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HOMOGENEITY OF VEGETATIVELY PROPAGATED VARIETIES

Paper from the Delegation of the
Netherlands

1. In a letter dated October 9, 1980, addressed to the Office of UPOV, the Delegation of the Netherlands presented a paper on Homogeneity of Vegetatively Propagated Varieties for discussion during the Sixteenth Session of the Technical Committee.
2. That paper is reproduced in the Annex to this document.

(Annex follows)

HOMOGENEITY OF VEGETATIVELY PROPAGATED VARIETIES
paper from the Delegation of the Netherlands

INTRODUCTION

According to Article 6(1)(c) of the Convention, the variety must be sufficiently homogeneous, having regard to the particular features of its sexual reproduction or vegetative propagation.

Information about the meaning of the word "sufficiently" is given on page 5 of the Revised General Introduction to the Guidelines for the Conduct of Tests for Distinctness, Homogeneity and Stability of New Varieties of Plants: acceptable maximum numbers of off-types are given in relation to the size of the sample.

This information is not sufficient to provide us with a clear and sharp borderline between homogeneous and heterogeneous under practical test circumstances. For in the first place it is not defined what precisely is an off-type and in the second place in most of the cases of vegetatively propagated varieties we have only one sample at our disposal and that is the sample sent in with the application by the breeder. This sample however can show a non-uniformity which is not caused or only partly caused by the heterogeneity of the original variety.

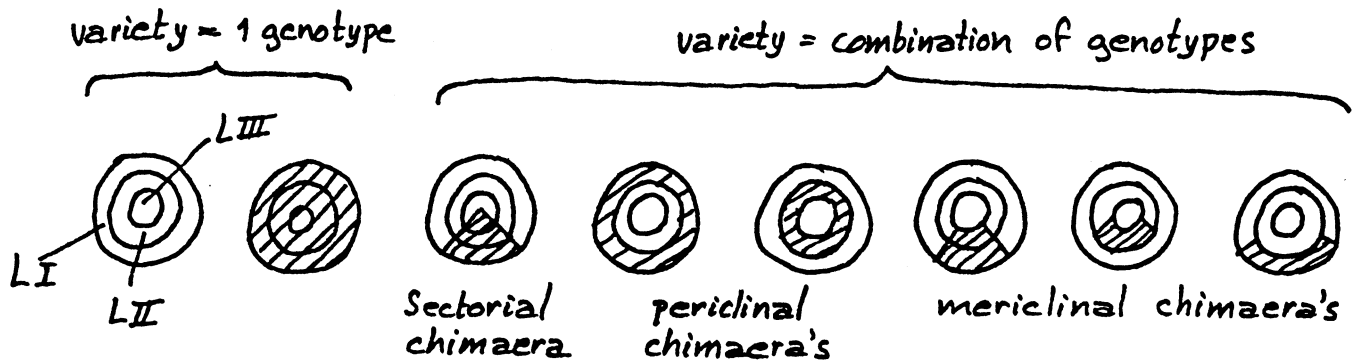
As background information this paper includes a survey of the methods of vegetative propagation and a schematic description of types of variety which may occur. The possible causes of non-uniformity in the application sample are then summarized. Finally a general scheme is given of standards which should be applied to the application sample in view of the assessing of the homogeneity of the variety. These standards should be given quantitatively in the test guidelines for the species concerned.

TYPES OF VARIETIES

In vegetatively propagated varieties a principal division can be made between varieties of which every individual plant consists of one genotype and varieties of which every individual consists of more than one genotype (chimaera). To make this grouping clear it should be understood that today it is generally accepted that the organisation of a shoot apex comprises an LI-, an LII- and an LIII-layer in the initial stage. These layers show mainly anticlinal cell divisions and for

that reason they remain discrete.

Types of varieties can be made schematically visible in the following way:



One type can change into another one by natural or artificial mutation, by rearrangement of the layers, by use of a wrong method of vegetative propagation or by insufficient selection during successive "generations" of vegetative propagation.

The type of the variety is not always self evident, even when the difference between the genotypes involved resides in a characteristic whose states are clearly visibly different.

Examples of clear combinations of different genotypes are varieties with variegated leaves (mostly sectorial or mericlinal chimaera's) or varieties with edged leaves (mostly periclinal chimaera's). It must be clear that the type of the variety in this respect is a part of the identity of the variety. Differences in the arrangement of the different genotypes can change the variety in another one or label a plant as an off-type.

Not all forms of variegation can be attributed to a combination of different genotypes. In the first place there exist species for which variegation is a normal mendelian character, secondly variegation can be caused by environment such as deficiencies or virus diseases. The well-known Rembrandt tulips owe their variegation to virus infection.

It should be recognized that the two different genotypes composing the chimaera can belong to different species or even to different genera as is the case with +Crataegomespilus dardarii = Crataegus monogyna + Mespilus germanica (NB x Crataemespilus grandiflora is a real hybrid between Crataegus oxyacantha and Mespilus germanica).

Finally it should be made clear that even in varieties consisting of one genotype modifications are possible which can be attributed to phenomena such as topophysis or cyclophysis. Examples of topophysis are especially common in conifers. Many prostrate varieties can only be maintained by taking lateral cuttings or grafts instead of terminal ones.

A well-known example of the effect of cyclophysis is the difference which can be observed between juvenil and adult plants (or varieties!) of *Hedera helix*.

METHODS OF PROPAGATION

Frequently there is a narrow connection between the method of propagation and the type of the variety. Often stability of the variety can be maintained only by choosing the right method of propagation.

The use of a wrong method or careless execution of a right method can cause heterogeneity in a variety. For this reason it is useful to summerize here the most important methods of vegetative propagation.

Natural methods. In a majority of cases nature employs generative methods of reproduction; nevertheless we meet also many natural types of vegetative propagation.

Examples are: Division (*Lemna*, succulents)

Shoots from roots or rhizomes (*Tilia*, *Carex*, *Matteucia*)

Daughter bulbs, cormlets, bulb scales (*Liliaceae*, *Amaryllidaceae*)

Bulbils (*Lilium*, *Asplenium*)

Artificial methods. Methods of vegetative propagation developed by man can be divided in two groups, namely the in-vivo methods and the in-vitro methods. The first group is mainly developed from the natural methods used by practical growers; the second group is developed in research laboratories.

In-vivo methods:

Division - Whole plants including their root system or root systems in dormancy are divided in two or more new plants. This method gives a low multiplication factor but no risk of an increased mutation frequency and is for this last reason suitable for the multiplication of chimaeras.

Layering - Rooting of stems or branches still connected to the motherplant, followed by seperation. This is also a method with a low multiplication factor, no risk of an increased mutation frequency and suitable for the multiplication of chimaeras.

Cuttings - Parts of stems, branches, leaves or roots are rooted in a special soil medium. The young plants are growing "on their own roots"- This method leads mostly to a high multiplication factor with almost no risk of an increased mutation frequency. Often periclinal or mericlinal chimaeras can not be propagated by root cuttings.

Grafting - Parts of stems or shoots are grafted on a rootstock; young plants are not growing on their own roots, sometimes later-on they do. There are many methods of grafting; the multiplication factor is usually lower than with propagation by cuttings. In rare cases there is a risk of initiating graftchimaeras. There is nearly no risk of an increased mutation frequency.

Budding - Grafting of a bud on a rootstock. The multiplication factor is high. Perhaps this method could lead to an increase of uncovering periclinal chimaeras.

In-vitro methods:

In-vitro cultures of plant material can for our purposes be divided into two groups: tissue cultures starting from whole organs and tissue cultures starting from parts of organs: explants.

A frequent example of the first case is the culture of meristems from stem tips leading to the formation of shoots from the terminal bud and possible axillary buds. This type of culture is frequently used to obtain virus-free material from virus infected plants but can also be used as a method of propagation, in some species even on a commercial scale.

In principle this method can be used for propagation of chimaeras although the abundance of mutations or the risk of uncovering is increased.

In the second case, where the culture is started from explants (parts of stems, leaves, bulbs, ovules etc.) there are two different ways of further development: either adventitious buds are formed directly from separate cells of the explant (Begonia!) or there is a stage in between, in the form of a callus. In both cases the new plant is derived from unorganized (monocellular or multicellular) matter. This method of propagation is not suitable for the multiplication of chimaeras but fits very well in breeding programs working with mutations initiated by artificial procedures such as radiation.

Per se this method often causes an increased frequency of mutations.

CAUSES OF NON-UNIFORMITY

The requirement for sufficient homogeneity means that the number of off-types in the variety must be restricted. Off-types are plants which are genealogically related to but which deviate from the plants forming the variety.

In practice the off-type will be recognized visually. However there are more ways in which non-uniformity can be caused. The following causes of non-uniformity can be distinguished:

- A. Variation in one or more characters caused solely by differences in the state of health of the plants involved and not by difference between genotypes.

This type of non-uniformity has nothing to do with the homogeneity of the variety but can, if present in the sample submitted, make it difficult or even impossible to obtain a true picture of the variety. For that reason this type of non-uniformity should be prevented by strong requirements about the state of health of the material submitted.

- B. Admixtures with plants which are not directly related in a genealogical way to the variety concerned.

In this case there is no question of a real heterogeneity of the variety. The mixing is usually a mistake made during propagation or just before or during the delivery of the material.

Another cause of admixture can be the supplanting of a graft by its rootstock.

- C. Heterogeneity caused by insufficiently continued selection ("primary" off-types).

In the case of vegetatively propagated plants this usually means that the material has been obtained from clearly different clones or that the variety originated as a mutation in another variety and the new material is still a mixture with the original material in the form of a complex chimaera.

In both cases the application is made too early and should be rejected if the number of off-types exceeds the limit set in the general introduction.

D. Heterogeneity caused by newly appearing mutations ("Secondary" off-types).

This type of heterogeneity is only acceptable to certain limits when it is thought possible to suppress by selection pressure the deviations from the identity aimed at.

In cases where this does not seem possible the application should be rejected on the score of an insufficient stability.

NB 1 !

Conclusion from the preceding subdivision:

Non-uniformity of a sample is caused by deviating plants.

A part of these deviating plants, the off-types, causes the heterogeneity of the variety.

NB 2 !

It is important to realize that off-types cannot only be caused by different states in one or more characters but, in the case of chimaerous varieties, also by quantitative or qualitative differences in the composition of the genotypes involved.

STANDARDS FOR THE SAMPLE

In order to be certain that judgement of non-uniformity of samples submitted, leads to a decision that will be independent of place and time, it is inevitable that precise requirements should be fixed about acceptable maximum numbers of diseased plants, mixed plants and genuine off-types. These numbers should be related to a fixed number of plants (or part of plants) to be submitted. For this reason the following requirements should be laid down in the guidelines concerned:

- A. Type of material to be sent in (cuttings, grafts, tubers, plants)
- B. Number of individuals
- C. Acceptable maximum number of plants failing to satisfy health requirements
- D. Acceptable maximum numbers of admixtures
- E. Acceptable maximum number of off-types caused by insufficient selection during breeding and/or propagation (primary off-types)
- F. Acceptable maximum number of off-types caused by newly appearing mutations (secondary off-types).
- G. Acceptable maximum total number of E + F

When the material submitted is multiplied by the authorities, the progenies of the separate plants should be maintained separately to make it possible to trace deviations observed in the trials to the original plants sent in.

EXAMPLES

	Carnation	Gerbera	Cymbidium
A. (type of material)	cuttings	young plants	budded plants
B. (identity sample)	60	6	2
C. (diseased)	9	0	0
D. (admixtures)	2	1	0
E. (prim.off-types)	2	1	0
F. (sec.off-types)	2	1	0
G. (E + F)	2	1	0

(End of Annex and of
document)