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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL WORKING PARTY FOR FRUIT CROPS

TECHNICAL WORKING PARTY FOR ORNAMENTAL PLANTS AND FOREST TREES

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CRITERIA FOR DETERMINING OFF-TYPE PLANTS

prepared by the Chairman of the
Technical Working Party for Ornamental Plants and Forest Trees,
with annexes provided by experts from France, Germany, Netherlands, New Zealand,
Republic of Korea and the United Kingdom

Background

1. At their thirty-seventh session, held in Hanover, Germany, from July 12 to 16, 2004 and thirty-fifth session, held in Marquardt (Potsdam), Germany from July 19 to 23, 2004, the Technical Working Party for Ornamental Plants and Forest Trees (TWO) and the Technical Working Party for Fruit Crops (TWF), respectively, considered document TWO/37/7-TWF/35/7 "Criteria for determining off-type plants", prepared by the Chairman of the TWO.

2. It was agreed by the TWO and the TWF that the Chairman should produce a draft document seeking to provide guidance on the criteria for determining off-type plants. As a basis for the drafting, information would be provided by experts from Australia (Melia), France (Lavandula), Germany (Regal Pelargonium), New Zealand (Hebe, Phormium) and the United Kingdom (Hebe) by the end of December 2004. The Chairman would also draw on the information provided in document TWO/37/7-TWF/35/7 and the information provided by the experts from the CPVO in document TWO/36/5, as well as other relevant UPOV documents. It was agreed that if a consensus could be reached on such guidance, the guidance should be incorporated as a section within document TGP/10. The TWO and TWF

agreed that it would not be appropriate to consider the development of different uniformity standards for variegated varieties. The TWF agreed that, with respect to fruit crops for which information might be provided, apple would be of particular interest, and experts were invited to send information to Mr. Barnaby by the end of December 2004.

3. The TWO noted the interest of the Technical Working Party on Automation and Computer Programs (TWC) and agreed that the TWC should be invited to consider document TWO/37/7-TWF/35/7 and the comments of the TWO as set out above. At its twenty-third session held from June 13 to 16 in Ottawa, Canada the TWC received a report from the Office of the Union (Office) on the background and contents of document TWO/37/7-TWF/35/7. It was agreed that a further report should be made at the twenty-fourth session of the TWC.

4. In accordance with the request of the TWO and TWF, various sources of information were used as a basis for developing this document. The information received from experts is provided as annexes to this document as follows:

Annex 1: France (Lavandula)
Annex 2: Germany (Regal pelargoniums)
Annex 3: Netherlands (Chimaeras)
Annex 4: New Zealand (Hebes)
Annex 5: Republic of Korea (Poinsettia and *Gymnocalycium mihanovichii*)
Annex 6: Republic of Korea (Pelargonium)
Annex 7: United Kingdom (Hebes)

Identifying Off-type Plants and Their Use in the Determination of Variety Uniformity

Introduction

5. The majority of ornamental varieties are vegetatively propagated and, in general, characteristics are assessed visually. For the determination of uniformity of varieties, individual plants that differ or do not meet the established description of characteristics for that variety are identified. Those differing plants are known as off-types and, according to a statistical basis, an acceptable number of these off-types is permissible, depending on the sample size, using a fixed population standard and acceptance probability (see document TG/1/3 “General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants” (General Introduction), Chapter 6.4.1.3)

6. The population standard component of the assessment of uniformity is relatively well understood and used widely. However, uncertainty and a lack of consistency can arise in the actual identification of the off-type plants. Creating a definitive model for identifying off-types is not possible, considering the very large range of ornamental genera within which examination of Distinctness, Uniformity and Stability (DUS) is required. What may prove helpful is to establish guidance with regard to best practice for the identification of off-type plants and their interpretation. Such guidance would not reduce the need for the DUS expert or examiner to exercise judgement and have a suitable level of experience for the genus concerned.

Determination of Off-Type Plants

7. It is helpful to make reference to the definition of an off-type for visually assessed varieties.

> "A plant is to be considered an off-type if it can be clearly distinguished from the variety in the expression of any characteristic of the whole or part of the plant that is used in the testing of distinctness, taking into consideration the particular features of its propagation." (General Introduction: Chapter 6.4.1.1)

That definition clarifies the importance of:

(a) the expression of any characteristic of the whole or part of the plant;
(b) the method of propagation; and
(c) the standard for distinctness between a candidate variety and any other variety, with respect to the identification of off-types plants in relation to plants of the variety under test

8. The definition in paragraph 7 requires that the whole plant or part of the plant is observed. It may be noted that a particular plant part might not be true-to-type, for example, a single green shoot where all the other shoots are red. The DUS examiner must decide whether one green shoot on a plant with many red shoots is an off-type. For Regal Pelargonium, the presence of non-fully purple upper petals is checked. If non-fully purple petals are present all over the plant and on all plants then this does not indicate a lack of uniformity. The occasional or inconsistent presence of non-fully purple upper petals would indicate questionable uniformity. For a red-bract poinsettia variety, the presence of non-red bract colors are observed. Plants that exhibit any green or white bract coloration are considered off-types, including those that also have some bracts with red coloration. This approach can also be applied to a whole-plant characteristic such as growth habit. When assessing whole-plant characteristics, the expert should be careful not to focus on the individual plant parts. An example could be a variety with a prostrate growth habit, but where some of the shoots are erect. Alternatively, there can be cases where individual plant parts appear true-to-type, but when put together they form a complete part of the plant that is not true-to-type. The small changes present on each individual plant part combine to form a more clearly observable difference. An example can be seen in apple coloration and patterning. The fruit color, color intensity, amount of overcolor and pattern of overcolor observed individually may all appear true-to-type, however, when the whole apple is observed, the overall impression may not be true-to-type. The definition of an off-type implies that any non true-to-type expression of a characteristic could make a plant an off-type. However, the definition clarifies that any off-type plant must be "clearly distinguishable".

9. In order to identify any plant as an off-type plant, that plant should be clearly distinguishable from the plants which form the description of the variety. In theory, the identified off-type plant, being clearly distinguishable from all other plants, could potentially form another variety. It is useful to keep this in mind: when an off-type plant is noted, the examiner needs to ask if it is clearly distinguishable in terms of the determination of distinctness. In many cases, this is a difficult task but the principles for the determination of distinctness should be applied to the identification of off-type plants for the assessment of uniformity.

10. The presence or absence of a non true-to-type expression of a characteristic may be enough to indicate off-type plants. In other cases, the presence or absence alone of an off-type expressions of a characteristic may not be sufficient and the frequency of the off-type expression may also require consideration. If there was only one plant with a green shoot in a variegated variety than that plant would be likely to be an off-type. However, if all plants had at least one green shoot then that may be a feature of the variety. The frequency of green shoots per plant becomes important where most plants have a few green shoots and where the plants with many green shoots are off-types. For *Phormium* (New Zealand Flax), there is large variation in the amount of leaf coloration within plants, usually observed as variable width of vertical banding or striping on the leaf blade. This variation is considered to be a feature of all known varieties and is seen on all plants. Therefore, it is not considered an indication of a uniformity problem. An off-type plant would be recorded if the color pattern changed, such as the complete absence of one or all of the bands. The uniformity of *Phormium* varieties is an example of where the variable expression is stable and is similar for all plants of the variety.

11. Related to the assessment of frequency of off-types is the frequency a particular characteristic is expressed. The laws of probability state that the more often something is observed, the increasing probability that a difference will appear. In practice, a variety that flowers several times in a season is more likely to express an off-type than a variety that only flowers once a season or only produces one flower per season, such as many bulbous crops. The use of population standards requires the counting of off-type plants and not of the number of characteristics with off-type expression. This approach may not be appropriate for crops such as those that only produce one flower per plant, once a year.

THE CANDIDATE VARIETY DESCRIPTION AND EXTERNAL INFLUENCES

12. An accurate and complete description of the variety and the experience and knowledge of the expert are of importance when identifying off-types plants. The variety description can be of assistance in establishing what the candidate looks like and may aid the epxert to judge whether any of the plants present are off-types. With this in mind, it may be good practice to assess uniformity after the description has been prepared in draft or final form.

13. In some instances an accurate variety description can be used to confirm what a variety should look like when plant morphology is altered by an external factor. Graft incompatibility in *Gymnocalycium mihanovichii* (Chin Cactus) can change the color of the scion. First impressions suggest that it is a variety uniformity problem but a description and understanding of the variety and the genus provides a cultural explanation. It may be necessary for the testing center to verify that this is correct. For *Hebe* and other shrubs, color change between leaves exposed to more or less light are often observed. Leaves on the inside of a bush may be less strongly variegated or a different shade of green. This is not necessarily due to variety uniformity but an environmental effect. Another example would be in hydrangea, where inconsistent sepal color across candidate plants may be considered to be an indication of questionable variety uniformity, however another probable explanation could be variation in the media pH per pot. A significant external influence could be the health and hygiene of the growing trial. Infestation by pest and disease could cause plant expression which results in plants appearing to be off-types.

Knowledge of what a variety looks like or supposed to look like is important to avoid confusing mixtures with off-types. To identify a plant as an off-type when that plant can be

identified as another variety may be considered unfair to the applicant. Mistakes do occur and incorrect plants can be supplied or made available for testing in error.

Guide for identifying off-types (for consideration)

1. Every DUS expert or examiner should have a clear view of what the candidate variety looks like. This is assisted by a complete and accurate description.
2. The principles for the determination of distinctness can be applied to determining off-type plants. Could the off-type plant potentially be a distinct variety?
3. Consider the number of plants observed, the number of growing cycles and timing of observations.
4. Consider whether a difference observed is of part of the plant or of the whole of the plant.
5. Consider the nature or type of expression that indicates a possible lack of uniformity.
6. Consider the frequency of the off-type expression.
7. Consider the cause of the off-type expression.
8. Consider any necessary actions to check or verify the assessment. This should include consultation with the breeder.

14. The TWF and TWO are requested to:

(a) comment on the discussion and examples; and

(b) consider whether it would be appropriate to seek to develop guidance on the determination of off-types for inclusion in the relevant TGP documents.

[Annexes follow]

ANNEX 1

FRANCE (LAVANDULA)

Criteria for determining "off type plants"
An example for Lavendula

Varieties from Lavendula genum are examined in GEVES, with a cooperation with New Zealand on behalf of national authorities (F.NL) and OCVV since 1978.

The species concerned are mostly : *L. angustifolia (lavender), L.x burnatii (lavendin), L. stoechas.*

These species are known to be allogamous. The vegetative propagation being easy, the varieties are mostly clones. The seed propagated materials are known to be not uniform (because of the allogamy and the difficulty to self the material). Nevertheless, two seeds propagated varieties have been recently applied and seems to be sufficiently uniform, as populations.

Methodology on how to determine off type plants is linked to the reproduction system of the species, the genetic structure of the variety and the method of propagation (seed or vegetatively propagated).

1. Vegetatively reproduced material: clones

Inside the DUS material, generally rooted cuttings grown in pod, and during the first DUS cycle, we group the plants as:

- conform plants
- doubtful plants
- off types plants

Plants are judged as off types if we observe, on at least one shoot, a vegetative characteristic or a flower color characteristic, which is able to be considered to give clearly distinction to such material. These off types shoots or floral stalks are mutations (generally return to mother plant).

Examples of clear distinct mutations which permit to class the plant as off type.

By experience, cuttings off such shoots give progenies:

- uniform and off type
- or non uniform, because of lack of stability, and off type.

Doubtful plants are examined during a second cycle through rooted cuttings progenies being observed by comparison to conform rooted cuttings progenies. These clones are compared between themselves.

If the doubtful progeny is conform to the conform progenies, the doubtful plant is finally considered as conform.

If the doubtful progeny is judged to be distinct from the conform progenies, the doubtful plant is finally considered as off type.

The characteristic for which we can consider a plant to be doubtful are, generally, such as:

- pilosity of calyx
- plant attitude, plant height
- slight differences in the color of flower organs

Having determined off type plants in the DUS material, we apply UPOV table of tolerance:

- x off types plants
- on the base of y plants observed

In our protocols y = 10.

2. Seed propagated material: populations

Inside the DUS material, coming from sowing, generally twenty plants, we group the plant during the first DUS cycle as:

- conform plants
- doubtful plants
- off type plants,

having a look at the continuous variation of the characteristics – population -.

Plants are judged as off types plants, if we observe, for at least one characteristic, a level of expression which allows the plant to be declared distinct from the population of plants (for qualitative characteristic generally one level is sufficient; quantitative characteristics it has to be checked).

Example: a color of a floral organe, or a slight difference on Floral organ pilosity.

Doubtful plants are examined during a second cycle by rooted cuttings progenies being observed by comparison to rooted cutting conform progenies. These clones are compared between themselves.

If the doubtful progeny is conform to the conform progenies, the doubtful plant is finally considered as conform.

If the doubtful progeny is judged to be distinct from the conform progenies, the doubtful plant is finally considered as off type.

The characteristics for which we can consider a plant to be doubtful are generally:

- pilosity of calyx
- plant attitude, plant height
- slight differences in the color of flower organs

Having determined off type plants in the DUS material, we apply UPOV label of tolerance:

- x off types plants
- on the base of y plants observed

Known protocols y = 10.

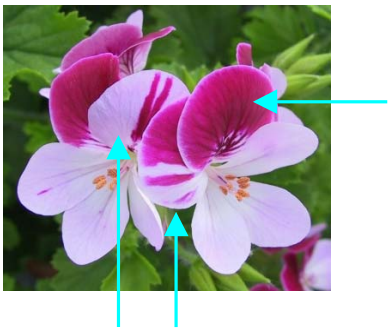
[Annex 2 follows]

ANNEX 2

GERMANY (REGAL PELARGONIUMS)

Criteria for Determining Off-Type Plants

In regal pelargoniums it can happen that on the upper petals the purple colour is sometimes not fully present.



No Off-type

Flowers with upper petals which are not fully purple are distributed all over the plant and occur on every plant of the variety. The number of flowers with upper petals which are not fully purple is approximately the same for every plant of the variety. (In the variety description it is mentioned that some flowers have upper petals which are not fully purple)

Off-type

(a) Flowers with upper petals which are not fully purple occur on only some plants of the variety. There are also plants with no such flowers.

(b) Flowers with upper petals which are not fully purple occur on all plants of the variety, but the number of such flowers is different. Plants with many of such flowers would be off-types, plants with only few of such flowers would be no off-types.

[Annex 3 follows]

ANNEX 3

NETHERLANDS (CHIMAERAS)

Chimaeras: How To Handle In Testing Uniformity?

CONTENT

1. INTRODUCTION

In some vegetative propagated ornamentals (e.g. tulip, rose and carnation), sometimes plants are observed showing a deviation that is expressed in a part of an organ only.
This phenomenon is originating mostly from a chimaerical structure of the plant part concerned.

Examples can be found as parts of flower petals showing a colour different from the ground colour (e.g. some red stripes on a white ground).

The phenomenon can vary in size from a tiny for instance red stripe on a petal to one or more totally red petals or even to a plant with red flowers only.

The question can arise easily whether very small deviations like tiny dots and small stripes could be serious enough to consider the whole plant as an off- type.

Yet another question that could arise whether it is fair to judge a species that is producing one flower only per growing cycle (e.g. tulip) in the same way as perpetual flowering species producing numerous flowers per growing season (e.g. gerbera).

There are not yet any UPOV or CPVO documents giving guidance on this subject in particular.

The aim of this study is to compose a directive on the base of the the current knowledge on chimaerical studies and on the applicable UPOV definitions (UPOV Convention 1991 and UPOV Introduction to the Technical Guidelines (TG1/3 and TGP7/1, 2002-2004) as well as the relevant parts of the reports of the Technical Working Party for Ornamental Plants and Forest Trees TWO).

Attention will be paid to the different features of off-types in Breeders'Rights trials as well as to the different histological structures in chimaeras.

After disussing the molecular background of mutation attention is paid to the methods of propagation of vegetatively propagated varieties in relation to their chimaerical structures.

The UPOV definitions of Variety and Off-type are given against which the extension of chimaerical structures is tested.

Finally after a discussion on a questionnaire during the last TWO meeting some conclusions are made.

In an appendix a step by step scheme is given how to handle in case of chimaerical off-types.

2. HETEROGENEITY IN VEGETATIVELY PROPAGATED PLANTS

In the case of vegetatively propagated material off-types are easy to assess, for variation in all characteristics is very narrow and off-types will manifest themselves clearly as a discontinuity in the material. In practice the off-type will be recognized visually.
If one or more off-types will occur we have to decide with which type(s) of heterogeneity we are concerned.

Admixtures: mixing with other plants which are not directly related in a genetic way to the variety concerned.

A situation that can be encountered quite easily in bulbous crops, during harvest and storage of large numbers of relatively small lots of bulbs mistakes can be made rather easily.

In this case there is no question of a real heterogeneity of the variety as the mixing is mostly a mistake of the breeder made before or during the delivery of the material.

Variation in one or more characteristics caused by differences in the state of health of the plants involved only and not by difference between genotypes.

It can be possible that this type of heterogeneity will not lead to rejection of the variety as far as in the case that enough plants have been left to prepare variety description.

Heterogeneity caused by insufficiently continued selection.

In the case of vegetative propagated plants this mostly means that the material is obtained from clearly different clones or that the variety originated as a mutation in another variety and the new material is still mixed in a chimaerical way with the original material.

In both cases the application is made too early and should be rejected if the number of off-types exceeds a fixed limit.

Heterogeneity caused by newly appearing mutations.

In this case the application should be rejected as well if the number of off-types exceeds the preset limits.

3. TYPES OF VARIETIES RELATED TO THEIR CHIMAERICAL STRUCTURE

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 variety consists of more than one genotype (chimaeras). To make this group clear it should be understood that the organization of a shoot apex comprises an LI-, an LII- and an LIII cell layer in the initial stage. These meristematical layers show mainly anticlinal (=parallel to the surface) cell divisions and for that reason they remain discrete.

Without the occurrence of any mutation the 3 layers will remain unchanged maintaining the same genotype thus showing a homogeneous phenotype.

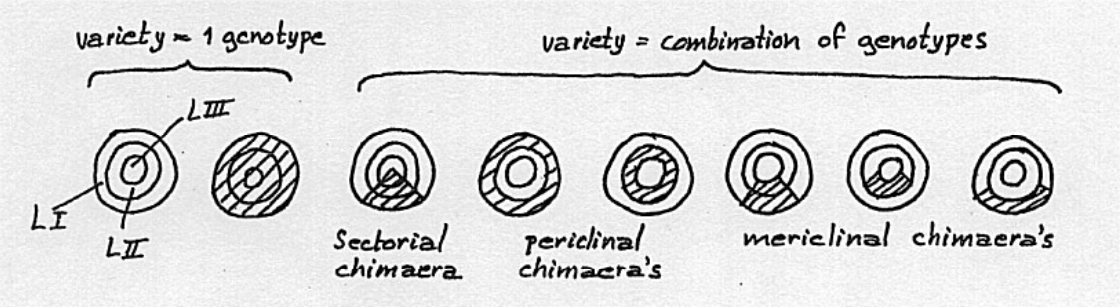
Once an occasional mutation has taken place and division of that particular mutated cell has occurred it could be possible that the effects of the mutation will become visible depending on the influence of the mutation on the phenotype of the plant and on the position of the layer containing the mutated tissue.

However one should realize that most mutations are probably recessive, lethal or are resulting in very subtle changes if any.

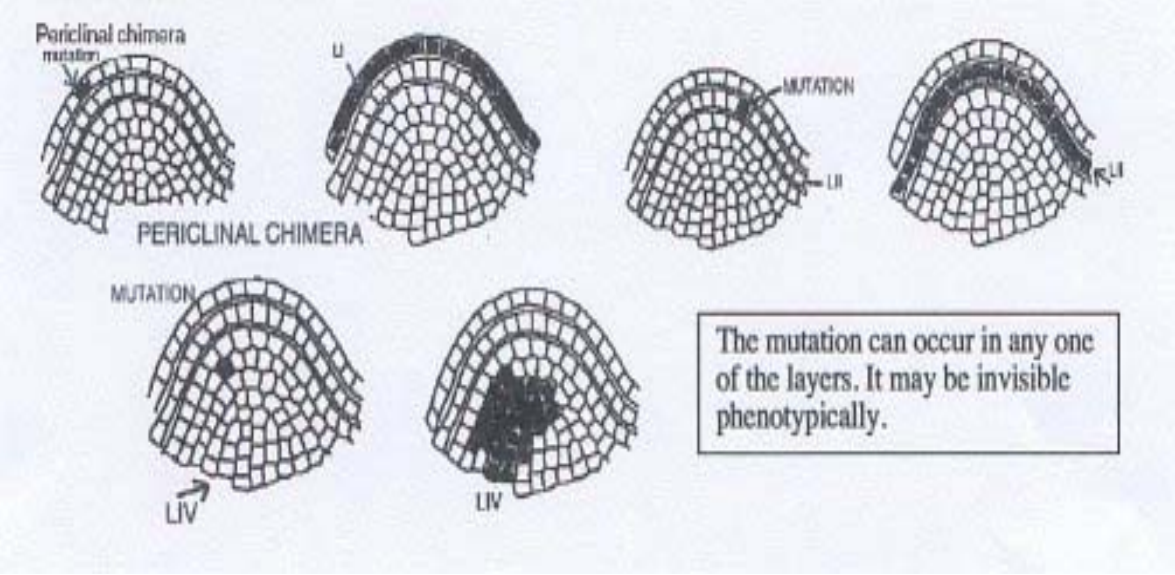
In the case the mutation is sited a the deep layer (LIII) part of the covering layer(s) has to be uncovered to make the mutation visible.

When affecting a striking characteristic like flower colour and if uncovered (if necessary) the effects can become very clear depending on the size of the mutated part.

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



As well as in a histological way:

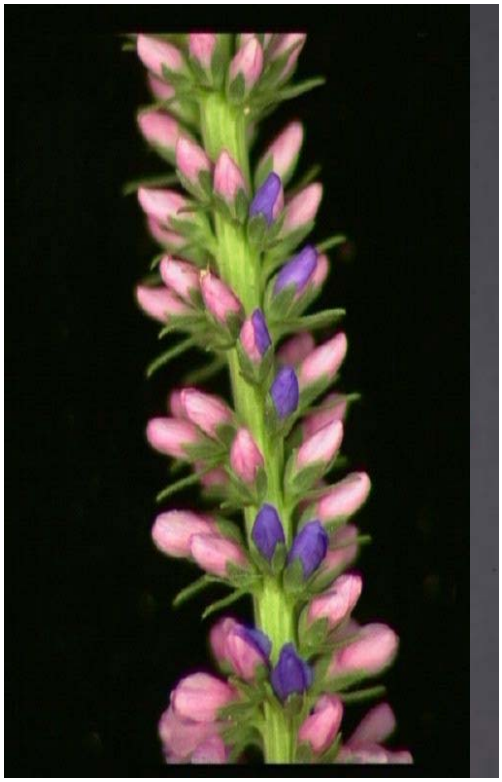


One type can change into another by natural or artificial mutation, by rearrangement of the layers, by use of a wrong method of vegetative propagation especially when using in-vitro propagation techniques.

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

Examples of clear combinations of different genotypes are varieties with variegated leaves or flowers (mostly sectorial or mericlonal chimaeras). In this case a mutation could have been occurred in the L II resulting in a total chlorophyll deficiency of the LII in parts of the leaf. By absence of chlorophyll at the mutated parts the leaves are showing now a white or yellowish color (the LI, epidermis is transparent) at the mutated parts concerned. Varieties with edged leaves can be the result of a chlorophyll deficiency mutation in monocotyls located in the LI resulting in a local absence of chlorophyll in the leaves but now showing white or yellow margins. Not all types of variegation can be attributed to a combination of different genotypes. In the first place there are species for which variegation is a normal Mendelian character, secondly variegation can be caused by the environment such as deficiencies or virus infection .

Differences in the arrangement of the different genotypes can change the variety in another one or label a plant as an off-type.



Probably sectorial (or mericlonal) chimaeras in Veronica and Tulipa

The same Tulipa viewed from above

4. MOLECULAR BACKGROUND OF MUTATIONS (SPORTS)

An attempt is made to explain mutations, without going to deeply into the genetics and biochemistry of it.

First realize that the genetic code exists in the form of long chains of DNA, made up of repeating "letters" of nucleotides: A (Adenine)-T (Thymine)- C (Cytosine)-G (Guanine). These are bound to a sugar based backbone.

The chain is a double helix, with "T" of one side always bonding to an "A" of the other side; "C" always bonding to "G". It can be imagined as looking like a ladder, with the sugar chain making up the vertical portions, and the –A-T- and –C-G- pairs making up the rungs.

In reading the DNA, 3- letter words (codons) form the language of the process. Each codon represents one specific amino acid, which may be incorporated into a protein.

Now to the possible "sports"

Deletion

In this case one of the letters is left out.

This can affect quite a long stretch of the code, since, by deleting a letter, you respell the words that follow, e.g.: Assume a sequence consists of these 4 codons (words); cat tac agt tag. If the first "a" were lost, it becomes ctt aca gtt ag? The gene is now read incorrectly.

Addition

In this case an extra letter is inserted. Tthis mutation may also affect the reading of many codons afterward. The sequence now becomes caG tta cag tta g??

Substitution

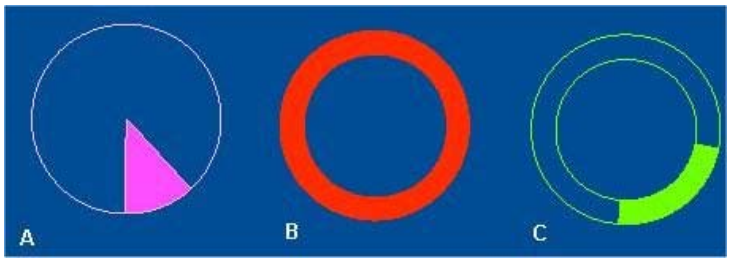
In this case, one letter is traded for another. Only the one codon will be misread; it has no effect on the following codons. But that codon change can remarkably affect the protein produced.

Transposon

The fourth kind of sport is fundamentally different from the first three. In this case, there is no net change; rather a part of existing code moves from an area of the DNA chain to another spot and reinserts itself. This is known as a transposon or nicknamed "jumping gene".

The following final two types of sports assume that there has been a mutation, due to one of the first 4 types, but that it did not affect every cell in the plant. If the cell in only one of the L1, L2 or L3 layers mutate we have chimeras; plants with 2 different genetic codes.

As we have seen the most common cases that can happen due to chimaerical situations are The sectorial chimaera, periclinal and mericlinal chimaera:



5. METHODS OF PROPAGATION

Frequently there is a narrow relation 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 summarize here the most important methods of vegetative propagation.

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

Examples are: Division (Lemna, succulents)
Shoots from roots or rhizomes (Tilia, Carex)
Daughter bulbs, cormlets, bulb scales (Liliaceae, Amaryllidaceae)
Bulbils (Lilium, Asplenium)

Artificial methods These methods of vegetative propagation can be divided in two groups, namely in-vivo methods and the in-vitro methods.

Mericlinal and sectorial chimaeras are by their very nature unstable and the likelihood of propagating plants with the same morphological pattern from these types is low especially in the case in-vitro methods are used but also for in-vivo methods like cutting, budding, grafting etc.

The application of in-vivo methods for propagating periclinal chaemeras can be successful because in this case a complete layer has been mutated.

In the case of chimaeras adventitious primordia derived separately from each of the 3 layers of the mothe rplant are carrying the same genetic information as the corresponding layers of the mother plant.

In our example the LII is showing a partly chlorophyll deficiency .
Primordia derived the roots (LIII tissue) can produce completely green plants instead of variegated plants.

In a very young primordium stage a new 3 layer system can be regenerated now carrying the genetic information of the LIII of the mother plant for all 3 layers and the chimaerical structure has disappeared.

Variegated Papaver orientale with a green "Back mutation" originating from adventitious root tissue (LIII layer).

6 . UPOV DEFINITIONS

Variety

Variety means a plant grouping within a single botanical taxon of the lowest known rank, which grouping, irrespective of whether the conditions for the grant of a breeders' right are fully met, can be defined by the expression of the characteristics resulting from a given genotype or combination of genotypes, distinguished from any other plant grouping by the expression of at least one of the said characteristics and considered as a unit with regard to its suitability for being propagated unchanged

Off-Type

A plant is considered to be an off-type if it can be clearly distinguished from the variety in the expression of any characteristic of the whole plant that is used in the testing of distinctness taking into consideration the particular features of its propagation. This definition makes it clear that in the assessment of uniformity, the standard for distinctness between off-types and a candidate variety is the same as for distinctness between a candidate variety and other varieties.

7. DEFINING OFF-TYPES DUE TO CHIMAERICAL STRUCTURES IN THE PLANTS OF THE SAMPLE

In the first place it is important to be sure that one deals with chimaeras and not with chimaera-like expressions caused by virus. In easily mutating ornamental crops the appearance of deviating colours on parts of the flowers can be striking.

In will be clear the identification samples will have to be free from virus and in some cases the plants are tested on the presence of virus before the plants are accepted by the testing authority.

In the case there is conviction on the presence of chimaeras the question should arise whether the chimaera found is originating from external factors present in the trial or not. Chimaerical structures can find their origin in different types of stress: chemical, physical or mechanical.

In the case the stress is mechanical by nature e.g. caused by insect bites or tearing of tissue and thus caused by external factors possibly present at the testing place one should certainly not label these chimeras automatically as off types that are counted in the assessment of the homogeneity!

Examples: split calyces in carnation, or minute stripes or dots in flowers of rose, gerbera or other ornamentals as the result of mechanical damage made by insects or mites in the early stages of flower development in particular.

As we will see hereafter in a TWO Questionnaire on off-types (TWO/37/7) many people have problems in considering small and very small plant parts off types.

Let us test the presence of small and very small off-types against the UPOV definition of off-type that as given above.

From the last sentence of the definition (The definition makes clear……other varieties) one could conclude that off-types can be very small in the size of their morphological expression as in ornamentals differences between varieties can be very small.

There is no grey area between the varieties. They are distinct or not and that can the already be the case by the presence of one minute stripe or dot in a flower.

So this sentence apparently is not supplying us with any tool to neglect off types that are very small in their expression!

The second question arising is how to handle in a situation where only a part of the plant is showing an off type

In the first sentence the wording “of any characteristic of the whole plant” seems to be open for more interpretations:

What is meant :

(a) with whole plant the expression of all organs of the plant used in testing are meant e.g the leaves, the flowers etc. or

(b) is meant the presence of the off-type on all the organs of the same order?

In the case b is meant we have no space to consider deviations due to chimaerical structures as off types as chimaeras seldom come to expression in the same way all over the plant. Moreover the interpretation under b of the word “whole” is looking rather artificial.

The outcome of the above discussion is that every off type irrespective of its size or local presence on the plant should be taken into consideration in determining the number of off-types.

To comply with the definition of off-type one could conclude:

All off-types – irrespective of the size off their expression on the part(s) of the plants tested should be taken into consideration.

It is important to exclude the possibility that mutations originating in the trial by mechanical influence are considered as off-types.

No answer has been given yet to the question whether it is fair to treat varieties of species producing only one flower (e.g. tulip) or numerous flowers (e.g.gerbera) during one growing cycle in the same way with respect to the number of off-types counted per plant per growing cycle.

Maybe a solution could be found by introducing a standard for each individual plant to be considered as an off type with respect to the number off flowers produced per growing cycle per plant.

A suggestion could be to use the same standard that is applied for the assessment of the homogeneity of the whole sample as a standard too to consider an individual plant as an off type.

In the case of tulip there will be no difference to the current situation as tulips are producing one flower only per season, whereas in gerbera that is producing over 35 flowers per plant per season the presence of more than 2 deviating flowers will label that plant as on off-type. 1 Or 2 off-types per plant in one growing cycle should be allowed in this concept.

8. HOMOGENEITY VERSUS STABILITY

Sometimes there is confusion on both terms

Homogeneity is observed on all the plants of a sample so on all the plants of the same generation.

Plants can be the same or different in their expression.

Normally in DUS testing of self pollinators and clones it is assumed that the stability is sufficient when all plants show the same expression and not sufficient when a certain number of off-types is counted.

In observing stability of the variety at least two generations of plants are needed.

In most heterogeneous samples propagation leads to a generation that is different from the former one, in particular with chimaerical off-types.

The two terms are often mixed:

In observing uniformity one generation is sufficient.

In observing stability two successive generations are necessary.

In predicting stability one generation can be sufficient sometimes, especially in case of chimaeras, as it is highly unlikely that the propagated plants show the same phenotype as the parent plant .

9. TWO QUESTIONNAIRE ON OFF-TYPES

At the thirty-sixth session of the TWO (Quito 2003) it was agreed that the Union office, in conjunction with the chairman of the TWO would prepare and issue a questionnaire seeking information on the proportion a plant which would need to be affected by a mutation or variation in order to be considered an off type, e.g. whether a single atypical leaf or petal would render the plant an off-type

The following questions were put:

1. *What practical difficulties (if any) have you experienced in determining what are off-type plants?*

The following answers were given:

A common problem identified in 8 responses is deciding when a variation observed in a plant becomes of importance. Is the variation observed sufficient to make the whole plant an off type? Can we say that a whole plant is an off-type when the observed change is on a single shoot, or flower or part of a petal?

This is in particular a problem with variegated leaves and bicolor flowers.

It is important to understand the potential natural variation within the crop or type (e.g. mutations), which can be difficult when there is limited crop experience or limited access to other plant material.

The question of suitable sample size for uniformity was raised. If large plant numbers are available, e.g. in commercial production blocks, a better sample for uniformity assessment might be obtained from these, rather than using the trial plants used for distinctness.

NL Comment:: Besides the arbitrariness of using commercial production blocks there is a practical problem: ornamental commercial production blocks seldom reach the full flower stage as the flowers are harvested already in the bud or semi bud/flower stage.

2. Type of material to be examined

Do you request a different type of material (e.g. propagation method, maturity, form, quality) for variegated varieties originating as mutations- compared to seedling varieties?

In general not. One respondent suggested that only material that will be used in commerce should be used.

NL answer: no

Comment: no material used in commerce should be used, the reason is mentioned under 1.

3. Quantity of material to be supplied

Do you request a different quantity of material to be supplied for variegated varietes or varieties originating as mutations-compared to seedling varieties?

Two respondents already do this for some crops and three are considering the possibility of doing so for certain types of varieties or species. Several respondents stated that greater numbers are requested for mutations and sometimes for varieties or species that have had previous or known problems.

The remainder follows the UPOV Test Guidelines where applicable.

NL answer: yes, in roses.

4. Number of growing cycles

Do you use a different number of growing cycles for variegated varieties or varieties originating as mutations?

There is a general agreement that a single growing cycle is acceptable for most ornamentals and, if there is any doubt or uncertainty after the first cycle, a second cycle is an option.

Two respondents routinely test mutations over two growing periods and two respondents stated that two growing cycles for variegated and some other types of variety may become standard.

NL: not as a matter of routine, in cases of doubt only
Comment: In varieties with variegated leaves (especially in woody plants) every plant will produce one or more green shoots sooner or later.

It seems to be unfair towards the applicant to do nothing more than just waiting for the appearance of a green shoot before rejecting the variety!
There should be a limit of no more than 2 years for the trial as a matter of routine in those cases.

5. Number of plants to be examined

Is the number of plants/parts of plants to be examined different for variegated varieties or varieties originating as mutations- compared to seedling varieties?

Generally not, however several respondents stated that more plants are examined for mutations.

NL: yes, occasionally (due to a larger plant sample)

6. Proportion of plants affected to be considered an off-type

What proportion of a plant affected by mutation or variegation (e.g. a single shoot on a plant, 10% of fruits on a tree etc.) would result in plant considered an off-type?

Any part of a plant that is variable could potentially make that whole plant an off-type. The possible cause of the variation, such as insect or mechanical damage, and importantly, the natural species should be taken into account. The difficulty is not so much in recording or identifying variation but in deciding the importance.

One respondent suggested that the wording of the plant characteristics is important. Whole or large part of plant variation is important for whole plant characteristics and smaller or lesser variations for plant part characteristics.

NL: any part

Comments on other answers: In many ornamentals different species have been crossed and moreover in nature they show a different way of propagation namely by seed . For that reason it seems less logical to look at the natural species. Moreover the method is demanding for subjectivity which we just try to avoid as much as possible. The answers on the wording of the characteristics should not be followed and the answer on "the whole or large part" should not be followed as well; as it is not objective at and is asking for trouble consequently.

7. Types of variation

Do you treat different mutations in different ways e.g. variations/mutations in basal shoots variations/mutations in stem shoots transient variations other?

The majority no, however one respondent stated (b) is the most important and two respondents indicated that differentiation could be made

NL answer: no

8. If you answered yes to question 7, please provide details of how each type of variation is treated.

From the three yes or possible responses in 7, statements suggested that variation in stem shoots could be more important than basal shoots. Variation in immature growth may be less important than in mature growth.

NL remark: *in perpetual flowering crops the first flush of flowering should not be used for DUS assessment. In NL that is already common praxis in roses and gerbera.*

9. Guidelines for determining off-types

Have you developed guidelines or protocols for the determinations of off-type plants?

Overall no, with three respondents stating that consideration is being given to this. New Zealand has written a procedure for the uniformity assessment of apple varieties from mutation.

10. Population standard

UPOV Test Guidelines specify a single population standard for a type of propagation. Do you consider that there should be a different population standard for: variegated varieties; varieties originating as mutations?

Two respondents wished no change to (a) or (b)
One respondent would consider the possibility
Three respondents ptoposed considerstion of new standards for (a) or (b)
Three respondents proposed new standard for (a) but not for (b)
One respondent proposed new standard for (b) but not (a)

NL answer: considering possibility

Comment; The instrument of the Population standard should not be used by case of presence of variegations or mutation but by case of the presence of mutability in the crop concerned.

11. Assessment of stability

Are there particular circumstances s where you make an assessment of stability by either growing a further generation, or by testing a new seed or plant stock of variegated varieties or varieties as mutation ?

General agreement is, as needed, propagating and/or growing a future generation or evaluating a second or more growing cycles is good practice. This would be in circumstances of doubt about uniformity and stability, such as the appearance of transient variation (variation on immature but not mature shoots) and variation in a small part of the plant.

10. CONCLUSIONS

Due to their instability and to the risk of misinterpretation chimaeras can give cause for difficulties with regard to their identification. One should be very careful in composing rules that must be as objective as possible.

1. It has to be absolutely sure that chimaerical off-types originating from mechanical factors occurring in the trial should be excluded from the assessment of off types in that particular trial.

2. As the official UPOV definition of Off-type seems to have left no room for a difference in interpretation of the importance of large and small off types/ chimeras (large and small) it seems to be clear that any chimaerical off-type from tiny to large has to be taken into

consideration. When combining 1 with 2 it could be advised to neglect the very small chimaeras (and by "very small" millimeters are meant indeed !).
The reason is the probability that these very small chimaerical structures could have caused by insects or mites in the trial uncovering a particular "L" layer.
By ignoring all extremely small chimaeras ANY risk is excluded.

3. Maybe some space is left within the UPOV definition concerning off types to handle species that are producing flowers only once a season in a different way from perpetual flowering species by using the same standard that is prescribed for counting the number of off types in the test but now taking into consideration the number of off-types produced per individual plant. This concept needs needs attention for a discussion in the TWO.

4. In some perpetual flowering crops (rose, gerbera) the first flush of flowering should not be used for DUS observations as it can not be considered as representative for the expression of the variety (e.g. rose, gerbera) during the whole growing cycle.

5. The use of a different population standard should be decided by case of the crop concerned only and in relation to the mutation rate of that crop.

6. In varieties owing their existence to a variegated leaf pattern only the "standard" for the duration of the trial should be fixed beforehand and it should be not longer than 2 years.

Apart from the strict UPOV definition that is restraining the development of more rules is has to be admitted that any other regulation or prescription will most probably lead to a species by species treatment that will not promote objectivity.

APPENDIX

STEP BY STEP APPROACH

HOW TO HANDLE IN DETECTING OFF-TYPES THAT ARE CHIMAERICAL BY NATURE

Be sure that the plants of the sample are free from pests and diseases from the moment of their arrival until the moment that the trial is closed. Plants showing any signs of pests or diseases should be refused rigorously. Before destructing the plants pictures of the symptoms should be taken as a piece of evidence of their presence.

In the case of observation of off-types during the trial it will be necessary to find out what is the nature of the off-type (admixture, pest or disease, chimaera or other).

In the case chimaeras are found indeed the greenhouse should be checked again on the presence of insects as a possible reason for the presence of the chimaeras. Insect damage during the trial normally could result in very small-sized chimaeras only

If the size of the chimaeras is very small (millimeters!) they should be neglected) for reasons mentioned above.

From the moment the chimaeras have been assessed all the individual plants of the sample should be controlled on the presence of chimaeras, their number as well as their size and shape should be recorded in a very careful way.

In some crops the first flush of flowers will not be taken into consideration for DUS observations as first flush is not always true to (the variety) type

In the case of doubt on the nature of the off-type (chimaera or not) not one could decide to propagate individual plants showing chimaeras. Before doing that it is advisable to contact the applicant asking him whether he is familiar with the phenomenon or not. That could prevent you a lot of work!

Pictures off the chimaerical plants should be taken.

Once regarded to be an off-type the current rules for reporting heterogeneity should be followed.

In the particular case that all plants of the sample are showing chimaeras and their expression is more or less the same for all the plants one could come to the conclusion that the sample is homogeneous for its heterogeneity.

It is to be advised to discuss these cases in the boards (CPVO, RvhK) as the question is open whether these varieties could be granted with rights or not.

Last but certainly not least do not hesitate to ask your colleagues for advise if you are not sure of your ground!

[Annex 4 follows]

ANNEX 4

NEW ZEALAND (HEBES)

Identification of Off-Types in Variegated *Hebe*

Hebe is a genus of around 100 species of small to large shrubs, the majority endemic to New Zealand. The broader *Hebe* grouping includes *Heliohebe* and hybrid ×*Heohebe.* The genus has been cultivated for over a century and can be found in many temperate countries, resulting in the breeding and selection of many varieties. A number of variegated varieties have been raised and these are popular in the market. It is the variegated varieties that are proving difficult to assess for uniformity and stability.

Plant Material Assessed

Eight plants of the variety are routinely used. Additional plants may be observed on a breeder's property or another site because a larger sample of 15 to 20 plants for uniformity assessment is useful. Using the uniformity standard of 1% and 95% probability, 1 off- type would be allowed for a sample of 8 plants and for 15-20 plants.

Determining off-type plants

The first step is to clearly established what the variety looks like, particularly the leaf colouration and variegation pattern. Only after this is it possible to observe characters on each plant and identify any that do not fit the variety as described. The process of identifying off-type characters and determining off-type plants include the type, cause and frequency of the off-type character.

The type of off-type character. A common variegation pattern in *Hebe* is a greenish midrib or centre part of the leaf and a lighter green or yellowish margin. When identifying off-type plants, it is helpful to be familiar with colouration and patterning of other variegated varieties in the genus. A common occurrence, observed across all variegated varieties of *Hebe* checked, is the variation in the amount of green and the amount of yellow/cream on each leaf. What seems to be important is the presence of the two colours on each leaf and not necessarily the amount. When looking at the candidate, the variation in the amount of colour on leaves may be of lesser importance than the presence of both colours. A plant with an all green or all yellow shoots would be an off-type, where a plant with some leaves with a broader margin colour than most, but still has both colours present, may not be.

Cause of the off-type character: Another factor to consider is the possible colour change between older and younger leaves, a maturity effect, and those exposed to more or less light. Leaves on the inside of a bush may be less strongly variegated. This is not necessarily due to variety uniformity but an environmental effect. In *Hebe*, every off-type character identified observed needs to be considered as to whether or not it really is a sign of variety uniformity or is it caused by maturity, environment or broader genus effect.

Frequency of the off-type character: The frequency of off-type characters on a single plant also requires a judgement. A shoot with a few completely green leaves is unlikely to make the plant an off-type where a completely green shoot or one with a high frequency of completely green leaves would be an off-type plant.

[Annex 5 follows]

ANNEX 5

REPUBLIC OF KOREA (POINSETTIA AND *GYMNOCALYCIUM MILHANOVICHII*)

Determining of Off-types

- Rep. Of Korea
- NSMO (National Seed Management Office)

Contents

- Determining Off-types in Poinsettia
- Determination of Uniformity in *Gymnocalycium mihanovichii*

1. Determining Off-types in Poinsettia

•Result

- This variety was rejected because that it was lake of uniformity in the bract color type.
- It was tested over two growing cycle and further generation propagated from the Off-type.

- Variety name : 'Fisson Jinglit'
  - The Subject of determination Off-types : Bract
  - Sample size : 50
  - Two growing cycles
  - The type of material to be examined : rooted cutting

- Appearance of off-types

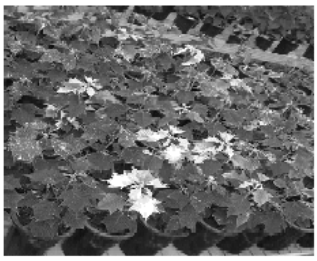


Fig. 1. Cultivation view

↑ Fig. 2. Normal(A) and off type(B,C,D)

Table. 1. Appearance rate of normal and off type

Pattern	Appearance plants(rate)
A	37(74%)
B	4(8%)
C, D	9(18%)
Total	50(100%)

ϖ Applicant' Opinion

- This variety was already registered in some other countries.
- The Off-type can be appeared by unsuitable circumstance. (temperature, fertilization and so on)

Assessment of uniformity by Further Generation Examination

- Procedure

Removal of main shoot ⇨ Appearance of lateral shoots
⇩
Cutting of lateral shoots ⇩ Inducement of Bract coloration
⇦ Assessment of Uniformity ⇧ Result sending to applicant

- Result

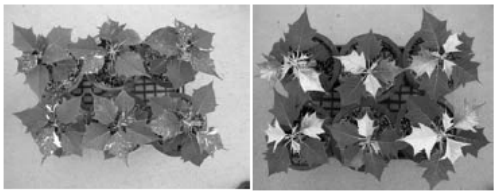


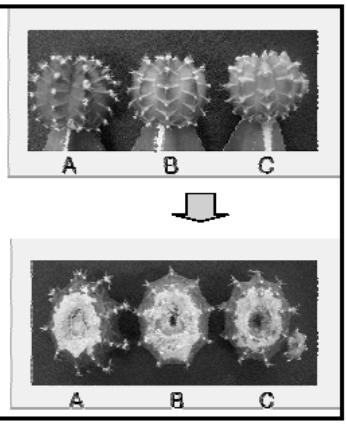
Fig. 3. Growth pattern of Further generation propagated from normal and off-type. (*Leaf : normal type, Right : off type)

2. Uniformity Determination of *Gymnocalycium mihanovichii*

•Result

- We confirmed the change of head coloration by grafting incompatibility.
- So, we determined that this variety have a uniformity.

- Change of Head Coloration by Grafting incompatibility



Assessment of uniformity by Examination of Grafting Incompatibility

- Procedure

Selection by Head Coloration ⇨ Separation of Head and stock
⇩
Examination of Grafting Incompatibility at Head Base
⇦ Assessment of Uniformity

ANNEX 6

REPUBLIC OF KOREA (PELARGONIUM)

Uniformity of Pelargonium

catalogue

applied variety

off-type 1

off-type 2

catalogue

applied variety

off-type 1

off-type 2

ANNEX 7

UNITED KINGDOM (HEBES)

Testing Uniformity in Variegated Hebes: Determination of Off-Types

Hebes are an important genus in commercial horticulture: there are currently over 300 different species and varieties on sale within the UK alone [as listed in the Plant Finder 2005-6], covering a large range of growth types, leaf and flower colours. Mostly they are small to medium sized garden shrubs, but they are also often produced for sale as container or pot plants intended for a more limited lifespan.

Varieties with variegated and or coloured foliage are particularly popular, and in many varieties the colour of the foliage changes with the seasons.

To date the variegation pattern observed is generally a cream or pale yellow margin with a green centre, but other types are also possible. A number of such varieties have been entered for DUS tests in the last ten years.

Testing uniformity

In testing uniformity of Hebes, as with any other variegated plant, the first point to establish is how the variegation pattern can vary within the plant, with the growth stage of the plant, or with the season. In some varieties the variegation pattern is more prominent at some times of year or at some growth stages, than at others; there can also be colour changes with foliage becoming [for example] pinker or more purple at certain times. This information should be provided by the applicant on the Technical Questionnaire.

During the test, any signs of apparent abnormalities which appear should be tagged and monitored to ensure they are not part of this expected pattern of change, a positional effect, or something transient caused by the environment. The typical behaviour of the genus, and in particular, other variegated varieties within it, should always be borne in mind.

Definition of an off-type

For the DUS test the minimum sample size is currently eight plants; using the uniformity standard of 1% and 95% probability, 1 off type is allowed.

The most common problems seen in variegated Hebes are the production of all-green or all-cream shoots, or shoots with foliage with a different type of variegation from the majority.

Experience in testing over the past ten years has shown that generally varieties fall into two categories: those which show no sign or almost no sign of any problem, and those where all or most plants produce large and/or multiple aberrant shoots very quickly.

Therefore in deciding whether a plant is an off-type or not, the quantity, vigour and frequency of the breaks and the behaviour of the variety as a whole should be considered.

A shoot with a few completely green leaves is unlikely to make the plant an offtype, but one or more completely green or differently coloured shoots, or a high frequency of smaller breaks, would result in an offtype plant.

[End of Annex 7 and of document]