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DRAFT

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/11

"EXAMINING STABILITY"

Document prepared by an expert from the European Community

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1. INTRODUCTION

The General Introduction (document TG/1/3) explains the following with regard to Stability:

"7.1 Requirements of the UPOV Convention

Article 6 (1)(d) of the 1961/1972 and 1978 Acts of the UPOV Convention require that a variety "must be stable in its essential characteristics, that is to say, it must remain true to its description after repeated reproduction or propagation or, where the breeder has defined a particular cycle of reproduction or multiplication, at the end of each cycle." Similarly, Article 9 of the 1991 Act of the UPOV Convention requires that a variety "shall be 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."

"7.2 Relevant / Essential Characteristics

The relevant or essential characteristics include at least all characteristics used for the examination of DUS or included in the variety description established at the date of grant of protection of that variety. Therefore, all obvious characteristics may be considered, irrespective of whether they appear in the Test Guidelines or not."

Thus it is clear that in the context of the UPOV Convention, references to Stability and its examination refer to the stability **of the variety itself**, after repeated propagation. It is important to be precise about this because in some crop sectors the word "stability" is used with other, slightly different meanings, most usually in the context of individual plants within a variety which are exhibiting significant mutations in some part of their structure during a single propagation cycle. Within the context of the UPOV Convention this would be treated as a uniformity problem, and the stability of the variety would not be examined further.

2. EXAMINATION OF STABILITY

2.1 Relationship between uniformity and stability

2.1.1 The General Introduction explains the following with regard to the examination of Stability:

"7.3.1.1 In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable. Furthermore, if the variety is not stable, material produced will not conform to the characteristics of the variety, and where the breeder is unable to provide material conforming to the characteristics of the variety, the breeder's right may be cancelled.

"7.3.1.2 Where appropriate, or in cases of doubt, stability may be tested, either by growing a further generation, or by testing a new seed or plant stock to ensure that it exhibits the same characteristics as those shown by the previous material supplied. Further guidance on the examination of stability is considered in document TGP/11, "Examining Stability.""

2.1.2 In stating that, "experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable" it is recognized that uniformity and stability are closely related. However, unless the stability of the variety is

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tested, either by growing a further generation, or by testing a new seed or plant stock, only the uniformity of the variety is assessed on a routine basis. In that respect, it is also recognized that there is a safeguard with respect to stability because, if a variety is not stable, material produced will not conform to the characteristics of the variety, and where the breeder is unable to provide material conforming to the characteristics of the variety, the breeder's right may be cancelled.

2.1.3 Whereas one should always be mindful of how the two issues of uniformity and stability are inter-related, unless the stability of the variety is tested by further propagation, only the uniformity of the variety is assessed on a routine basis.

2.2 Practical aspects to consider for the examination of stability

2.2.1 If the number of off-types in a variety is already clearly outside the permitted tolerances during a single test, the variety will be judged not uniform (as outlined in TGP/10/1 "Examining Uniformity". In this case the stability of the variety is not examined.

2.2.2 For cases which are not clear-cut, in order to be certain whether the stability criterion has been met by a candidate variety, the testing authority should decide whether to continue testing for stability once D and U are established. This further continuation of testing should be conducted either: (i) based on the same sample (but after the propagation cycle) or (ii) by testing a new seed or plant stock to ensure that it exhibits the same characteristic as those shown by the initial plant material supplied. By this stage the testing authority should have already established a draft variety description of the candidate variety, so that in the judgement of stability there is a clear and fixed idea of what constitutes a representative plant of that variety.

2.2.3 The additional (one or more) independent growing periods would not require the utilisation of reference varieties, nor would it require as great an emphasis being placed on the observation of the expression of the relevant characteristics since this has already been established in the "D" and "U" part of the examination. The greater part of the work has in fact to be dedicated to the correct propagation of the candidate variety. This could be undertaken by the examination authority, but in doubtful cases, in order to respect a specific manner of the reproduction or propagation of the sample which is obtained after the subsequent cycle of reproduction or propagation.

2.3 Method of propagation and the testing of stability

2.3.1 The genetic shift of a plant variety in the successive generations is generally affected by its mode of propagation. Therefore, the mode propagation needs to be considered before testing stability. The mode of propagation in plant varieties can be grouped into two broad categories: a) asexual and b) sexual.

Asexually propagated varieties

2.3.2 In asexually (cf. vegetatively) propagated varieties, stability can be determined from the observed uniformity of the relevant characteristics. When the relevant characteristics are found to be sufficiently uniform then they can be deemed to be sufficiently stable as well.

This is ascertained from the fact that there is no recombination phase in the asexual mode of propagation and all plants will eventually breed true and therefore, they should be stable in their successive generations. In asexually propagated varieties, stability is strongly linked with its uniformity and therefore, a separate test for stability generally is not warranted.

2.3.3 This determination will hold good if there are no mutations or soma-clonal variations during the asexual reproduction phase. For example sometimes in variegated varieties, there is a tendency that the "variegated" characteristic reverts back to "normal" after repeated propagation. It is generally determined by the prior knowledge and experience about the characteristics and can be tested by growing the variegated variety through successive asexual reproduction cycles. Stability can be determined by checking that the number of off-types in the repeated propagation is within limits.

2.3.4 Alternatively, a sample of a variety may appear to be uniform, but there are indications that the variety may not be stable. Examples might be where the patterning of the flowers varies within plants, but each plant appears to show the same variation; where there are different types of variegation in the foliage within plants, but each plant exhibits the same variation; or where there are non-significant tiny late mutations [e.g. small flower stripes] but in higher than usual numbers for the species and plant type. In cases of doubt, in these situations the stability of the variety is practically tested by re-propagating the plants for a further test, and checking that [i] the new sample repeats the same within-plant patterning as the original, and [ii] that the variety has not moved in the expression of its overall characteristics.

Sexually propagated varieties

2.3.5 In sexually (cf. seed) propagated crops the situation is different, since varieties produced this way undergo meiotic division. There is a recombination phase of the parental genes, whereupon the male and female gametes fuse together to form the new embryo of the next generation. In the sexual reproduction process, the new embryos undergo genetic recombination depending on the level of allelic similarity or homozygosity of their immediate parents. Hence, the stability of a sexually propagated plant variety is directly linked with its level of homozygosity and is not subject to the issues of within-plant instability to be found with asexually propagated varieties.

2.3.6 Plant breeders often release new varieties from advanced generations such as the F7 or F8 generation. At this level a sufficient level of homozygosity (98 to 99%) is already achieved in the varieties. Theoretically, 100% homozygosity cannot be reached by conventional plant breeding techniques (except for double haploidy); therefore, a small amount of genetic shift is always expected in successive generations of seed propagated crops. This genetic shift is smaller in self-pollinated or nearly self-pollinated varieties and higher in out crossing or open-pollinated varieties.

2.3.7 As a result of the above, a direct test is often required to test stability in sexually reproducing varieties propagated through seeds. The three following scenarios can be envisaged, with the first two being applicable to non-hybrid varieties and the third one for hybrid varieties:

(*a*) Testing stability as part of the comparative trial: Two generations of seeds of the candidate variety are sown along with the other reference varieties in the comparative trial. Ideally, the seeds are from two successive generations, designated as G1 and G2. For

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trialing purposes, G1 or the first generation of the candidate variety is compared against the reference varieties (R1, R2, R3....) to examine its distinctness and uniformity. For testing stability, G2 or the second generation of the candidate is compared against the first generation G1 to establish that there is <u>no difference</u> between them in their <u>relevant characteristics</u>. So the test of stability is basically <u>a test for similarity between both generations</u>. The following diagram will illustrate the situation:



(b) Testing stability in a separate trial: Sometimes it is not possible to plant the second generation of seed at the time of sowing the comparative trial. In that situation the comparative trial is used only for determining distinctness and uniformity. Seeds of the candidate variety is harvested at the end of the trial and planted in the next season along with the original seed of the candidate. The second trial does not include the reference varieties and the only objective is to look at the <u>similarity of the two generations in their relevant characteristics</u>.



The double headed arrow showing similarity between two generations of the candidate to establish stability.

(c) Testing stability in hybrids: Hybrids are produced by crossing two or more parental lines; they cannot breed true because of their heterozygous genetic make-up. Therefore, two generations of hybrids cannot be produced from two successive generations of seeds. All hybrids have a definitive cycle of propagation from their parental lines and according to the different acts of the UPOV conventions (UPOV 1978, Article 6 and UPOV 1991, Article 9) they should be stable at the end of each cycle of propagation. For example, a single cross hybrid (F1) developed in the first year (Year 1) should be compared against the same single cross hybrid (F1) produced in the second year from the same parental lines (P1 and P2). This can be demonstrated by the following diagram:



The double headed arrow showing <u>similarity between the F1 hybrids produced in</u> <u>two different years to establish stability</u>. For double and triple cross hybrids, F1 from two different reproduction cycles (which may take more than one year) should be compared in a similar way. In the particular case of hybrid varieties, when plant material does not conform to the variety description and consequently the stability criterion is questionable, then in addition to an examination of the hybrid variety itself, one can also try to draw a conclusion by assessing the stability of its parent lines if these are made available by the applicant during the DUS test.

2.4 Conclusion

2.4.1 The stability criterion can be concluded on by an assumption based on the uniformity of the variety, or in case of doubt, by testing it directly through the re-propagation of the candidate variety or via one of its subsequent generations.

2.4.2 Once the relevant authority is satisfied that the candidate variety fulfils the stability criterion subsequent to the finalising of the DUS test, then on technical grounds it can be awarded plant breeders' rights.

2.4.3 If the plant material does not conform to the characteristics of the candidate variety after repeated reproduction of propagation then it has to be considered that the variety is not stable and the breeders' rights shall not be granted.

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