Essentially Derived Varieties in Ornamentals

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Abstract

The concept of Essentially derived varieties (EDVs) was introduced in the UPOV 1991 act to protect the interests of the breeder of the initial variety. When a variety is considered as an EDV, authorisation for commercial exploitation is needed from the breeder of the initial variety. There is considerable debate going on about which approaches to use for determining essential derivation and also which thresholds should be used in the different plant species. For determining whether a variety should be considered essentially derived from an existing variety two conceptually different approaches can be taken. The first one is based on genetic conformity, the second is more a forensic approach. For the implementation of the EDV concept using the conformity approach it is important that similarities between unrelated varieties can clearly be separated from essentially derived varieties. In the forensic approach the high genetic similarity between original variety and mutant is taken as a starting point. The basic idea is to calculate the probability that a second, putatively derived, variety would have a profile identical to the initial variety, given an independent breeding history. Both approaches will be illustrated and ways to implement the EDV concept will be discussed.

INTRODUCTION

In the international convention for the protection of new varieties of plants of 1991 the concept of 'essentially derived varieties' (EDV) was introduced to protect the interests of the breeder of the initial variety. The International Union for the Protection of new varieties of Plants (UPOV) defines an essentially derived variety in the 1991 act. as follows: a variety shall be deemed to be essentially derived from another variety ("the initial variety") when (i) it is predominantly derived from the initial variety, or from a variety that is itself predominantly derived from the initial variety, while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety, (ii) it is clearly distinguishable from the initial variety, and (iii) except for the differences which result from the act of derivation, it conforms to the initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety (UPOV, 1991). The UPOV (1991) act gives examples of how essentially derived varieties may be obtained. These include the selection of a natural or induced mutant, or of a somaclonal variant, the selection of a variant individual from plants of the initial variety, backcrossing, or transformation by genetic engineering.

Most ornamental species are outbreeders and varieties are maintained vegetatively. Crosses result in diverse plant material that can be easily distinguished, the more so when several founding fathers were used. Repeated backcrossing is not (often) used in ornamental breeding. This reduces the ways that EDVs can be made in ornamentals to somaclonal variant, induced or natural mutants, and GMOs. The latter is currently not an issue in Europe. For both somaclonal variants and mutants the genetic similarity between initial variety and derivatives will be very high (close to 1.0). The morphological differences result from just one or a very few changes in the DNA of the initial variety, which are unlikely to be detected using molecular markers. Mutants are a common phenomenon among many ornamental plant species. Usually, such mutants or 'sports' are discovered

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during the multiplication phase. The discoverer may apply for Plant Breeders Rights (PBR) and when granted exploit these rights. It is up to the breeder of the initial variety to show that the new variety is essentially derived from his initial variety. To establish this, molecular markers may be used. In this paper we explore the possibilities of implementing the EDV concept in ornamentals taking rose as an example.

There are two principally different ways to determine whether a variety may be essentially derived from an initial variety. The first one is based on genetic conformity, the second on a type of forensic approach. For the implementation of the EDV concept using the conformity approach it is important that genetic similarities between unrelated varieties can be clearly separated from essentially derived varieties. In the forensic approach the high genetic similarity between original variety and mutant is taken as a starting point. The basic idea is to calculate the probability that a second, putatively derived, variety would have a profile identical to the initial variety, given an independent breeding history. Both approaches will be illustrated and ways to implement the EDV concept discussed.

CONFORMITY APPROACH

The conformity approach was evaluated by the International Association of Plant Breeders (ASSINSEL) and the International Seed Federation (ISF) on a crop by crop basis. Statistical aspects of identifying putative essentially derived varieties using this approach have been described (Van Eeuwijk and Law, 2004; Van Eeuwijk and Baril, 2001). A clear protocol for the assessment of essential derivation has been produced by ASSINSEL/FIS (2005). In this document a protocol for obtaining threshold levels for EDV using the tail principle is described (see also Van Eeuwijk and Law, 2004). To obtain a threshold level a reference set of varieties is genotyped using molecular markers and pairwise similarities are calculated. This reference set is specific for a certain group of varieties within a species, e.g. the hybrid tea roses. The threshold should be chosen at a pairwise similarity that separates the EDV from the non-EDV pairs. To facilitate this use can be made of materials that are considered clear EDVs such as mutants in the case of roses (Vosman et al., 2004) or clear non-EDV cases such as a BC_1 in the case of *Calluna* (Borchert et al., 2008). *Calluna vulgaris* has a very narrow gene pool, resulting in high genetic similarities between varieties. In the *Calluna* example a reference set was constructed consisting of non-ambiguous EDV and clear non-EDV cases for proof of concept. The non-ambiguous cases were indicated by the breeding companies involved. After testing a threshold was provided by a Dice value of 0.98. This value prevents BC1 individuals from being categorized as EDV.

Vosman et al. (2004) studied the possibilities for introduction of the EDV concept in roses using AFLP. They found that the pair-wise Jaccard similarities between original varieties and derived mutants were close to one (>0.96), whereas all similarities between original varieties were below 0.80, with 75% of the non-mutant similarities even being below 0.50. This enables a clear separation between original varieties and mutants. That the pair-wise Jaccard similarity was not 1 was explained by errors in the scoring of the AFLPs (experimental errors). Based on the results presented in that paper, a safe separation line between EDVs and non-EDVs could be drawn at a Jaccard genetic similarity of 0.95. This threshold of 0.95 would allow some variation in genetic similarities resulting from experimental errors as well as from the existence of original varieties that are closer to each other. The threshold would also fit to the results obtained by Debener et al. (2000) and by De Riek et al. (2001).

FORENSIC APPROACH

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. This will make it possible to assign a putatively derived (mutant) variety to the initial variety, provided that initial varieties all show clearly different genotypes. In addition, when a marker system is used that detects highly polymorphic markers only a small set of

markers will be needed, which would make the approach very affordable. The basic idea is to calculate the probability that a second, putative derived, variety has an identical profile as the first, protected, initial, variety, while assuming an independent breeding history. The principle was first illustrated to identify grapevine varieties (Ibañez, 2001; Ibañez and Van Eeuwijk, 2003). Vosman et al. (2003) used this approach, which is based on forensic science, for the detection of mutant varieties in hybrid tea rose using microsatellite markers. Microsatellite markers have the advantage of being co-dominant, highly polymorphic, multi-allelic markers. Depending on the germplasm evaluated and markers selected, this generally means that a high degree of discrimination can be reached with just a few markers. The isolation and characterization of microsatellite markers for rose was described previously (Esselink et al., 2003; Vosman et al., 2001). 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 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. For each locus, allelic phenotypes were assessed, and the frequency with which the allelic phenotype was observed within the collection of 407 varieties determined (Table 1).

Using the data presented in Table 1 it can be calculated that with just three markers (RhO517, RhAB40 and RhB303) the chance of obtaining identical profiles is around 0.3%. Using all markers this chance is less than 1 in 10^6. This of course is all under the assumption that the markers are unlinked and that within the rose gene pool used by the breeders there is random mating. For the first assumption there is at this moment only partly support as not all markers used have been mapped, but using only markers mapping to different linkage groups the chance of finding identical profiles by chance is already lower than 10^4. The second assumption can be addressed by looking for substructure in the set of varieties used. Such substructure was not found (Smulders et al., 2009).

DISCUSSION AND CONCLUSIONS

Following the introduction of the EDV concept in the UPOV act 1991 several studies have been carried out to provide procedures on how to assess essential derivation and also to set thresholds for it. These have resulted in a paper by ASSINSEL/ISF (2005) describing protocols and experiences obtained in several crop species. In ornamentals only a small number of studies are available and these show that the procedure as proposed by ASSINSEL/ISF (2005) can be applied to these crops as well. As genetic variation within crop species differs widely, the setting of thresholds needs to be done on a crop by crop basis, for which the procedure as developed for lettuce (ASSINSEL/ISF, 2005; Van Eeuwijk and Law, 2004) can be applied.

In ornamentals the most common type of EDV are mutants or ‘sports’. For these the genetic similarity between initial variety and derived variety is very high (almost 1). Molecular marker profiles of initial and derived varieties will therefore be identical. To detect such situations one can also use the forensic approach to establish essential derivation. In this paper it was shown that chances of obtaining identical profiles just by chance are extremely small and 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. The advantage of this approach is that the number of markers that need to be interrogated is small and costs associated to this consequently much lower than in the conformity approach.

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Tables

Table 1. Locus, linkage group, number of alleles, number of allelic phenotypes and frequency of the most common allelic phenotypes, in a set of 407 different hybrid rose varieties (described by Smulders et al., 2009).

Locus	Linkage group	Number of alleles	Number of allelic phenotypes	Frequency of most common allelic phenotype
RhAB15	2	6	28	0.29
RhAB201	5	4	15	0.23
RhAB22	6	7	23	0.31
RhAB40	4	9	79	0.19
RhB303	n.d.	6	37	0.12
RhD221	4	6	32	0.31
RhE2b	6	7	32	0.37
RhEO506	2	6	34	0.20
RhM405	n.d.	4	9	0.4
RhO517	1	5	27	0.12
RhP519	7.	6	32	0.22

n.d.: not determined.