A microsatellite database for carnation

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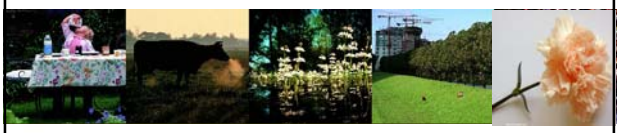

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Carnation project:

- Part of the methods development programme for plant breeder's rights research in the Netherlands
  - Develop, improve and evaluate methods that might be used for future DUS testing
- Content determined by LNV, Plantum and NAKtuinbouw
- Executed by PRI in collaboration with NAKtuinbouw

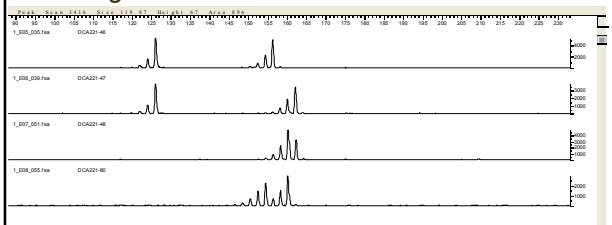
Carnation project:

- Select a set of high quality, highly discriminative STMS markers
- Produce a set of 3 - 4 multiplexes that will facilitate efficient, cost effective genotyping
- Characterize approx. 150 varieties to show possibilities (material provided by the breeders, collected by NAKtuinbouw)

Microsatellite markers for database

- Different requirements compared to breeding
- Robust and reliable markers needed
- Not all markers are suitable > selection of markers

Testing markers



Stutter peaks, duplicated loci, no polymorphism

Selection of microsatellite loci

- Available set of 43 microsatellite loci
- Selection of final loci on test set of 12 varieties
- Out of these 43 microsatellite loci 13 were selected for further testing on whole sample set
  - 5 publicly available markers
  - 8 markers available from PRI

Characteristics of selected markers

Multiplex	Locus	Repeat motif	Number of alleles
1	DCD224 (Fam)	$(CTT)_{10}$	7
	DCD105 (Hex)	$(TCT)_{26}$	9
	DC14 (Ned)	$(GAT)_7$ $(GCC)_7$	5
2	DC16 (Fam)	$(TGA)_{19}$	8
	DCF005 (Hex)	$(TGTTTGT)_3$	8
	DINMADSBOX (Ned)	$(TA)_7$	6[1]
3	DC12 (Fam)	$(AACCT)_3$ $(CGG)_9$	2
	DINCARACC (Hex)	$(TA)_8$	7
	DC09 (Ned)	$(TGA)_{11}$ $(GAT)_5$	10
	DC22 (Fam)*	$(TCT)_{15}$	3
4	DC06 (Fam)	$(AAC)_6$	5
	DC10 (Hex)	$(GCG)_{13-2}$	7
	DC27 (Ned)	$(CGG)_5$	7

Carnation; crop specific properties

- Several species contributed to carnation: *D. caryophyllus* , but also amongst others *D. plumarius, D. caesius, D. chinensis*
- Varieties are diploid, triploid or tetraploid but also aneuploids can be present. Garden varieties may even be hexaploid

Plant material used:

- 12 varieties which were used as reference variety in DUS test of 2005
- 133 varieties provided by 5 breeding companies
  - Original varieties and mutant groups
- 27 varieties were analyzed in duplex

Ploidy level assessment

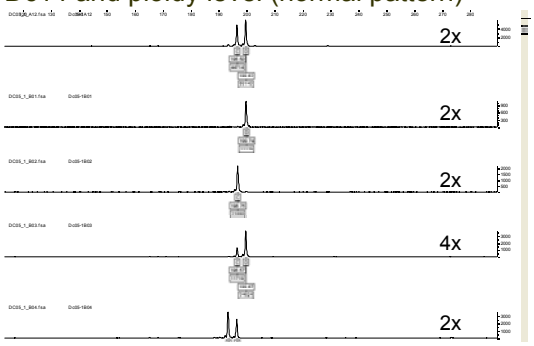
Leave material from all 133 varieties obtained from the breeding companies was analyzed for ploidy level using flow cytometry

- Results:
  - Diploids: 103 (ratio 0.21-0.24)
  - Triploids: 5 (ratio 0.33-0.34)
  - Tetraploids: 25 (0.46-0.52)

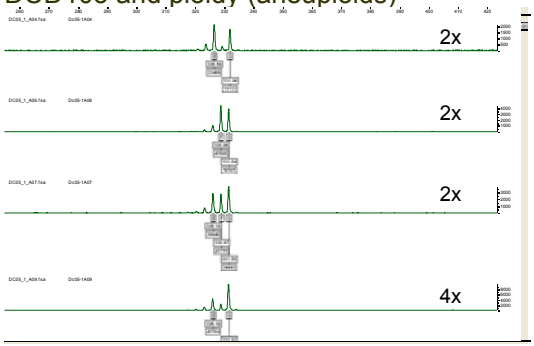
Microsatellite analyses

- Standard multiplex PCR assay, reproducibility test and repeated analyses of failed samples
- Check of samples with unexpected number of alleles
- Dominant scoring of the SSR markers into allelic phenotypes
- Building 1/ 0 excel file
- Similarity matrix (Jaccard) and Tree (UPGMA)

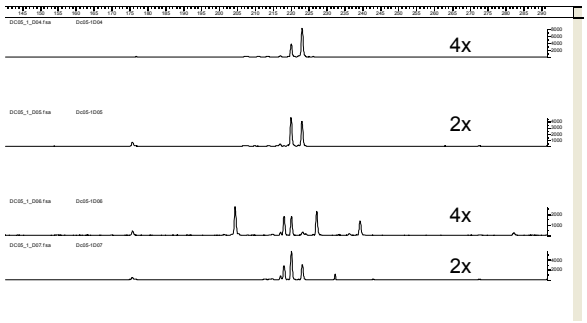
Dc14 and ploidy level (normal pattern)



DCD105 and ploidy (aneuploids)

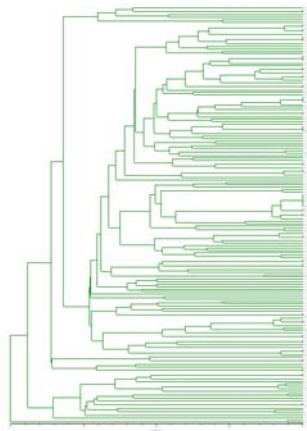


DC09 and ploidy (aneuploids)



Number of allelic phenotypes per locus

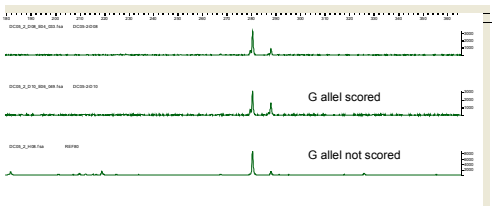
Locus	Number of allelic phenotypes	PIC value based on allelic phenotypes	Frequency of most common allelic phenotype	Number of different alleles in most common allelic phenotype
DCD224	20	0.77	0.42	2
DCD105	17	0.79	0.32	2
DC14	10	0.79	0.35	1
DC16	12	0.52	0.67	1
DCF005	15	0.70	0.45	1
DINMADSBOX	15	0.79	0.35	2
DC12	3	0.63	0.44	2
DC9	17	0.78	0.33	1
DINCARACC	14	0.71	0.49	1
DC22	5	0.42	0.74	1
DC6	9	0.64	0.54	1
DC27	16	0.61	0.55	1
DC10	10	0.34	0.81	1



Threshold for Identification based on duplo's

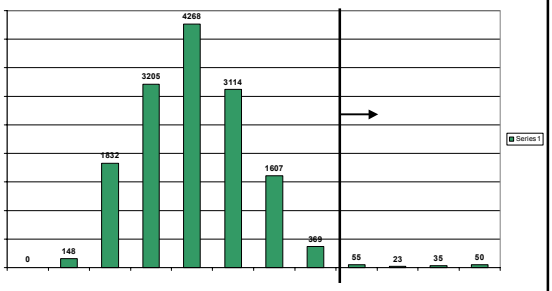
- Differences between duplicated samples
  - Selective amplification of alleles, stutter peaks, constant bands and threshold for peaks in allele designation
- Analysis of duplicated samples (27)
  - Identical patterns: 16
  - One allele difference: 10
  - Two alleles difference: 1

Differential amplification DC10 Barbara mutants



Allele deleted from dbase

Similarity distribution (Jaccard)



Analysis of dataset

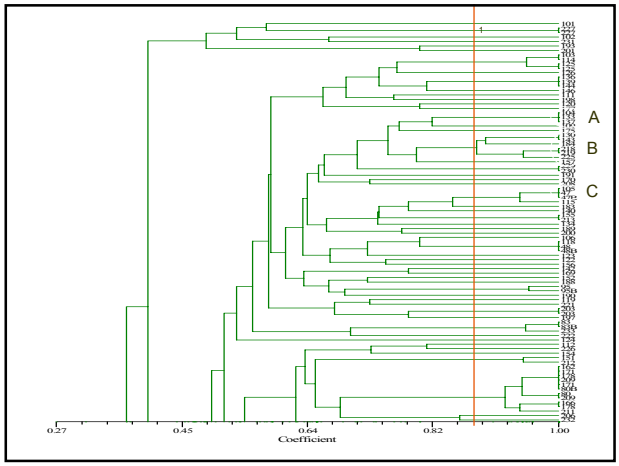
Analysis without prior knowledge of relationships between samples

- Varieties with two or less alleles difference joined into groups
- Analysis of the dataset for the consequences of this threshold

Analysis of dataset (excluding duplicates)

Based on threshold 19 groups were identified:

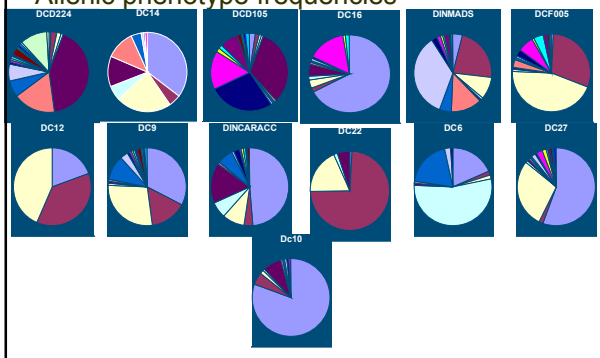
- Six groups consisted of a variety put into dataset twice or were also present in testset
- Ten known mutant groups were identified
- 3 groups that could not be explained with the information available
  - Analysis of new material from varieties gave identical results



Discriminative power of markers

- 172 varieties tested
- 118 potentially different
- 111 unique pattern (94%)
- Pairwise > 99.9 % can be distinguished

Allerlic phenotype frequencies



Discriminative power of markers

- Highest probability of identical profile can be calculated based on frequency of most common phenotype from each marker
  - Upper probability of identical pattern in unrelated samples
    1 : 625552

Conclusions:

- Genetic variation among carnation varieties seems sufficient to distinguish among varieties for identification purposes using the selected markers
- Some varieties showed more than the expected number of alleles. This may be caused by aneuploidy
- To account for technical variation an threshold of two alleles was used.
- With the threshold used 3 groups of varieties were found that could not be explained with the information available

People involved

Plant Research International

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- Danny Esselink
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- Lysbeth Hof
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