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IDENTIFICATION OF CARNATION VARIETIES

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411

IDENTIFICATION OF CARNATION VARIETIES

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Introduction

Microsatellites are highly polymorphic, tandemly repeated DNA sequences with a basic repeat unit (or'core'sequence) of 2-8 base pairs, such as GA, CTT or GATA. The polymorphism found in this type of repetitive DNA is largely due to variations in the copy number of the basic repeat unit. For example, one variety may contain 10 copies of the repeat whereas others may contain 11 or 12. These differences in repeat length occur more frequently than base pair mutations. They can be analysed using the sequence tagged microsatellite site (STMS) approach. In this PCR-based approach specific pairs of primers that are complementary to unique flanking DNA sequences, are utilised to amplify the fragment containing the microsatellite from the genome of a particular variety.

Microsatellites are frequently used for identification of varieties. The reason for this is that they show the level of polymorphism needed to distinguish between the often closely related varieties. Sequence tagged microsatellites are co-dominant markers, i.e. all alleles present in heterozygous individuals will be visible. Often they are multiallelic and as many as 28 different alleles have been reported for some microsatellites in the scientific literature. STMS markers can be reproducibly obtained in different laboratories, as was shown in an experiment conducted in an EU Framework III project. As they are PCR based markers their analysis can be automated. The data generated from the analysis of STMS markers are in a form that can be stored easily as discrete allele lengths (number of base pairs/nucleotides) in a simple spreadsheet. Finally, they can be recorded efficiently and cost-effectively which makes them the ideal markersystem for plant variety identification.

As already mentioned, the only prerequisite for creating a STMS marker is the availability of primers based on the flanking sequences for the PCR amplification of the microsatellite. In principle there are two ways to obtain these sequences. First, they can be extracted from a sequence database, such as EMBL or Genbank. These databases contain information on microsatellites that were sequenced accidentally because they were present in, or close to, a gene that was under study for another reason. This way of obtaining the necessary sequence information is fast and cheap. However, for only a limited number of well studied species there is sufficient sequence information available.

In all other cases one has to clone and sequence DNA containing the microsatellites, which is a costly and time consuming process. However, efficient procedures for the development of libraries specifically enriched for microsatellites have been developed. In this report results obtained with microsatellites obtained from a database as well as own isolates are discussed.

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Carnation microsatellites from the EMBL/Genbank databases

In the EMBL/Genbank database only a limited number (37) of *Dianthus* sequences was present (Dec 1996). Di- and trinucleotide repeats with six or more repeat units and tetranucleotide repeats with four or more repeat units were selected. In all cases 1 mismatch was allowed. This resulted in three dinucleotide repeats, five trinucleotide repeats and one tetranucleotide repeat. Six microsatellites found in the database were tested on a set of 12 carnation varieties. Tests were performed on a standard sequencing gel and PCR products were made visible by silverstaining. Three out of the six were monomorphic, whereas the other 3 were capable of discriminating among 9 out of the 12 varieties, showing 5, 6 and 7 alleles each (see the first six entries in Table 1).

Carnation microsatellites isolated from a DNA library

For isolation use was made of an enrichment procedure (essentially according to Karagyosov et al. NAR 21:3911-3912), which involves the hybridization towards synthetic oligonucleotides complementary to the microsatellite motif. The procedure was tested for several di, tri and tetranucleotide repeats. The procedure resulted in 12% of the fragments containing a microsatellite, which is an enrichment of 25- 625 times. Of these sequences an average of 35% can be used to develop a STMS marker. Table 1 shows the results obtained with 16 of such microsatellite markers on a set of 12 varieties. The number of alleles detected varied between 2 and 6. However, even with these additional 16 microsatellite markers it was not possible to discriminate between the test varieties 'Yellow dust sim' and Scania. The two varieties differed by only one microsatellite from the variety 'White sim'. This suggests a relationship between these three varieties. This could not be tested further, since information on the breeding history of these varieties is not available.

Discriminating power

For a large discriminating power the most important factors are the number of different alleles detected for a certain locus and the distribution of the alleles over the varieties that are under study. For example, when only one primer pair for locus DCF005 was tested on 98 carnation varieties, 14 varieties turned out to have a unique pattern. The remaining varieties could be divided in 15 groups, containing 2 to 27 varieties. For this analysis use was made of fluorescent PCR amplification products which were resolved on a ALFexpress DNA sequencer. Also of importance is the distribution of the loci over the genome. They should as far as possible be equally distributed. From a cost point of view the discriminating power per PCR reaction is important. This is affected by the number of alleles that is found for a certain locus and the number of loci that can be multiplexed (i.e. amplified and analysed together).

Storage of data

In contrast to RFLP, RAPD or multilocus fingerprinting data, it is relatively easy to store microsatellite data in a database. In the near future, information per carnation locus will be stored as the number of repeats that is present in a particular allele or as discrete allele

413

length (number of base pairs/ nucleotides). For reference, allelic ladders can be constructed, making the whole procedure of determining the identity of a given allele independent of the exact electrophoretic system and the molecular weight reference markers used in a particular laboratory. A database containing this information might be useful for selecting reference varieties for PBR testing trials of carnation. Also, since the number of registered varieties increases linearly over time, and because of this it is impossible to check efficiently each newly submitted variety against all old varieties, molecular markers will help the registration authorities to know immediately which varieties in the existing catalogue are similar to the newly submitted varieties, and will aid in the planting of suitable controls in the greenhouse (trials) for phenotypic scoring.

Locus	Repeat	Alleles	Size
DCAMCRBSY	(CAA)17	7	143
DCCARACC	(TA)8	6	227
DCDIA30	(TA)7-1	1	163
DCFHT	(ATT)5	1	164
DCGSTA	(T)23-1	1	179
DCMADSBOX	(TA)7	5	136
DCA221	(CT)35	4	155
DCB109	(CA)26	5	124
DCB131	(TG)33	3	228
DCB134	(GT)28	4	193
DCB135	(T)27-2(GT)28	4	194
DCB140	(TG)40	4	174
DCD010	(CTT)24-2	6	184
DCD102	(GA)20(GAA)18	5	163
DCD105	(TCT)26	3	240
DCD217	(AAG)23	2	201
DCD224a	(CTT)10	6	137
DCE210	(AAC)18	6	158
DCE218	(T)65-10	2	173
DCF005	(TGTTTGT)5	3	186
DCF107	(CAAA)15	2	204
DCF115	(A)56-10	2	161

Table1: Characteristics of the STMS loci used for identification of Carnation varieties. The numbers of alleles given were determined on a set of 12 varieties. The first six loci were derived from entries in the EMBL/Genbank databases. The other loci were obtained from the characterisation of a DNA library enriched for microsatellites. Size (in base pairs) was calculated from the sequenced fragment. Repeat indicates the main repetitve stretch, with mismatches of the perfect repeat indicated as '-'.

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