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TGP/13 Draft 1
ORIGINAL: English
DATE: November 13, 2002

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

Associated Document
to the
General Introduction to the Examination
of Distinctness, Uniformity and Stability and the
Development of Harmonized Descriptions of New Varieties of Plants (document TG/1/3)

DOCUMENT TGP/13

"GUIDANCE FOR NEW TYPES AND SPECIES"

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to be considered by the

Technical Working Party for Ornamental Plants and Forest Trees (TWO), at its thirty-fifth session to be held in Quito, from November 18 to 22, 2002

Technical Working Party for Fruit Crops (TWF), at its thirty-third session to be held in San Carlos de Bariloche, Argentina, from November 25 to 29, 2002

GENERAL GUIDANCE FOR NEW SPECIES AND TYPES OF VARIETIES

I. INTRODUCTION

1. The 1991 UPOV Convention provides for protection of the whole plant kingdom. Testing authorities, especially in the ornamental crops, are being faced with more and more new candidate varieties that represent the first application for protection within a plant genus or species. Sophisticated genetic techniques such as cell selection, protoplast fusion and widecrossing, along with new intercrossing and multiplication methods are continually becoming available to breeders, often transposed from more advanced breeding or multiplication systems used in other species. As a result, novel variety types are increasingly being introduced, such as complex hybrid variety systems with varying levels of plant-to-plant uniformity, highly uniform micropropagated clonal varieties derived from within sexually reproduced and more variable populations or more variable seed propagated varieties produced in hitherto highly uniform vegetatively propagated species. We now have the situation in several crop species where it is possible to have several different variety types being produced at the one time, each requiring a different standard of uniformity.

2. A testing authority may therefore receive a request to test a candidate variety of a new species or of a novel variety type within an existing species with which they have no previous experience. In this situation, the first step should be to determine whether or not a UPOV test guideline exists for the species or for the new candidate variety type. If it does not, then a search should be made in document TGP/5, "Experience and Cooperation in DUS Testing," for other UPOV member States that have testing experience with the species or the new variety type. If this also has no result, it will be necessary to establish a new testing system to determine the distinctness, uniformity and stability of the candidate variety and to prepare a new test guideline. In the preparation of a new test guideline, guidance on the key issues to be addressed can be obtained from document TGP/7, "Development of Test Guidelines" and it could also be helpful to discuss testing with neighbouring countries. The DUS testing of new species and variety types does not differ in principle from the testing of any variety. The difference is in the level of testing experience and the amount known about that new species or variety type.

3. Before preparation of the new guideline and certainly before testing begins, it is important to learn as much about the new species as possible and to collect relevant information. Possible sources of information include botanical literature, trade and industry publications, trade catalogues, national research institutes, amateur plant collectors and the breeder. The main determinant of the plant breeding and multiplication systems utilized for variety production within any particular plant species is usually the natural mode of reproduction of that species combined with the ability of the plant breeder to control and modify this. The breeder may therefore be the most important and possibly the only source of information and plant material source and it is therefore advisable to develop a good level of cooperation with the breeder.

4. For the granting of the breeder's right, the method of propagation of any variety has implications for the technical examination of its compliance with all the DUS criteria, but in particular for the consideration of uniformity. It is important to examine the naturally occurring reproductive system of the new candidate variety as this will influence the way in

which it will have to be examined to determine whether it is distinct, sufficiently uniform and stable. It is therefore useful to briefly outline the four main types of plant reproductive systems and variety types which result from the different natural methods of reproduction adopted by plants, together with artificial hybridization systems, and to suggest the appropriate levels of uniformity which might reasonably be expected.

II. REPRODUCTIVE SYSTEMS AND VARIETY TYPES

Vegetatively Propagated Varieties – Clones

5. In vegetatively propagated varieties, the variety is produced by cloning a single plant. Once the initial cross or selection has been completed and the desirable phenotype identified, this can then be propagated vegetatively without further substantive genetic change. Vegetative propagation has the advantage for the plant breeding industry that plants propagated in this way, without recourse to the sexual cycle, are normally more uniform and more phenotypically stable than those reproduced sexually. Variety maintenance and multiplication is less complex than other forms of propagation except in certain species where deviations from description can easily result from natural somatic mutation. Indeed, in some crops many new protected varieties continue to arise in this way from spontaneous mutation. In some micropropagation systems there have also been problems with unacceptable levels of somaclonal variation and regeneration protocols have had to be carefully developed to try to avoid this.

6. A vegetatively propagated variety is derived from a single plant. Member plants of that variety should be phenotypically similar and varieties can be expected to exhibit reduced plant-to-plant variability, showing higher levels of uniformity and stability for all characteristics than other methods of propagation.

Self-Fertilized Varieties – Lines

7. For the plant breeding industry the main advantage of self-fertilization is that it confers a much greater degree of phenotypic uniformity in varieties than does cross-fertilization. Varieties of self-fertilized species tend towards homozygosity, at least in their main descriptor characteristics, which are positively selected for uniformity by the plant breeder. They can, as a result, more easily be maintained true to their description across repeated cycles of sexual multiplication as the male and female gametes are of increasingly similar (if not identical) genotypic makeup. Variety multiplication is therefore relatively straightforward provided initial selection and maintenance have been correctly carried out.

8. Self-fertilized varieties tend towards homozygosity and member plants will be phenotypically similar and, certainly similar for their main descriptor characteristics. Varieties should show low plant-to-plant variability and may be expected to have a high level of uniformity and stability.

Cross-fertilized Varieties – Populations

9. From the point of view of the natural environment, the cross-fertilized plant species are those which most easily can employ the variability brought about by sexual reproduction to adapt to environmental or cultural requirements. However, this plasticity and readiness to respond to the pressures of natural selection reflects a lack of uniformity and stability and

means that multiplication and maintenance of cross-fertilized varieties true to their description is much more difficult.

10. Varieties are made up of populations of heterozygous and genetically different plants and are kept within certain descriptive ranges either by continuous re-selection or, more usually, by production of what is termed a synthetic variety. In the case of a synthetic variety, for example in forage grasses, a number of similar and desirable plants are selected and placed in a totally intercrossing environment (polycross). The aim is to produce a population of plants in the F1, which will be in genetic equilibrium and will be able to maintain the essential identity of the variety across a limited number of further generations in an open-pollinated system with a degree of isolation for the seed crop.

11. Cross-fertilized varieties are derived from populations of selected plants. In all subsequent generations the member plants, all of which are heterozygous, will show a range of expression for most characteristics that will be determined by the make-up of the initial founder plants. Varieties will therefore inherently show plant-to-plant variability and the permitted limits of this variation for any variety must be defined by statistical means in comparison with similar types or species. In maintenance and multiplication, such varieties are kept stable and true to description only with considerable care.

Hybrid Varieties

12. A hybrid variety results from the inducement of exclusive cross-fertilization between its component parental lines. This can be brought about by either manual (hand pollination, detasseling), chemical (gametocide) or genetic (self-incompatibility, male sterility) means. These may be used in various controlled crossing systems such as a two or three-way cross, inbred hybrid, top cross or a hybrid between two cross-fertilized varieties. The objective of the breeder is to utilize the heterosis or hybrid vigor which is expressed to most effect in the F1 generation to produce a favorable advantage in yield or some other desirable characteristic for the resultant progeny or hybrid variety. This heterosis is particularly well expressed in the case of crosses between inbred lines but can also be shown to advantage within other systems.

13. The hybrid variety produced from a single cross between two inbred lines is normally highly uniform because, in the F1 generation, all progeny are of the same predetermined, though heterozygous, genotype. The fact that heterozygosity is evident is of no consequence as there is normally no further multiplication beyond the F1 and the variety is maintained by repeatedly returning to a controlled cross of the parental lines. With heterozygous parents, e.g. multiple cross hybrids or hybrids between cross-fertilized varieties, the situation with respect to uniformity is as for cross-fertilized varieties except that, once again, as no further multiplication of the hybrid itself is intended, there is no need to consider any influence of genetic equilibrium upon stability across future generations.

14. Hybrid varieties are produced from a controlled cross between selected parents with the progeny representing the hybrid variety. While heterozygosity is present, phenotypic similarity and plant-to-plant uniformity is very high in the case of single-cross inbreds. In other hybrids, the level of uniformity has to be considered according to the uniformity of the parents. The need to achieve a satisfactory level of uniformity for distinctness purposes must be given careful consideration where a range of hybrid systems exist within the one crop. Acceptance of a very low level of uniformity in complex hybrids could greatly inhibit the scope for distinctness between new candidate varieties. For this reason, characterization of parent lines has been used in some situations in determining the distinctness of hybrid

varieties. Where this approach is not used it will be necessary to give careful consideration to preserving the scope for development of new varieties. While uniformity remains a prerequisite of the hybrid variety it is not considered in respect of stability as multiplication is based upon maintenance of the parental lines and repeated enactment of the controlled crossing scheme to produce the hybrid.

III. TESTING DISTINCTNESS

15. It is necessary to establish that the new candidate variety is distinct from any other varieties of common knowledge. The selection of similar varieties for inclusion in the growing trial for distinctness comparisons with a candidate variety is the usual first step in the DUS examination. In this context, it is important to ensure that the term variety is clearly understood and it is important to keep in mind the definition of a variety in the UPOV Convention. Document TGP/3, "Varieties of Common Knowledge" should also be used to assist in understanding the meaning of the term common knowledge.

16. In completion of the technical questionnaire, the breeder has the first important role to play, providing information on the origin, maintenance and reproduction of the variety and a preliminary description on the most important characteristics, often for use by the testing authority for grouping purposes in advance of field-testing. Information is often provided on the most similar varieties to the candidate variety which can be useful to confirm the descriptive information given for grouping purposes and can also be used to direct the testing authority towards the most appropriate varieties for inclusion in field-testing. It is advisable to check that the information supplied by the breeder about the most similar varieties is accurate. This can be carried out using a preliminary examination of test plant material, or when a growing trial has been completed and the essential descriptive characteristics of the new candidate variety have been determined.

17. The testing of candidate varieties in new species or of new variety types follows the same principles as for the testing of all varieties. The testing of new species and types may differ from other variety testing in the determination of varieties of common knowledge as there may or may not be other similar varieties in existence.

18. It can be useful to examine the breeding origin of the candidate variety to increase information about the new species or new variety type. The origin of a variety may be able to indicate the probability of varieties of common knowledge. The following are four scenarios:

(i) Clonal reproduction from a seedling or mutation, originating from a population in the wild of a species not thought to be in cultivation. It is probable that there are no varieties of common knowledge. The authority should prepare a description and assess uniformity and stability according to the principles for testing the first varieties of a new genus species or variety type.

(ii) Clonal reproduction from a seedling in a population of a species in horticulture. The species may be in commercial cultivation but possibly only exists as a heterogeneous group of seedlings. It is less probable that there are varieties of common knowledge because the species is known to exist only as a heterogeneous group of seedlings. The heterogeneous seedlings do not conform to a single description, are not uniform and cannot be considered as varieties. As a result these seedlings cannot be the subjects of DUS testing because plant-to-plant comparisons can only be made between varieties. If the seedlings have a level of

uniformity in at least the main characteristics and have conformity to a single description, then these seedlings possibly could be treated as varieties for DUS testing.

(iii) Clonal selection for a desired attribute or form, possibly originating from mutation, from a species in horticulture. Other clonal forms may have been selected in the species and exist in commerce as named or unnamed varieties. The candidate variety should be compared with the other varieties existing in commerce and with any other appropriate material. The new variety should be distinct from the other varieties and uniform not only for the distinct characteristic but for all characteristics.

(iv) Seed propagated variety selected for a particular character from a population. Other seed propagated varieties, whether named or unnamed, could exist in commerce. The new seed propagated variety selected for a particular character should be compared with other similar seed propagated varieties, whether named or unnamed. The new variety should be distinct from the other seed propagated varieties and uniformity assessed according to the reproductive system of the variety and the applicable UPOV uniformity method.

19. Each of the above scenarios gives some information about the species and provides an indication as to whether or not varieties of common knowledge could exist. For further information on the determination of distinctness in various crop types, reference should be made to document TGP/9, “Examining Distinctness” and to individual crop Test Guidelines.

IV. TESTING UNIFORMITY

20. For testing authorities, the determination of the level of uniformity required of the first new candidate variety resulting from a new breeding method or representing the first application for protection within a plant species is very important as this variety will normally set the future uniformity standard for its type. However, there is a difficulty as in these circumstances there will be little or no past experience of the level of uniformity to be reasonably expected and no or few comparable varieties will be available for use for reference purposes.

21. While there is a general acceptance that the first variety of a new type must, to some degree, be allowed to set the standard for uniformity, it is important that this is set at an appropriate level. If the standard is set too high then an unreasonably high target might be set for the future which could hinder breeding development. The higher the degree of uniformity for a characteristic or variety, the less scope there is for new variety selection. However, if the uniformity standard is set too high it may become almost unattainable thereby preventing the development of new varieties. The examination system must therefore always strive to find the optimal balance between these two goals.

22. Alternatively, if too low a standard is accepted this could also prevent further breeding progress as the first variety would have encompassed too large a proportion of the possible variability available within the type or species.

23. The main determinant of the plant breeding and multiplication systems utilized for variety production within any particular plant species is the natural mode of reproduction of that species and the ability of the plant breeder to control and modify this. At the early stages of development of the breeding history of most plant species the natural reproductive systems in place have therefore normally dictated what is considered to be sufficient uniformity.

Therefore, in considering the standards to be required for uniformity of a new variety type in an already exploited crop or in a new plant species, it is important that full consideration is given to the influence of the inherent natural reproductive system of the crop species. It is important that efforts are made to understand the natural genetics and reproductive methodology of the type and species to which the new variety belongs to be able to have a realistic expectation of the level of uniformity that might reasonably be expected. This must be taken together with the modifying effects of the breeding and maintenance strategies employed by the breeder which must be studied to be sure that these will result in a true variety entity which will be able to be accurately described and either maintained or repeatedly produced true to its description over a period of years. Guidance can often be obtained from parallel breeding developments in other similar or related crop species.

24. Article 8 of the UPOV Convention uses the phrases "*sufficiently uniform*" and "*subject to the variation which may be expected from the particular features of its propagation,*" and so introduces the concept of an acceptable level of uniformity which is dependent upon the genetic make-up and system of propagation under consideration.

25. With vegetatively propagated, self-fertilized and single cross in-bred hybrid variety types, plant-to-plant uniformity of the phenotype can, within reason, be expected for most characteristics with some additional allowances for the occurrence of inbreds in the hybrids. The assessment of uniformity is normally carried out by the use of population standards. Firstly, the essential state of the variety should be defined either as a whole or across specific descriptor characteristics. Once the variety is clearly defined and described, any individual plants that do not conform to the variety description are identified as off-types. Depending upon the population standard appropriate for the species under examination, the sample size used for the examination of uniformity is determined together with the maximum allowable number of off-types within the sample to allow the variety to meet the uniformity criterion.

26. For the continuously expressed and measured characteristics normally used in cross-fertilized varieties or, when relevant, multiple cross hybrid populations, it is not appropriate to use population standards. Thus, it is not a fixed level of uniformity that is required of such types but a relative uniformity in comparison with other similar varieties. The spread of the normal distribution for each of the measured characteristics, as represented statistically by the standard deviation (SD), is normally employed to determine compliance with the uniformity criterion. For the candidate variety, the distribution of the individual plant data for each characteristic is examined alongside similar data for known and comparable reference varieties of similar type and must fall within tolerances based upon predetermined statistical probability levels.

27. The question then arises of how uniformity can be assessed if the new variety is the first in a new species or of a new type and, as a consequence, there are no other comparable varieties. In these circumstances, uniformity will need to be determined by another method. The level of uniformity required of a candidate variety can sometimes be based upon past experience of what is known to be attainable by the breeding method used and has been shown to have been successful in the maintenance and multiplication of varieties of a similar type in other related species. Therefore, the following approaches may assist:

(a) Consideration of the levels of uniformity achieved in closely related species;

(b) Consideration of the level of natural uniformity in the family;

(c) Consulting with the breeder in order to understand the breeding method;

(d) Considering the possibility of further improving the variety's uniformity, for example whether the breeder could easily remove more off-types;

(d) Based on available knowledge, considering how uniform a cross-pollinated variety from the new species could be.

28. For further information on the setting of uniformity standards in various crop types, reference should be made to document TGP/10, "Examining Uniformity" and to individual crop Test Guidelines.

V. INTERSPECIFIC AND INTERGENERIC VARIETIES

29. Interspecific or intergeneric hybrid varieties are a special type of new species. The DUS testing of these varieties are carried out following the same principles as for any variety. The main difference is in the determination of the character list or test guideline used. It is helpful to first look at the parents.

30. The parents may be well known but the resulting hybrid is new. An example is plumcots (*Prunus salicina* x *P. armeniaca*). For this example, UPOV Test Guidelines exist for both the parents and could be used separately or in combination. The first step in determining whether an existing guideline would be appropriate would be to assess the variety characteristics and determine if the variety is more like one parent than the other. If one parent has strongly influenced the variety characteristics, then that parent's guideline might be used. If a low number of characteristics or states do not correspond to the guideline selected, then the other parent's guideline is also available as a source of possibly more appropriate characteristics to enable a full variety description. It may become necessary to prepare a new national guideline for the hybrid, and potentially a new UPOV guideline if the interspecific or intergeneric hybrid varieties will be grown also in other UPOV member States.

31. Some UPOV Guidelines are prepared to include the testing of all varieties in a genus. When a guideline is prepared for a genus, it is usually most suited for a few species from within that genus where there is testing experience. This should not limit its use and genus based guidelines are suitable for the testing of interspecific varieties.

32. Should the parents have no test guidelines associated with them, it becomes necessary to prepare a new guideline and testing method for the hybrid using the principles in this document and in document TGP/7, "Development of Test Guidelines."

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