposon-based molecular markers for grapevine species and cultivars identification. Tree Genet. Genom. 6, 451–466.

Delgado-Benarroch, L., Causier, B., Weiss, J., Egea-Cortines., M., 2009. FORMOSA controls cell division and expansion during floral development in *Antirrhinum majus*. Planta 229, 1219–1229.

De Riek, J., Debener, T., 2009. Present Use of Molecular Markers in Ornamental Breeding. In: XXIII International EUCARPIA Symposium, Section Ornamentals, Colourful Breeding and Genetics-Part II 855, pp. 77–84.

Feng, X., Wilson, Y., Bowers, J., Kennaway, R., Bangham, A., Hannah, A., Coen, E., Hudson, A., 2009. Evolution of allometry in Antirrhinum. Plant Cell 21, 2999–3007.

Feschotte, C., Jiang, N., Wessler, S.R., 2002. Plant transposable elements: where genetics meets genomics. Nat. Rev. Genet. 3, 329–341.

Feschotte, C., Swamy, L., Wessler, S.R., 2003. Genome-wide analysis of mariner-like transposable elements in rice reveals complex relationships with Stowaway miniature inverted repeat transposable elements (MITEs). Genetics 163, 747–758.

Jimenez, J.F., Sanchez-Gomez, P., Guemes, J., Werner, O., Rossello, J.A., 2002. Genetic variability in a narrow endemic snapdragon (*Antirrhinum subbaeticum*, Scrophulariaceae) using RAPD markers. Heredity 89, 387–393.

Kalendar, R., Flavell, A.J., Ellis, T.H.N., Sjakste, T., Moisy, C., Schulman, A.H., 2011. Analysis of plant diversity with retrotransposon-based molecular markers. Heredity 106, 520–530.

Kwon, S.J., Park, K.C., Kim, J.H., Lee, J.K., Kim, N.S., 2005. Rim 2/Hipa CACTA transposon display; A new genetic marker technique in Oryza species. BMC Genet. 6, 15.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and clustal X version 2.0. Bioinformatics 23, 2947–2948.

Mateu-Andres, I., 1999. Allozymic variation and divergence in three species of Antirrhinum L-(Scrophulariaceae-Antirrhineae). Bot. J. Linn. Soc. 131, 187–199.

Monden, Y., Naito, K., Okumoto, Y., Saito, H., Oki, N., Tsukiyama, T., Ideta, O., Nakazaki, T., Wessler, S.R., Tanisaka, T., 2009. High potential of a transposon mPing as a marker system in japonica × japonica cross in rice. DNA Res. 16, 131–140.

Schwarz-Sommer, Z., de Andrade, S.E., Berndtgen, R., Lonnig, W.E., Muller, A., Nindl, I., Stuber, K., Wunder, J., Saedler, H., Gubitz, T., Borking, A., Golz, J.F., Ritter, E., Hudson, A., 2003. A linkage map of an F(2) hybrid population of *Antirrhinum majus* and *A. molle*. Genetics 163, 699–710.

Schwarz-Sommer, Z., Gubitz, T., Weiss, J., Gomez-di-Marco, P., Delgado-Benarroch, L., Hudson, A., Egea-Cortines, M., 2010. A molecular recombination map of *Antirrhinum majus*. BMC Plant Biol. 10, 275.

Schwinn, K., Venail, J., Shang, Y.J., Mackay, S., Alm, V., Butelli, E., Oyama, R., Bailey, P., Davies, K., Martin, C., 2006. A small family of MYB-regulatory genes controls floral pigmentation intensity and patterning in the genus Antirrhinum. Plant Cell 18, 831–851.

Takahashi, H., Akagi, H., Mori, K., Sato, K., Takeda, K., 2006. Genomic distribution of MITEs in barley determined by MITE-AFLP mapping. Genome 49, 1616–1620.

Vargas, P., Rossello, J.A., Oyama, R., Guemes, J., 2004. Molecular evidence for naturalness of genera in the tribe Antirrhineae (Scrophulariaceae) and three independent evolutionary lineages from the New World and the Old. Plant Syst. Evol. 249, 151–172.

Vogel, J., Garvin, D., Mockler, T., Schmutz, J., Rokhsar, D., Bevan, M., Barry, K., Lucas, S., Harmon-Smith, M., Lail, K., 2010. Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 463, 763–768.

Ward, J.H., 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. 58, 236.

Wilson, Y., Hudson, A., 2011. The evolutionary history of Antirrhinum suggests that ancestral phenotype combinations survived repeated hybridizations. Plant J. 66, 1032–1043.