

Plant genetic resources and molecular markers: variety registration in a new era

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Abstract

Variety registration is an important area of plant genetic resource characterization and utilization. Within the European Union, varieties must be included on a National List (NL) of a member state or on the Common Catalogue (a compilation of the NLs of the member states) before seed can be sold. This requires a series of tests and trials which assess if the variety is distinct, uniform and stable (DUS) and if it has sufficient value for cultivation and use (VCU). The same DUS criteria are also used world-wide for the granting of Plant Breeders' Rights (PBR), an intellectual property protection system. Both DUS and VCU are currently assessed primarily using field-based trials. However, the potential use of biochemical and molecular markers for DUS purposes is being actively investigated and such markers could have an important role to play in maintaining the quality and scope of PBR in an environment where the increasing number of countries involved in DUS testing and the number of variety comparisons to be made are causing logistical difficulties. More recently, given firstly the increase in the availability of markers from expressed regions of the genome, and secondly the possibilities raised by detailed DNA sequencing programmes and the association of markers (particularly single nucleotide polymorphisms, SNPs) with specific genes, the prospects for a more molecular approach to VCU are also being discussed. This paper reviews the current situation with regard to the use of molecular markers for DUS and VCU testing and considers future prospects for variety registration in the 21st century, '-omics', era.

Keywords: DUS; molecular markers; variety registration; VCU

Introduction and background

Variety registration can mean different things in different parts of the world. In this paper, it will be taken to mean (i) the processes whereby the marketing of seed of varieties within the member states of the European Union (EU) is controlled, and (ii) the granting of Plant Breeders' Rights (PBR) to breeders of new varieties.

The marketing of seed within the EU is an important function, and as such is subject to a number of controls. These controls are mostly contained within Commission Directives 2002/53/EC, 2002/55/EC, 72/180/EC and 72/168/EC. These say in brief that before seed of most

major agricultural and vegetable varieties can be sold in the EU, the variety must be included on a National List (NL) of a member state or on the Common Catalogue (a compilation of the NLs of the member states). Varieties are examined for eligibility for inclusion on a NL by a series of tests and trials which assess if the variety is distinct, uniform and stable (DUS) and if it has sufficient value for cultivation and use (VCU).

DUS tests for NL purposes must comply with the relevant EC Directives. However, the same DUS criteria that are used for NL testing are also used for the granting of PBR, a world-wide intellectual property protection system designed to assist plant breeders to recoup their investment in the development of new varieties (Mauria, 2000). Whilst not all crops are included in NL testing—for example there are no national lists for

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ornamental species—varieties of all crops are eligible for PBR. Although PBR systems are part of national legislation, there is now an EU-wide protection system operated by the Community Plant Variety Office (CPVO). In addition, there has been much recent world-wide interest in PBR systems generally, due largely to developments within the World Trade Organization (WTO). Countries that are signatories to the WTO also agree to abide by the conditions of TRIPS (trade-related intellectual property systems) and one condition of TRIPS is that an 'effective system' for the protection of new plant varieties exists. WTO members are thus establishing (or have already established) systems for PBR, and most of them are adopting PBR based on the principles established by the International Union for the Protection of New Varieties of Plants (UPOV, see www.upov.int for more information).

UPOV is based in Geneva and currently (February 2003) has more than 50 members. One of the primary functions of UPOV is to co-ordinate the legal and technical aspects of PBR, and to this end, there is a UPOV Convention (latest version 1991). The corner-stone of the UPOV PBR system is that in order to qualify for protection, a newly bred plant variety has to be shown to be new, distinct from others 'of common knowledge' and sufficiently uniform and stable in the characteristics used to demonstrate distinctness. An important activity of UPOV is to produce Guidelines for conducting this DUS testing in a wide range of crops, and there are now UPOV Guidelines for more than 250 different species (agricultural and horticultural, including vegetables, fruits and ornamentals) with others in development. The Guidelines contain lists of characteristics that can be used for DUS testing of a given species, as well as instructions on how to record these characteristics. A list of 'Example Varieties' which demonstrate the various states (or 'UPOV Notes') of a characteristic is also included.

The second part of NL testing—VCU—has no such international co-ordination. According to the EC Directives, the VCU of a variety is satisfactory if its 'qualities ... offer a clear improvement...'. This improvement must be in 'cultivation, or as regards the uses which can be made of the crops or the products derived therefrom...'. The qualities are 'taken as a whole...', so that deficiencies in one area can be compensated by strengths in others.

At the moment, both DUS and VCU for NL purposes are assessed in the EU member states using a series of replicated field tests and trials. Greenhouse and laboratory tests are also used where appropriate. NL testing takes a minimum of two years and requires considerable resources, not least in land. Most VCU assessment requires the growing of replicated variety trials at several

sites at different locations within a country. For example, there are currently seven sites per year for VCU testing of winter oilseed rape in the UK. Again, the recent DUS trials for oilseed rape in the UK alone have in excess of 1400 plots, occupying over 1.5 ha. The characteristics used in both DUS and VCU testing are described in the relevant Directives, as well as in appropriate UPOV Guidelines, CPVO Protocols and national documentation. Typically, for DUS, the characteristics are field-based observations of morphological aspects of the phenotype. For example in oilseed rape, features such as leaf colour, the dentation of the leaf margin, the colour of flower petals, plant height at full flowering, time taken to 10% flowering and cotyledon width/length are typical characteristics. These are a mixture of continuously and discontinuously expressed descriptors, and which after recording, at least in a species such as oilseed rape, are analysed statistically in order to make distinctness decisions. The characteristics are also used to produce the variety description, which is the legal 'definition' of the variety. For VCU, agronomically significant characteristics that are oriented towards improved production, such as yield and specific disease resistances, are very important, along with particular attributes, qualities and value for end-use, e.g. in wheat, resistance to lodging, specific weight, Hagberg Falling Number. Again there has to be statistical analysis of the data from the various trials and, usually, there is some kind of weighting system for the characteristics, to take account of the need to consider the qualities of a variety as a whole.

It is difficult to argue with the view that taken overall, the NL system has been largely successful. It has allowed a series of improved varieties to be utilized by European farmers for many years and has undoubtedly contributed to the success of EU agriculture and the range of food available to EU consumers. PBR, although it has its critics, certainly helps to maintain an active and diverse plant breeding industry, which in turn contributes to the variation available (Koebner *et al.*, 2003). However, the registration of new varieties of cultivated plants of all kinds is not generally regarded as an exercise in plant genetic resource characterization. The main purpose of the process is to deliver or at least make available to the end-user, farmer or grower and ultimately consumer, the benefits of plant breeding through selection and refinement of plant genetic resource(s). By the same token, these registered genetic resources are amongst the best characterized available. The DUS process establishes the uniqueness of the material, ensuring that new varieties are genuinely different, and the VCU process evaluates its utility. The outcome of the integration of these different kinds of data provides a substantial description that allows identification and discrimination of any given variety from others, in a way which

germplasm bank managers would find valuable were their accessions described to a similar degree.

This is an interesting perspective from which to view the variety registration process, because it encourages questioning of the purpose and thus value of such descriptions and the processes which give rise to them. This in turn helps to proscribe the research necessary to improve the systems. For example, VCU testing evaluates the 'utility' of the genetic resource—precisely what this utility is can be modified by examining the process. In the past, for arable crop species at least, this has meant their suitability for some role in intensive production systems. More recently though, policy-makers, partly in response to public and other pressures, have taken an interest in altering the nature of developed agriculture to improve its 'sustainability'. The detailed scientific meaning of this remains to emerge, but clearly appropriate genetic resources can be expected to play a part in its success as a policy (e.g. Humphreys, 2003). The VCU part of variety registration could therefore be a powerful tool for policy-makers in ensuring that the appropriate genetic resources are available to deliver their policy objectives. Again, the success of modern molecular biology and genetics in dissecting plant genetic resources and enabling their improvement calls into question the continuation of the use of phenotypic characteristics as the sole basis of variety description and protection. In addition, there is much interest in reducing the time, resources, land use and hence cost, of variety registration systems. Molecular markers have a significant role to play in all of these areas, and the following considers some of these issues further.

Molecular markers and variety registration

There has been relatively little activity to date on the possible applications of molecular markers in VCU testing. However, the situation in DUS is very different (Cooke and Reeves, 1998; Donini *et al.*, 2000a). We will argue and demonstrate that the two processes can be linked in the future. The objectives are to produce a registration system that is rapid, cost-effective and as objective as possible, using varietal characteristics that can be readily assessed and are not unduly influenced by the environment.

Although as mentioned above DUS testing currently employs mostly visually observable characteristics that are expressions of the phenotype of a variety, there is much interest in the potential uses of molecular markers. To this end, there has been a UPOV Working Party on Biochemical and Molecular Techniques (BMT) since 1993/94. For many years prior to that, the use of biochemical markers for DUS purposes had been pursued,

and progress was finally made (Cooke, 1995; Jones *et al.*, in press). As a result, prescribed biochemical markers (seed proteins, isozymes) are included in the UPOV Guidelines of certain crops (e.g. wheat, barley, maize), albeit as additional characteristics only to be used in particular circumstances. This is not entirely logical, and one of the roles of the BMT is to co-ordinate activities related to the applications of markers to ensure that the maximum benefits are obtained.

At the moment, the formal position of both UPOV and plant breeders' organizations such as the International Seed Federation is that markers may not be used for DUS testing and the granting of PBR. However, it is generally becoming recognized that molecular markers have an important future role in DUS testing and related activities. Most interest within the BMT is now focused on microsatellites (simple sequence repeats, SSRs) as the marker of choice, although there is interest in amplified fragment length polymorphisms (AFLPs) in some quarters (Donini *et al.*, 2000a) and emerging techniques (e.g. SNPs; Koebner *et al.*, 2001; Rafalski, 2002) are kept under review. Notwithstanding the choice of marker, the BMT has raised various issues, including:

- The number of markers that should be used.
- Whether or not the distribution of the markers within the genome is important.
- Whether or not it is important that the markers are mapped.
- Whether or not it is preferable to use markers that relate to expressed regions of the genome.
- Are standardized methods of marker analysis available?
- Are the suggested markers publicly available?
- Whilst it is relatively well documented that markers can discriminate between varieties (and thus might be able to demonstrate 'D'), what about the 'U' and 'S' aspects?
- Would the use of markers inevitably reduce the 'minimum distance' between distinct varieties?

Clearly many of these questions are also of relevance to plant genetic resource characterization more generally. The answers to some of them are now beginning to emerge. For instance, a recently completed EU-funded project produced a tested and standardized protocol for the analysis of an agreed set of SSRs that could be used in wheat and tomato varieties for identification and related applications (Bredemeijer *et al.*, 2002; Röder *et al.*, 2002). This project also produced a database containing SSR profiles at *ca* 20 loci of 500 varieties of each crop. The SSRs could be reliably analysed using a range of detection platforms, and procedures were put in place to assure the quality of data production.

Furthermore, information about the uniformity of varieties with respect to SSRs was obtained (e.g. Fig. 1), which has been complemented by more recent and ongoing research (Cooke *et al.*, in press). The approaches taken within this project, and the lessons learned, were an important step forward with regard to the use of markers for DUS testing and for genetic resource evaluation more generally, demonstrating that centrally maintained molecular profile databases can be produced and populated with good quality data from different countries, using a standard protocol. This indicates that molecular markers could begin to address some of the difficulties associated with existing variety testing, e.g. the limited extent of the reference collections that are used, which in turn is of relevance to the analysis of large germplasm collections.

There is emerging a general view that whilst there may be no absolute requirement that markers are mapped and distributed as evenly as possible throughout the genome (and certainly no such information is available for the majority of the morphological characteristics currently in use in DUS testing), nevertheless it is probably preferable to use mapped markers, the distribution of which is known. However, this does depend, as does the number of markers required, on how markers will be utilized in DUS testing. For example, it is possible that markers could be used merely as additional characters, in much the same way as storage proteins and isozymes are currently used in some species. It is unlikely that this will happen, since it does not make best use of the potential advantages of molecular markers. Much more probable is that markers will be used to replace certain morphological characters, or to estimate the relationship between varieties by some agreed type of distance measure (Law *et al.*, 1999). In this way, markers could thus be used to

rationalize the reference collections and/or identify closely related varieties before field trials are sown. Again, this may have useful implications for the management of germplasm collections.

On this basis, provisional models for the potential use of SSRs in revised DUS testing systems have been proposed, e.g. for roses in the Netherlands and for wheat in the UK (Fig. 2). Other options are also possible.

The likely outcomes of the current debates within UPOV and elsewhere are unclear. However, the discussions are having the interesting effect of making people question the purpose of DUS testing. The paramount question should not be, 'do DNA markers replicate the existing system?'; but rather, 'can DNA markers provide a scientifically more credible system?'. At present, the use of DNA markers is being examined in the context of replicating the existing approaches based on morphological phenotype. No attempt is being made to question whether this morphological 'Gold Standard' was, or is now, anything more than a pragmatic approach to infra-specific taxonomy adopted in the past because no other cost-effective tools were available. If the purpose of DUS testing is simply to find some way by which to declare two varieties different regardless of the biological meaning of that difference, then the current system is entirely satisfactory. If, on the other hand, PBR is a means of rewarding the outcome of a scientifically based plant breeding exercise by establishing an effective 'zone of protection' around a variety, based on relatedness and taking associations between varieties into account, then the system should be based on metrics which reflect these associations (Law *et al.*, 1999). Morphological characteristics and the way in which they are currently applied cannot do this adequately, a point which is emphasized when the concept of the

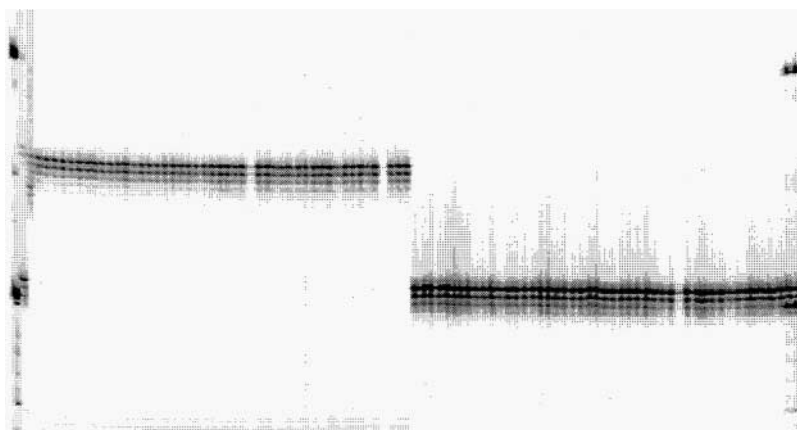


Fig. 1. An example of the use of DNA microsatellites to analyse and distinguish between varieties. Two wheat varieties are shown, with 48 individuals of each, analysed using the marker WMS408. Markers such as this would be ideal for DUS testing purposes, as the different alleles can be easily and reliably scored, there is clear polymorphism between varieties and uniformity within varieties. (Data kindly supplied by Dr Vince Lea and Susan Freeman, NIAB.)

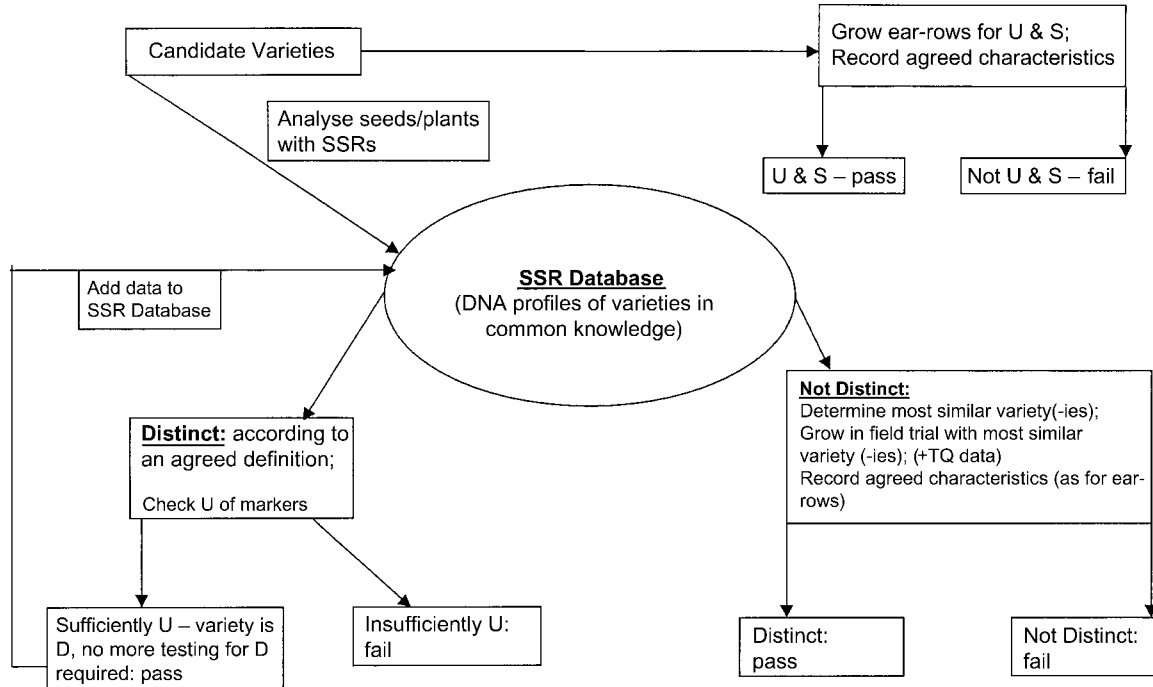


Fig. 2. A possible model for future DUS testing, based on a centralized database of DNA profiles of varieties.

dependent or Essentially Derived Variety (EDV) is incorporated. This was introduced into the UPOV Convention in 1991, and uses the relationships between varieties in order to partition the ownership of rights held therein. A variety can be considered to be essentially derived from another (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 variety in the expression of the essential characteristics that result from the genotypes or combination of genotypes of the initial variety (UPOV Convention, 1991)

Essential derivation is therefore concerned with defining the boundary of ownership around an existing variety. If a new variety were found to fall within this boundary, then it would be deemed to be essentially derived (dependent) from the first variety. If, on the other hand, a new variety exceeded the boundary, then it would be a new (independent) variety (see Fig. 3). Both types of variety are eligible for PBR, but EDVs will

be subject to other licensing arrangements and agreements as well.

Although the concept of the EDV is important to the EU (see Council Regulation 2100/94), to the plant breeding industry, to UPOV, and more widely, its definition is unclear. With the introduction of new breeding techniques such as genetic engineering the ‘distances’ between varieties are arguably narrowing. There is a need to measure these distances accurately and because of this need, the EU has funded a research project (Molecular and Other Markers for the Establishment of Essential Derivation in Crop Plants, MMEDV) which will generate data on distances between varieties and use these data to provide a framework for the establishment of essential derivation in a scientifically credible manner. Output from the project will assist the relevant authorities and the plant breeding industry in all matters relating to essential derivation and will help in the processes of variety testing.

Again there is an obvious relevance to plant genetic resource characterization, where it will be of interest to be able to estimate the relatedness between accessions, the diversity of the collection (distance between accessions) and the extent to which they have been utilized, e.g. in the production of new varieties.

The future—variety registration in the ‘-omics’ era

Most research undertaken to date into the uses of molecular markers for variety registration has utilized

Divergence (distance) from initial variety, X	Minimum		Limit
	----- ----- distance		----- ----->Distance of EDV
Type of variety:	X* = plagiarized X	X' = new, EDV (dependent)	Y = new variety (independent)
Differentiation from X	Insignificant changes	Clearly distinct, but retains 'essential characteristics'	Clearly distinct
Eligibility for PBR	No	Yes (essentially derived)	Yes

Fig. 3. A visual depiction of the essential derivation (ED) concept. To be distinct, a new variety has to exceed the 'minimum distance' threshold (X'), but could still be dependent on the initial variety (X) unless it also exceeds the threshold distance for independence (Y). Research is currently under way to help to define the concept of distance in relation to DUS testing and to provide tools to establish these thresholds.

anonymous markers, that is markers (such as SSRs) derived from parts of the genome that are either non-functional, or whose functions are unknown. This is not a criticism. These markers have been shown to be very useful and can clearly be used, *inter alia*, to distinguish between and identify varieties, and to assess genetic diversity (Law *et al.*, 1998, 1999; Cooke and Reeves, 1998; Donini *et al.*, 2000a, 2000b; Koebner *et al.*, 2003). However, given that the 21st century is becoming the genomics era, with the characterization of key genes and their functions becoming more commonplace, along with the opportunity to study the products of these genes (proteomics) and their functions (metabolomics), it is opportune to speculate how these *-omics* technologies might impact on variety registration in the future.

There are now many sources from which 'functional' markers, or at least markers from regions of the genome that are known to be expressed and therefore can be assumed to have some function(s), can be obtained. These include, for instance, markers linked to specific traits, markers for known genes, those (e.g. some SSRs) derived from expressed sequence tags, and those (primarily SNPs) that are emerging from various projects around the world (Eujayl *et al.*, 2001; Kota *et al.*, 2001; Kanazin *et al.*, 2002; Kantety *et al.*, 2002; Rafalski, 2002; Vigouroux *et al.*, 2002). There are some fairly obvious possible uses for such markers. For instance, a marker for a GM trait could be analysed instead of the trait, if this were difficult or expensive to test. The same could apply to disease resistance genes (for example see Werner *et al.*, 2000 with regard to barley yellow mosaic virus) and potentially also to quality traits such as malting quality in barley (e.g. Marquez-Cedillo *et al.*, 2000). However, with a sufficient number of markers, the applications could be wider, for example assessing those features of a variety that made it more or less suitable for sustainable production or for use in an organic or low input system, or for growing in particular

regions (Humphreys, 2003). Such factors could be part of a newly focused VCU testing system which would be less production-orientated. In addition, this approach might also have the added benefit of being able to assess more agronomically significant characteristics in the DUS context. Traditionally, and for good reasons, DUS testing has not used performance characteristics. The environmental effects on such characteristics are usually large and they are not capable of precise description, thus making variety description impossible. But the use of a marker for performance characteristics is a different thing and would overcome such objections. In turn, this would enable a clear link to be made between the DUS and VCU aspects of registration. Other potential benefits follow on from this linkage. The emphasis of registration would be moved away from the field and into the laboratory and computer, with attendant effects on costs and resource requirements. The effects of registration on, for example, genetic diversity, would be easier to assess, and this could also contribute to the environmental impact assessment of varieties. In addition, a marker-based registration system would be more flexible and able to adapt to changing requirements, be they agricultural, environmental or political. The value of such approaches also has much relevance to the characterization and utilization of plant genetic resources in general. Recognition of this synergy would establish variety registration firmly where it belongs, as a legitimate part of plant genetic resource management and evaluation.

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