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THE USE OF MICROSATELLITES FOR IDENTIFYING PUTATIVE EDV'S IN ROSE

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THE USE OF MICROSATELLITES FOR IDENTIFYING PUTATIVE EDV'S IN ROSE

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Introduction

1. Mutants are a common phenomenon among many ornamental plant species. Usually, such mutants or 'sports' are detected in the multiplication phase. The discoverer can obtain Plant Breeders' Rights for such mutants when they are shown to be distinct from all existing varieties, including the original variety. To protect the interests of the breeder of the original variety the International Union for the Protection of New Varieties of Plants (UPOV) has introduced the concept of 'essentially derived varieties' (EDV). In the UPOV act 1991, mutation is mentioned as one of the possible ways to obtain an EDV. As mutants usually are the result of just very few changes in the genetic makeup of a variety, the genetic similarity between original variety and mutant will be high (very close to 100%). As it is unlikely that molecular marker systems will pick up mutant loci, it can be expected that genetic profiles of initial and derived (mutant) varieties will be identical, except for technical errors. In this paper we present a procedure, based on forensic science, to support decisions with regard to the reversal of the burden of proof in essential derivation disputes. The basic idea is to calculate the probability that a second, putative derived, variety has an identical profile as the first, protected, initial, variety, given an independent breeding history. The principle is illustrated for the detection of mutant varieties in hybrid tea rose using microsatellite markers.

Microsatellite markers

2. Microsatellite markers have the advantage of being co-dominant, highly polymorphic, multi-allelic markers. Depending on germplasm evaluated and marker selected, this generally means that a high degree of discrimination can be reached with just a few markers. Recently, the isolation and characterization of microsatellite markers for rose was described (Esselink *et al* 2003; Vosman *et al* 2001). From this set of markers two of the most informative and best scorable markers (RhB303 and RhO517) were selected.

3. As the rose varieties under study are tetraploid, the use of microsatellites does not give full disclosure of the genotypes. For example, a variety exhibiting the d and e alleles for locus 2, can have either 3 copies of allele d and one copy of allele e, or 2 of each, or one copy of d and 3 of e. Thus, the observations on the microsatellite loci are still in a sort of phenotypic form, where the phenotype consists of a collection of observed allele peaks, without details on the actual allelic composition. Becher *et al* (2000) introduced the term allelic phenotype to describe such a profile of allelic peaks.

4. To thoroughly characterize the RhB303 and RhO517 their allelic phenotypes were determined in 367 hybrid tea varieties (e.g. excluding mutants).

5. For each marker, the probability of finding an identical profile for a putative derived variety given the profile of an initial variety, P, was estimated by the relative frequency with which the locus profile was observed within the collection of 367 varieties. The variance for

this probability of concurrence of profiles for initial and derived varieties was estimated by the binomial variance $\text{var}(\hat{P}) = \frac{\hat{P}(1-\hat{P})}{n}$. Because 367 varieties were included, the variances for individual profile probabilities were small.

To compare the microsatellite loci with each other with respect to discriminative power, we calculated for each locus a diversity measure as follows $J' = \frac{n\log(n) - \sum_{i=1}^{k} f_i \log(f_i)}{n\log(k)}$, with n the number of varieties, k the number of allelic phenotypes per locus, and f_i the frequency for the allelic phenotype i. *J'* takes values between 0 and 1, and becomes larger with more allelic phenotypes per locus, i.e. k larger, and a more uniform distribution of phenotypes.

6. The loci were very similar with respect to the number of allelic phenotypes and the distribution across varieties, as can be seen in table 1.

Table 1: Characterization of the markers RhB303 and RhO517 using of 367 hybrid tea varieties (excluding mutants)

Marker	Number of alleles	Number of allelic phenotypes	J'
RhB303	6	31	0.87
RhO517	5	27	0.90

7. The frequency distribution of the different allelic phenotypes in the 367 hybrid tea varieties shows that both markers have a rather uniform distribution, with different allelic phenotypes present in frequencies between 0 -13% for RhB303 and 0 -10% for RhO517.

Detecting EDVs

8. To evaluate the markers in their ability of detecting putative EDVs, a set of 83 rose varieties was used. These consisted of 12 known mutant series (37 varieties in total; Table 2), a set of 44 red, and 2 yellow rose varieties. Leaf material was obtained from the breeding companies or from the Centre for Genetic Resources the Netherlands (CGN). These 83 varieties were genotyped using the same two markers and were included in the estimation procedure for the profile probabilities.

Table 2: Plant material used from different mutant groups

Mutant groups	Variety identifiers							
Leonidas	g[1]	1	2	3	4	50		
Edith Piaf	g[2]	5	6					
Pretty Woman	g[3]	7	8					
Vivaldi	g[4]	9	10	11	12	14		
Prophyta	g[5]	13	15	16	45	46	47	48	79
Femma	g[6]	17	18	19	20	21	24	65	

Pistache	g[7]	22	23				
Jazz	g[8]	25	26				
Renate	g[9]	27	28	29	30		
Surprise	g[10]	31	32	38			
Lydia	g[11]	34	35	36	37	44	
Frisco	g[12]	39	40	41	42	43	49
?	g[13]	51	70				

9. Individual loci (markers) can be combined to provide a more powerful discrimination of essential derivation and non-essential derivation cases. When loci are independent, the estimate for the probability of occurrence of a particular multi-locus profile is just the product of the estimated probabilities for individual loci, $\prod_l \hat{P}_l$, where l refers to the locus. The corresponding variance is approx. $Var(\prod_l \hat{P}_l) = (\prod_l P_l)^2 \left(\prod_l \left[1 + \frac{Var(\hat{P}_l)}{P_l^2} \right] - 1 \right)$ (Evett and Weir 1998, Box 5.6).

10. For evaluation of this formula, the population parameters should be replaced by their estimates.

11. We combined the markers RhB303 and RhO517 to estimate probabilities and corresponding 0.95 upper limits for identical profiles on the basis of chance.

12. The upper limit for the highest probability is around 1%. This is a promising finding, because it means that with just 2 well-chosen microsatellite loci, probabilities of identity by chance are so low that it appears justified to ask for a reversal of the burden of proof in essential derivation disputes.

Conclusion

13. Allelic phenotypes observed for microsatellite loci in roses provide a sufficient means for triggering a reversal of the burden of proof in essential derivation disputes with respect to protected varieties and mutants. Observations on a few well-chosen loci result in probabilities for identity by chance that will be rather hard to explain on the basis of independent breeding programs for the protected initial variety and the putatively derived variety. A similar result was obtained for the use of micro satellites in vegetatively propagated seedless grape varieties (Ibañez, J. and Van Eeuwijk, 2003).

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