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POSSIBLE FUTURE ROLES FOR MOLECULAR TECHNIQUES IN THE IDENTIFICATION AND REGISTRATION OF NEW PLANT CULTIVARS

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POSSIBLE FUTURE ROLES FOR MOLECULAR TECHNIQUES IN THE IDENTIFICATION AND REGISTRATION OF NEW PLANT CULTIVARS

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Abstract

Rapid advances are taking place in genetic studies across the plant sciences and biochemical and molecular methods are now available for the identification and description of plant genotypes and cultivars in a number of crops. However, the identification of natural genotypes or of existing cultivars which are already registered and protected is rather different from the *de novo* registration and granting of Plant Breeders' Rights to a new cultivar and there are important issues to be considered.

At present, the basis for most technical examinations for the grant of a breeder's right usually involves a growing test to determine the morphology of the component plants of a new candidate cultivar, in comparison with appropriate reference cultivars to establish its distinctness and uniformity and stability (DUS). Increasingly, for some crops, in circumstances where there have been problems in determining distinctness using routine morphological characteristics, the use of biochemical characteristics, examined by electrophoresis, has become accepted by UPOV (The International Union for the Protection of New Varieties of Plants) to provide supporting evidence of distinctness, provided the normal uniformity standards are met and there is a good understanding of the genetics involved.

As yet the potential for molecular techniques has not been fully explored and their use is still under discussion at a technical level within UPOV. Therefore, they have not yet been accepted for determination of the distinctness of new cultivars, although, especially in the consideration of essential derivation, it is recognised that they are likely to have an important future role in the determination of genetic distance.

1. Introduction

If the present contribution which molecular biology is making to advances in plant breeding bears full fruit then in future we should begin to see a rather more complex situation taking shape with respect to the protection of plant cultivars and seed marketing.

The intellectual property protection provided for the cultivar as a whole (the UPOV system of Plant Breeders' Rights), with which we are familiar, will be joined by a further degree of patent protection for the various genes which have been incorporated into the cultivar. This, in itself, poses no real problem and is not so different from the situation which currently exists in the automotive or computer industries where commercial products have within them a collection of patents and agreements for various components about which the eventual purchaser knows and cares little.

The 1991 UPOV Convention has provided for this sort of situation. The concept of Essential Derivation allows for fair levels of protection for both the holder of Plant Breeders' Rights of the original cultivar and the breeder of the new cultivar with its inherent, perhaps small, genetic 'improvements' - provided it is distinct, uniform and stable. Also, although outside the UPOV system, the intellectual property considerations for the gene constructs used to 'improve' the cultivar are protectable through patenting.

The more difficult question, however, is how we can move from the current system of examination for cultivar protection based almost exclusively upon the appearance, morphology and phenotype of the cultivar to the types of systems which will be required in this developing situation. The challenge that must be faced on the technical side is harness the new biochemical and molecular techniques to be able to clearly identify the new cultivar and determine whether the DUS and other conditions required for its protection, as set out in Articles 1,7 and 14 of the UPOV Convention, have been met.

2. Genotype, phenotype and expression

In considering the technical aspects of examining cultivars for their eligibility for the award of Plant Breeders' Rights there are several key words and phrases across Articles 1,7 and 14 of the 1991 UPOV Convention which should be briefly examined: 'genotype' (and phenotype), 'expression of characteristics', 'clearly distinguishable' and 'variety'. To properly consider the meaning of these terms in a cultivar protection context, it is first necessary to examine the relationship between genome, genotype and phenotype. One interpretation of the progression through from the DNA level to the complexity of whole-plant organisation is diagrammatically depicted below:

Interpretation of genome, genotype and phenotype as relevant to cultivar protection

HERITABLE DNA **(NUCLEAR / CYTOPLASMIC)**	**GENOME**
NUCLEOTIDE SEQUENCES **(NUCLEAR / CYTOPLASMIC)**	**GENOTYPE**
PRODUCTS OF GENE EXPRESSION **PROTEINS** **ENZYMES** **BIOSTRUCTURES,** **BIOCOMPOUNDS** **PLANT MORPHOLOGY** **PHYSIOLOGY**	**PHENOTYPE**

To establish the distinctness of a new candidate cultivar (variety), Article 7 of the 1991 Convention only requires it to be **'clearly distinguishable'** from others. The former 1978 Convention, at Article 6(1)(a), made the proviso that the characteristics which would permit a cultivar to be defined and distinguished must be capable of precise recognition and

description. The new 1991 Convention makes no such statement and thus can be interpreted as leaving the way clear for multivariate or genetic distance measurements using a range of different characteristics and techniques.

Distinctness is, of course, also linked to the concept of cultivar (variety) which in Article 1 is, in particular, **'defined by the expression of the characteristics resulting from a given genotype or genotypes'**. On one interpretation of this statement, distinctness would only be able to be shown between cultivars using characteristics known to derive from the expressed parts of the genome, that is, after the level of organisation of DNA into recognisable genes. However, other heritable, and perhaps indirectly expressed areas of the genome, may also be considered to contribute to the definition and essential identity of a cultivar. All such organised areas, expressed either directly or indirectly, can be considered as contributing to the genotype that determines the phenotype. This may be as measurable morphological or physiological traits or as hidden, possibly polygenic, contributors to the overall phenotype in characteristics such as yield, plant height, production of intermediate metabolites or control and organisation of cell function.

The concept of Essential Derivation at Article 14 of the Convention indicates that an essentially derived cultivar (variety) must be **'predominately derived'** from, yet remain clearly distinguishable from, the initial cultivar from which it is derived. Further, however, it must also **'conform to the initial variety in the expression of the essential characteristics….'.** Here, in particular**,** there is a clear opportunity for the application of molecular techniques to the measurement of genetic distance and determination of the degree of 'relatedness' between cultivars

While there are clearly opportunities for the application of biochemical and molecular techniques, the above interpretations- and these are still under debate within UPOV - have implications for the way in which the new techniques might be applied. This is especially relevant to some of the more basic DNA fingerprinting techniques that consider genomic organisational and structural differences without knowledge of their function.

3. Recent technological developments

From the point of view of their registration, the potential ease with which cultivars can be identified in the laboratory using electrophoretic, DNA fingerprinting or gene-probing techniques makes their use potentially very attractive. Identification can be achieved within a few hours using these techniques and characteristics, in contrast to several months or even several growing seasons in the field using conventional morphological or physiological characteristics. There are now a large number of scientific papers covering the use of a range of techniques for the identification of differences between cultivars at the genomic level. These techniques are also being used for various purposes including gene mapping and the study of evolutionary relationships across a wide range of crops. The fact that these laboratory-based characteristics are also generally independent of environmental influence and are therefore particularly amenable to application within descriptive databases is also a great attraction for breeders and testing authorities alike.

In 1993, UPOV, recognising the potential implications of the new biochemical and molecular techniques for cultivar protection, set up a technical group to examine some of the issues involved and this 'Working Group on Biochemical and Molecular Techniques' continues to meet regularly. The Group's role is to consider the various methods available and, with due regard to the principles outlined in the UPOV Convention, consider how these might in future

supplement or replace conventional morphological observations. The present thinking within this Group and within the various UPOV Technical Working Parties is that there may be considerable potential benefits for certain crops if the inherent technical and philosophical difficulties can be overcome.

It is worth reviewing the various types of biochemical and molecular methods now under discussion alongside the position concerning conventional morphological characteristics.

3.1 Morphological observations

There is no doubt that in the past these characteristics have been closest to the way in which the plant propagation and seeds industries consider cultivar identity. However, this may not be the case in the future with the insertion of new genetic components into cultivars. The industry may wish to be sure that certain genes conferring useful agronomic attributes are in the cultivar and may also begin to identify with these.

Part of the problem now facing registration authorities with morphological observations is the history of having in the past granted protection to cultivars on the basis of several different principles. The word 'important' with respect to distinctness in Article 6 of the 1978 UPOV Convention whose definition caused much discussion has now been dropped and we are left simply with 'clearly distinguishable' in Article 7 of the 1991 Convention.

However, problems still remain because of past interpretation of the meaning of 'important' in two different senses. Substantial polygenic differences across major tracts of the genome and small single gene differences have been confused and given equivalent status in the granting of protection. For example, on the one hand 'important' has been used to describe characteristics used only for descriptive and DUS purposes like stipule shape in peas or flower colour in potatoes. On the other hand the commercial importance of certain single gene characteristics as the resistance to *Bremia* in lettuce or variation in flower colour in various ornamentals has also been widely accepted. Other basic and polygenic characteristics such as plant height are also examined across a range of crops and can have importance in both descriptive and commercial contexts.

Thus, while DUS can still be easily, and perhaps most usefully, determined with the morphological characteristics, it will now be difficult to sort out the problems of Essential Derivation and genetic distance using such characteristics alone. It is perhaps in this area that the DNA profiling and gene probing methods may be of considerable potential in the future – possibly used in conjunction with the existing morphological characters.

3.2 Electrophoresis - protein typing

For the various electrophoretic methods available for examining plant proteins or enzymes the situation is not very far removed from that with conventional morphological characteristics. The question of expression is not an issue as these proteins and enzymes are clearly phenotypic.

However, the first adoption by UPOV in 1994 of standardised electrophoretic methods into the Guidelines, for glutenins in wheat and hordeins in barley, came about only after considerable discussion and involved the setting of a very important basic principle. This extended the philosophy in Article 1 of the Convention concerning 'expression of the characteristics resulting from a given genotype….' and linked the application of electrophoresis in cultivar registration to a clear understanding of the genetic basis for the differences obtained on the electrophoretic gels. Thus it was agreed that while gel patterns or

band 'barcodes' might be perfectly satisfactory for simple identification of existing pre-registered cultivars, a proper genetic interpretation of these bands was necessary for the *de novo* registration of new cultivars.

This principle needs to be adhered to if muddled thinking is to be avoided. It provides a useful firm foundation that has already allowed progress with the application of several different electrophoretic methods in the DUS examination of cultivars in a range of crops.

3.3 DNA profiling

There are many definitions across this whole area of technology, but in this paper the term DNA profiling is confined to an examination of the genomic DNA organisation and structure without any interpretative effort. There are now large numbers of scientific papers covering the use of a range of methods such as RFLP, RAPD, AFLP, STMS and other PCR based techniques for various purposes including gene mapping and the study of evolutionary relationships across a range of crops. Most interestingly, many workers are now specifically addressing the problem of identification of cultivars or genotypes using these molecular techniques and systems, many of these outlining potential for use in routine tests for the identification of differences between cultivars at the genomic level.

Such DNA profiling methods have already shown their excellent potential for use in straightforward cultivar and plant identification. Also, from the point of view of cultivar protection, the ease with which cultivars can be identified in the laboratory has attractions for both breeders and testing authorities. Although the costs of individual identifications remain high they are probably no greater than those involved in a full morphological examination and, most significantly for certain crops, can provide a stable and more environmentally independent identity for the cultivar.

However, the fact that most DNA profiling methods do not identify the presence of genes but simply characterise sequences of the genome as a method of cultivar classification means that individual gene expression is not explored and interpretation of the function of the sequence is not provided. Such methods may therefore be inappropriate for use on their own for cultivar registration and protection, except in the provision of supporting information on genetic distance for any decision on distinctness. They may, however, be useful in the consideration of Essential Derivation, provided mapping has been carried out to show good distribution across the genome and allow a robust measurement of genetic distance. These issues are all currently under consideration by UPOV, particularly within the Working Group on Biochemical and Molecular Techniques.

3.4 Gene probing

Taking the philosophy on genetic interpretation which has now been established for electrophoresis, it is possible that some of the DNA profiling methodologies which simply examine genomic structure may not be entirely appropriate for use on their own for plant cultivar protection

However, where the genome is probed specifically for the presence of, or differences in, recognised genes where the genetic interpretation is understood or that have an expression in the phenotype, then a different situation can be considered to exist. With such gene probing, the presence or otherwise of selected genes within a cultivar or differences in the DNA make-up of these genes can be determined. In these circumstances there seems little reason why the results shoult not be used for DUS purposes. Gene mapping can be used to determine whether these traits are well spread across the genome and so able to provide robust

measurements of genetic distance. This is an exciting development and may allow a more sophisticated approach than in current cultivar protection systems. However, certain principles should be maintained and no short-cuts taken with certain non-interpretative methods which amount to no more than a superficial 'bar-coding' of cultivars. This could spoil the exciting potential for progress offered in this area.

There is also the possibility of determining DUS, perhaps in an Essentially Derived situation, on the basis of the insertion of a novel gene into a cultivar. In this area and the associated role of investigation of patent protection for novel genes in different cultivars lie further exciting possibilities offered by new DNA probing technologies

4. Cultivar identification and cultivar registration

There are clearly technical considerations across protein electrophoresis, DNA profiling and gene probing which are common both to their more straightforward use for identification of protected and pre-registered cultivars and to their adoption for registration purposes and granting of protection to new cultivars. These factors include the obvious and more straightforward points such as the need for standardised methodologies, proof of reproducibility between laboratories, agreed interpretation of information from gels, cultivar uniformity and robust measurement of genetic distance.

To move from the use of these techniques for identification to adopt them for registration and cultivar protection may seem to some to be an entirely logical step but there are several additional factors with regard to **cultivar registration** which are not implicated in simple **cultivar identification**. Many of these are technical issues which should be relatively easy to resolve in time but there are also more complex issues involving the relationships between genome, genotype expression and phenotype which still require much detailed discussion. Most significant of all, however, may be consideration of the overall effects, through wholesale use of inappropriate techniques, of reducing or minimum distance between cultivars and the knock-on effects this could have upon cultivar protection. This is an issue that has already stimulated considerable debate between breeders and registration authorities and has yet to be fully resolved.

It is clear that some key examinations for morphological uniformity will probably continue to be essential as it is difficult to establish the necessary firm links between uniformity in protein or genome composition and uniformity in plant morphology. To start with, the new techniques will probably have to be used alongside morphological examinations and, of course, there are cost implications in this. Additional costs would arise, not only in cultivar registration itself, but also later in seed certification in the maintenance and multiplication of the cultivar true to its biochemical or molecular description.

The UPOV philosophy of using electrophoretic characteristics only to provide supporting evidence when morphological characteristics alone have proved insufficient to show the distinctness of a cultivar represents a commonsense approach which could also be taken with molecular techniques. The need for these more complex and expensive examinations is then confined to cases where they represent the only means of establishing distinctness. Thus a commercial decision can be taken by the breeder on whether the market potential of the cultivar warrants the extra cost of registration by sophisticated methodology and indeed the further extra costs which may be incurred during maintenance.

5. Conclusions

There has undoubtedly been a tendency over the years, certainly in some crops where breeding is very active and/or the gene-pool small, for testing authorities to take smaller and smaller morphological differences in certain characteristics into account for distinctness. It is of course desirable that the breeder of an improved cultivar should be able to achieve registration for his innovation. However, the responsibility also rests with the testing authority to provide sufficient protection for existing cultivars. In this context it is significant that the discussions as to whether or not a candidate cultivar is "clearly distinguishable" from an already protected cultivar usually involve a dialogue between the testing authority and the breeder of the new cultivar. The breeder of the existing cultivar, whose interests must be protected, is usually totally unaware of any possible infringement of his right. I believe that testing authorities must therefore take a conservative line in the examination of claimed innovation and take care not to erode the intellectual property protection of existing varieties when striving to reward genuine new innovation. This is a difficult balance that could be upset by an over-eager embracing of molecular techniques. This is especially so if the view is allowed to gain favour that the Essential Derivation concept will in future allow a more liberal interpretation of distinctness thresholds.

Plant Breeders' Rights is only of value to the breeder, who, after all, largely pays for the system, if it provides him with a realistic protection of his innovation for a long enough period during which to obtain reasonable recompense, including profit, for his R&D expenditure in the breeding of the cultivar. The concept of "minimum distance", with all its attendant problems, therefore remains an issue for continuing debate and I personally believe that distinctness should not be reduced simply to differences in a few nucleotide base-pairs. Care must be taken that the proliferation of techniques and methods that can identify variation within existing cultivars and reveal quite small differences at either the DNA, genotype or phenotype level does not lead to a situation where the term "clearly distinguishable" becomes devalued.

It is also important that confusion does not arise from too academic a consideration of the issue of the "so-called" expressed - and non-expressed parts of the genome. What is important is that anything identified on a gel should have a clearly understood genetic basis before being used to establish distinctness. Cultivar uniformity and stability must also be established for the trait involved.

The best way to achieve a balance between protecting innovation and yet allowing the opportunity for further advancement would be for some consideration of minimum (genetic) distance to be included in the determination of whether cultivars are distinct, although this may not be legally defensible within the UPOV Convention which only requires a new candidate cultivar to be "clearly distinguishable. Minimum distance should be considered as a minimum difference in or change required in the total genotype of one protected cultivar before protection can be granted to another similar cultivar. ". However, rather than being subjective and inconsistent across characteristics and crops, as at present, this may in future have to be given a more formal statistical identity. It probably cannot, however, be considered simply in terms of quantitative genetics because of the differing economic importance of certain resulting phenotypic differences, for example, disease resistance genes. Over the past thirty years since the signing of the first UPOV Convention in 1961 we have, using phenotypic distinctness, continued to allow both polygenic and single gene characteristics to confer distinctness upon cultivars depending upon the perceived 'importance' of the characteristic. History cannot be re-written and we are therefore stuck with this anomaly.

Molecular biology can be of tremendous assistance in the evaluation of genetic distance or minimum distance between cultivars. This potential must not be wasted by shortsighted adoption of methods that simply examine band pattern differences on gels without considering genetic interpretation, gene expression or the robustness of the genetic distance measurements produced. To this end more development work is needed in the mapping of the markers probed for in various crops to ensure a good independence across the genome. This must be fully researched before we step into widespread application of molecular techniques for DUS determination.

Discussions are still ongoing within UPOV on future application of molecular techniques but a personal summary of the possible future applications for these different methodologies, alongside conventional morphological methods, is presented below:

Summary of possible and most appropriate applications of various technologies across intellectual property protection systems for plant cultivars

	Cultivar DUS and Registration	**Cultivar Essential Derivation**	**Gene Patent Protection**
Morphological observations		**?**	**?**
Protein electrophoresis	(1)	**?**	**?**
DNA profiling	**?**	(2)	**X**
Gene probing	(3)	**?**	

(1) Assuming genetic interpretation of gel band patterns is known.
(2) Assuming good distribution of markers across the genome.
(3) Assuming genetic interpretation is known and the traits are uniform within the cultivar

In summary, for **cultivar identification,** when the problem is simply determining whether a given seed sample is of the cultivar stated or even to establish the identity of an unknown sample by comparison with cultivars from an established reference collection, then molecular techniques will provide cost effective and rapid methods. However, this is quite different from the situation in **cultivar registration** where the identity and description of a reputedly novel selection must be established *de novo* and its distinctness, uniformity and stability proven. Here the principles of genetic interpretation of the differences between cultivars and an understanding of the functional role or phenotypic expression of these differences are also important. Molecular techniques that satisfy the above principles should have a significant future role to play in cultivar protection.

The basis for most technical examinations for the grant Plant Breeders' Rights is still a comparison of the morphology of the component plants of a candidate cultivar with appropriate reference cultivars to establish its distinctness and its uniformity and stability. Increasingly, for specific crops, in circumstances where there have been problems in determining distinctness using morphological characteristics, the use of electrophoretic characteristics to provide supporting evidence of distinctness is now becoming accepted, provided there is sufficient within-cultivar uniformity and a good understanding of the genetics involved. The possible use of molecular techniques is still under discussion at a technical level but their use has not yet been accepted within the UPOV system because the full implications of their use for the principles of variety protection are still unclear.

The views expressed in this paper are personal opinions and do not represent a policy statement on behalf of either the UK Testing Authorities or UPOV.

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