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DUS testing for plant variety protection: Some researchable issues

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Abstract

In order to implement effectively Protection of Plant Varieties and Farmers' Rights Act, 2001 testing of new varieties for Distinctiveness (D), Uniformity (U) and Stability (S) is essential. Based on field and laboratory trials along with the most similar variety a new variety is compared for the characteristics which describe the variety. Distinctiveness assessment of a new variety apparently looks easy but actually it is not so particularly in case of crop species maintained /reproduced through often cross pollination and cross pollination owing to presence of plants with varied expression in some characteristics. Similarly, uniformity assessment of a variety ensures its genetic purity. The crop specific DUS test guideline has been developed and followed for plant variety assessment to register a new variety. Further there is enough scope to comprehend the guidelines for the benefit of plant variety examiners. To make the comparison scientific and valid some statistical procedures are used. Plant varieties differ genetically and the differences are expressed in the form of phenotypes (characteristics). The level of difference depends not only on the genetic constitution of the characteristics but also on the sample size, level of confidence, reject numbers etc. These are required to be studied in each plant species keeping in mind the overall variation available among the varieties in a plant species and easiness in the registration process. In view of influence of growing environment on the quantitative characteristics there is need to identify a separate set of example varieties for proper description and assess distinctiveness of new varieties for its protection.

Key words: Distinctiveness, uniformity, plant variety, example variety, PVP

Introduction

The application of Intellectual Property Rights (IPRs) to new varieties of plants is a relatively recent phenomenon. The key objective of Plant Variety Protection (PVP), a form of IPR, is to stimulate plant variety innovations. PVP has become well established in developed countries over the last three decades or so. Till the early 1990s, PVP remained almost exclusively a feature of developed countries. While developing countries recognized the importance of varietal improvement for agricultural productivity growth, they generally relied on research by public sector institutions at the national and international level for the development of new varieties.

Article 27 (3) (b) of the Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPs) deals with plant variety protection and states that "Members shall provide for the protection of plant varieties either by patents or by an effective *sui generis* system or by any combination thereof". Having research and variety development both in the public and private sectors, traditional communities and farmers conserving landraces/folk varieties and conserving plant genetic resources for food, fibre and feed, India has adopted a novel *sui generis* system providing the establishment of an effective system for the protection of plant varieties. The Government of India enacted the legislation on "*Protection of Plant Varieties and Farmers' Rights (PPV & FR)* Act in 2001 and the "*Protection of Plant Varieties and Farmers' Rights* Rules (2003)" was notified (Anonymous, 2004).

Under the Act, a new variety shall be registered if it conforms to the criteria of Novelty, Distinctiveness, Uniformity and Stability (DUS). The new variety must be clearly distinguishable by one or more essential characteristics from any other variety whose existence is a matter of common knowledge at the time when the protection is applied for. The variety is deemed uniform if subject to the variation that may be expected

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from the particular features of its propagation, is sufficiently uniform in its relevant characteristics. The variety is deemed to be stable if its relevant characteristics remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle. However, the Act provides for registration of extant/already available variety(s) under extant categories under which varieties of common knowledge; varieties notified under Seeds Act, 1966 and farmers 'varieties can also be protected.

Accurate morphological and agronomic descriptions of cultivars and varieties have been the basis of tests for DUS within worldwide PVP systems (Smith and Smith, 1989). Indian guidelines have been developed for DUS testing in different crops. Accordingly, the plant morphological characteristics listed in the crop specific DUS guideline are useful for characterizing varieties. There are no empirical studies to decide many of such issues. There is a need to find out the scientific bases for sole reliance on the UPOV guidelines in our national guidelines. There is also a need to redefine our concepts for distinctiveness, and uniformity on a scientific base in open pollinated crop varieties and composites due to inbuilt variability in these entities.

Testing distinctiveness

Study of distinctiveness is necessary for describing a variety with a set of characteristics and to distinguish a new variety from all other known varieties. The PPV&FR Act provides that a variety that is to be registered is to express distinctiveness as compared to other morphologically similar varieties for any essential characteristic. As per the Act, an essential characteristic means such heritable trait of a plant variety which is governed by the expression of one or more genes that contribute to the principal features, performance or value of the plant variety. Uniformity assessment for characteristics within a variety is required to avoid impurity/contamination from other varieties. Since the uniformity of a variety is dependent on a defined description of it, defining a variety for its characteristic state of expression is very important. In absence of a distinct character state, the testing for DUS of a variety loses its strength.

Lack of uniformity in a variety indicates lack of expression/distinctness of a particular character state in a given variety. In case of existence of more than one form of a characteristic in a variety, can the variety be described appropriately? In other words, whether a candidate variety can be considered to be distinct by using a characteristic which is not uniform. The existence of more than one character form/state of any particular variety could arise due to complex nature of the characteristics and the genetic background/ pedigree of the variety, its breeding history, and its method of multiplication. It is highly unexpected in case of purely self-pollinated crops and single cross hybrids but could be possible in open pollinated varieties and hybrids involving more than two parents of cross pollinated species (Thang 2006, unpublished data on maize).

Though the permissible limits of off-types for uniformity assessment in all crops and varietal types in general are available, no mention for a particular variety in which a characteristic has many forms of expression. It may also be possible that the candidate variety is uniform for a set of characteristics but a similar reference variety is not uniform or vice-versa. There could be the following possibilities to deal such situations.

Absence of absolute uniformity in a characteristic and no overlap between the varieties

Example	Candidate variety (X)	Reference variety (Y)
i)	Form A (100%)	Form B (50%):C (50%)
ii)	Form A (50%):B (50%)	Form C (50%)
iii)	Form A (50%):B (50%)	Form C (50%):D (50%)

In i) Form A is 100% present in the candidate variety and it is absent in the reference variety (it is a case of distinctness where alternate form of state of expression is not available: e.g. Blue Rose); and in ii) and iii) absolute uniformity is absent in the candidate variety. Therefore, still the candidate variety is clearly distinguishable in all these situations. Though absolute uniformity is not required in routine DUS characteristics but scope of distinctiveness between two similar varieties may be reduced to a greater extent.

Absence of absolute uniformity in a characteristic but overlap between the varieties

To assess for **sufficient uniformity** within different types of variety(s) e.g. self-pollinated/cross-pollinated/ synthetic/vegetative etc. the approaches namely off-types, and relative tolerance limit are used.

a) Off-types

If a variety is uniform for one (e.g. form A) it would be recognized as distinct from a variety which was uniform for a different form (e.g. form B).

Example	Candidate variety (X)	Reference variety (Y)
i)	Form A	Form A (50%):B (50%)
ii)	Form A (50%):B (50%)	Form A (50%)
iii)	Form A (50%):B (50%)	Form B (50%):C (50%)

In the above cases the candidate and reference variety cannot be considered clearly distinguishable. For example i) certain plants of form B of variety Y would be off-types in variety X but plants of variety in X in Y would always be considered to be the variety. It would not be justified to accept a variety which would not always be considered to be an off-type in another variety. Therefore, it may be proposed that where uniformity is assessed using the concept of off-types, distinctness must only be determined on characteristics for which there is sufficient uniformity in the varieties. It is a general practice. However, the question is if the variety is indeed distinct for, lets say, few characteristics but not uniform in other characteristics: will the variety be registrable?

Development of "uniform" form of a characteristic in a non-uniform existing variety would be desirable in some situations. For example, in case of susceptible type to a greater extent, using purposive breeding and maintenance breeding (normally back cross) the resistant types are developed then the new variety may be considered as an EDV (provided the recurrent genome content exceeds beyond a permissible threshold limit) and in such case uniformity of the characteristic has to be maintained to pass through not only the test of uniformity but for distinctness.

b) Relative tolerance limits

If a variety possesses the characteristics with a range of expression two possible situations may arise.

i) Different range of expression but same mean value:

Case	Candidate variety (X)		Reference variety (Y)	
	Range	Mean	Range	Mean
1	68-82	75	63-87	75
2	63-87	75	68-82	75

In both the examples the candidate variety would not be considered distinctively different from the reference variety as both have the same mean values for the measured characteristic.

ii) Different range of expression and different mean value:

Case	Candidate variety (X)		Reference variety (Y)	
	Range	Mean	Range	Mean
1	66-76	71	68-82	75
2	68-74	71	68-82	75
3	68-80	74	68-82	75

For case 1 it is accepted that X and Y are clearly distinguishable provided the range of expression is within the acceptable limits for relative uniformity. For Case 2, X has been selected entirely from within the variability in Y. Possibly it is an EDV. The candidate variety must be sufficiently uniform if Y is accepted as uniform. However, the key consideration is whether X and Y are clearly distinguishable. In case of not claiming the candidate variety as an EDV, then at least some plants in X required to be proved to be different from at least some plants in Y. It is not an usual test in DUS examination. Example 3 illustrates where variety X and Y would probably not be distinct because the difference would not be sufficient.

Therefore, in cases of meeting uniformity requirement in characteristics, distinctness can be established by different mean values without regarding that the range of expression in one variety is contained entirely within the other.

Testing uniformity

A variety is considered uniform if, subject to the variation that may be expected from the particular features of its propagation, it is sufficiently uniform in its essential characteristics (article 15.3(c) of the PPV & FR Act, 2001). The variation shown by a variety must be as low as possible to permit accurate description and assessment of distinctness of a variety. To be considered uniform and to ensure stability, it is essential that the variety shows limited variation of its essential characteristics. However, a certain tolerance in variation, which varies according to the reproductive system of a variety, is also required. It means that the level of uniformity required for varieties in vegetatively propagated, self-pollinated, often-cross pollinated, cross-pollinated crops, inbred lines, synthetic varieties and hybrid varieties would be different. In self-pollinated and vegetatively propagated varieties, where all the individual plants of a variety are expected to be quite similar, it is possible to assess uniformity by the number of off-type plants. However, in cross-pollinated crop varieties, where the variation within a variety is larger because of its inherent reproductive systems, the individual plants would not be very similar to each other and it is not possible to visualize which plants should be considered as off-types. Therefore, a norm of relative uniformity is used for cross-pollinated varieties in which the basis of assessment is the variation within the candidate variety in comparison to the variation in comparable variety. Or otherwise if the range of variation of a particular characteristic is well within the range normally expressed in the varieties of common knowledge of that particular crop species, the variety may be considered to be uniform.

Assessment of uniformity is based on the number of off-types in the variety for vegetatively propagated and self-pollinated varieties. A off type plant is one that can be clearly distinguished from the all other plants in the variety in the expression of any characteristic that is used in the examination of distinctness. Most of the characteristics of vegetatively propagated and self-pollinated varieties are observed visually. However, measurements of individual plant or parts of plant followed by statistical analysis are done to judge whether or not a plant is an off-type. The acceptance number of off-types tolerated in a variety is usually based on a fixed population standard and acceptance probability. The population standard can be expressed as the percentage of off-types to be accepted if all individuals of the variety could be examined. The probability of correctly accepting a uniform variety is called acceptance probability. Population standard and acceptance probability to be used for assessing the uniformity have been stated in the National DUS Test Guidelines of the respective species (Table 1). In most of the crops acceptance probability of 95% has been suggested. However, population standard varies depending on the features of seed multiplication/propagation of a variety. The number of off-types, should not unless indicated otherwise in the appropriate Test Guideline, exceed the tolerance level for a variety to be eligible for protection. In case of acceptance probability of >95% and population standard of 1%, the maximum allowed off-types in the range of sample size is as below (UPOV, 2016).

Sample size	Maximum number of off-types
≤ 5	0
6-35	1
36-82	2
83-137	3
138-198	4
199-262	5
263-329	6
330-399	7

Keeping the acceptance probability of 95% and varying the population standard (say 0.1, 1, 2, 3, 5% etc.) the maximum number of off-types would be different. A critical study of Table 1 indicates that an appropriate number of off-types are required to be mentioned in each species.

The sample size has an important role not only for making the test scientific and precise but also to keep the cost for testing at minimum. However, the larger the sample size with same acceptance probability, it would tend to have proportionally less probability of Type II errors. On the other hand, small sample size results in high probabilities of accepting non-uniform varieties. The sample size should therefore be chosen to give an acceptably low level of Type II errors. However, small increases in the sample size may not always be advantageous and the largest sample sizes in the range of sample sizes should be used.

Also, it is essential to further study the hybrids of different types in both self and cross- pollinated crop species to reach an appropriate number of off-types harmonizing the genetic purity standards of varieties and hybrid seed being distributed in active commerce.

Therefore, in view of the general understanding on the requirements for testing of uniformity in plant varieties and the issues namely, 1) Character type: qualitative/ pseudo-qualitative/quantitative, 2) Mating system: self-pollinated/cross-pollinated/vegetatively propagated, 3) Type of variety: pure line/open pollinated/hybrid/synthetic/composite and 4) Type of hybrid: single cross/double cross/three-way cross, there is enough scope to relook into the uniformity standards in a given variety, the appropriate sample size and probability level in each crop species. It may be also be appropriate to harmonize the uniformity analysis with the standard followed in genetic purity

Table 1. Sample size and reject number in different population standard and acceptance probability recommended in uniformity assessment of field and vegetable crops as notified by PPV&FR Authority, Govt. of India

Crop	Sample size	Reject number
Population standard (0.1%) and acceptance probability (95%)		
Rice		
Whole plot	1500	4
Single panicle row	50	2
Linseed	100	2
Sesame	100	3
Population standard (0.5%) and acceptance probability (95%)		
Green gram	250	4
Cotton	300	6
Population standard (1%) and acceptance probability (95%)		
Chickpea	100	3
Groundnut	300	4
Maize		
Inbred and single cross hybrid	100	3
Variety and other hybrid	100	6
Pearl millet		
Inbred and single cross hybrid	100	3
Variety and other hybrid	100	6
Wheat		
Whole plot	1000	2
Single panicle rows	100	3
Jute	400	4
Sunflower		
Hybrid and parental line	100	3
Variety	100	5
Sorghum		
Whole plot	100	6
Single ear row	100	6
Population standard (1%) and acceptance probability (95%)		
Potato	120	2
Brinjal	150	4
Tomato	150	4
Okra	180	3
Cauliflower	150	2
Cabbage	150	2
Onion	100	3
Garlic	200	3
Population standard (2%) and acceptance probability (95%)		
Rapeseed (*Brassica rapa*), Indian mustard, Karan Rai (*B. carinata*), Gobhi Sarson (*B. napus*) Parental line	700	10
Population Standard (5%) and acceptance probability (95%)		
Rapeseed (*B. rapa*), Indian mustard, Karan Rai (*B. carinata*), Gobhi Sarson (*B. napus*) variety and hybrid	700	25
Castor		
Hybrid	<15%	
Variety and parental line	< 5%	

analysis of varieties in commercial seed samples.

Example varieties

Example varieties are those varieties that are required to illustrate, show and indicate the state of expression for a particular characteristic, which is otherwise not well known and understood by a plant variety examiner. Usually it is more required in case of characteristics that are influenced by environmental factors. The quantitative characteristics or measured characteristics are more prone to environmental fluctuations during DUS test trials under open field conditions. Usually the set of example varieties are indicated against each characteristic state in each crop based DUS test guideline. There are situations that a given example variety shows variations in its characteristic expression in different agro-ecological conditions. Comparison among varieties in such situations keeping the expression of example variety as reference/ standard one would invite a definite problem in properly describing a candidate variety. Therefore, it is essential to study such characteristics across the agro-ecological situations and identify the varieties that show stable expression in characteristic state in a particular location/zone.

For example, a candidate variety is grown in two locations along with an example variety (Fig. 1). Leaf blade length varies in the two locations for both the candidate and example variety. The measurement of leaf length classifies variety in different states. In the present case, at Location A, the candidate variety is scored as medium with 20 cm leaf blade length while at location B the candidate variety scored as long with 30 cm length of leaf blade if the earlier scale is followed. On the other hand if a suitable example variety is found to respond the varied location factors in location B though the scale is changed the note of

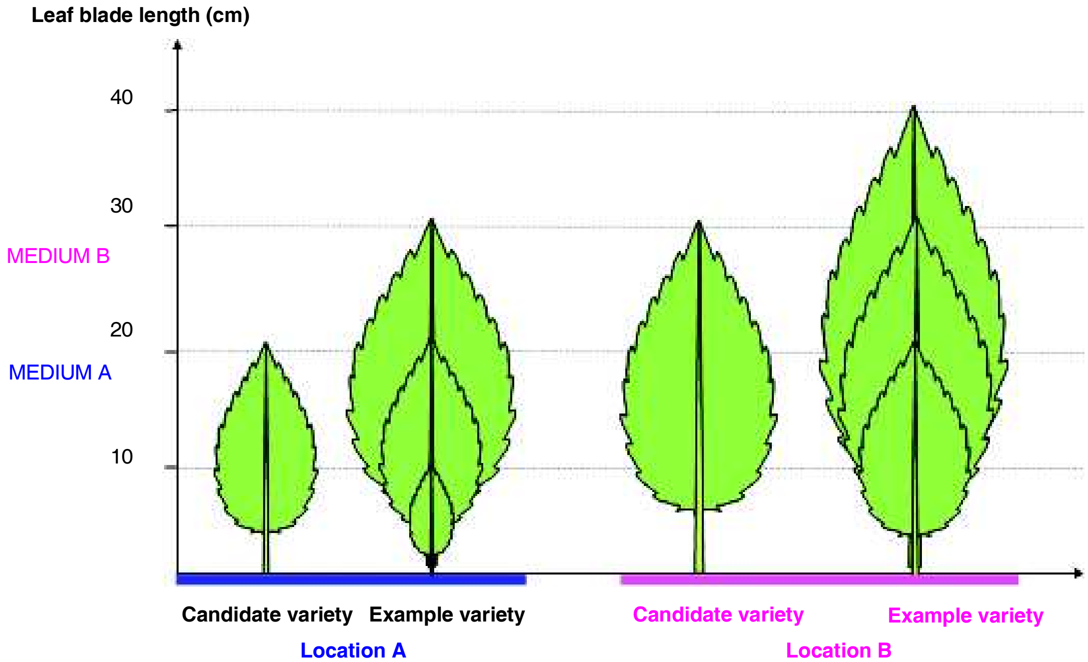

Fig. 1. Example varieties in two location

the candidate variety would not. Therefore, a location specific set of example varieties would solve the problem. It may also be important to think about whether the single set of example variety would be useful to describe all different kinds of varieties available in a particular crop species.

Conclusion

In conclusion, there is enough scope of understanding the various components related to distinctiveness assessment in plant varieties maintained and multiplied following different method. The area of uniformity or homogeneity assessment is equally important considering its importance in varietal purity. The statistical analysis of data related to characteristics is still to be relooked for making the uniformity testing procedure unambiguous. The issue of example varieties in the respective DUS Test Guideline is to be understood in its real spirit. Example varieties may not be necessary in all the characteristics. However, there is further scope of identifying a separate set of example varieties for different agro-ecological areas for proper description of plant varieties. In India now a huge database on varietal characteristics is available in many plant species grown in more than one location for many years. A systematic and critical analysis of such data would answer to many practical questions with respect to DUS testing for an effective plant variety protection system.

Declaration

The authors declare no conflict of interest.

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