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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/9

"EXAMINING DISTINCTNESS"

Document prepared by the Office of the Union

to be considered by the

Technical Working Party for Vegetables (TWV),
at its thirty-eighth session, to be held in Seoul, from June 7 to 11, 2004

Technical Working Party on Automation and Computer Programs (TWC).
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SECTION 1: INTRODUCTION

To be developed after all other sections completed.

SECTION 2: SELECTING VARIETIES FOR THE GROWING TRIAL

Having established the appropriate variety collection for the assessment of distinctness (for further details on variety collections see TGP/4), it is necessary to determine which of the varieties in the collection should be included in the growing trial. The following means of selecting varieties from the variety collection to be included in the growing trial are explained in this secton:

(i) use of grouping characteristics (see section 2.1)

(ii) use of phenotypic distance (see section 2.2).

2.1 Grouping characteristics

2.1.1 Introduction

2.1.1.1 [1]The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics. The information provided by the applicant in the Technical Questionnaire of the application form is the basis for allocating the variety (see Chapter 10 of the TG Template included in Annex I of TGP/7) to the corresponding group in the collection.

2.1.1.2 The General Introduction (TG/1/3) sets out the function of grouping characteristics (see TG/1/3, section 4.8. Functional Categorization of Characteristics) as follows:

> "1. Characteristics in which the documented states of expression, even where recorded at different locations, can be used to select, either individually or in combination with other such characteristics, varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness.
>
> "2. Characteristics in which the documented states of expression, even where recorded at different locations, can be used, either individually or in combination with other such characteristics, to organize the growing trial so that similar varieties are grouped together."

2.1.1.3 The UPOV Test Guidelines provides a list of grouping characteristics. Nevertheless, [2]the number of grouping characteristics is not fixed. If there are only a few characteristics which satisfy the criteria these are all likely to be selected as grouping characteristics. However, if there are many characteristics which fulfill the criteria these might not all be selected as grouping characteristics in the Test Guidelines. In the latter case, a selection of the most efficient characteristics might be made.

[1] First sentence from TGP/7, TG Template, section 5.1
[2] From TGP/7, GN 13.2

2.1.2 Selecting Grouping Characteristics

The General Introduction (TG/1/3) set out the criteria (see TG/1/3, section 4.8. Functional Categorization of Characteristics) for the selection of grouping characteristics as follows:

> "1. (a) Qualitative characteristics or
>
> (b) Quantitative or pseudo-qualitative characteristics which provide useful discrimination between the varieties of common knowledge from documented states of expression recorded at different locations.
>
> "2. Must be useful for functions 1 and 2 (see paragraph 4 above).
>
> "3. Should be an asterisked characteristic and/or included in the Technical Questionnaire or application form."

2.1.3 Using grouping characteristics

2.1.3.1 Once the set of grouping characteristics has been selected, the collection of varieties is divided into groups using the different states of expression of these characteristic.

2.1.3.2 The following theoretical example is presented for illustration in the use of grouping characteristics:

Grouping characteristics:

(i) Leaf shape: with states of expression lanceolate (1); elliptic (2)

(ii) Flower color: with states of expression white (1); purple (2)

using these two characteristics four groups can be formed

GROUP 1: Leaf shape lanceolate and flower color white
GROUP 2: Leaf shape lanceolate and flower color purple
GROUP 3: Leaf shape elliptic and flower color white
GROUP 4: Leaf shape elliptic and flower color purple

An example for wheat, using the characteristics presented in TG/1/3 is presented.

Wheat: In the Test Guidelines for wheat (document TG/3/11) the grouping characteristics indicated in Section V are:

(i) Straw: pith in cross section (half way between base of ear and stem node below) (characteristic 10)
(ii) Ear: color (characteristic 16)
(iii) Awns or scurs: presence (characteristic 14)
(iv) Seasonal type (characteristic 26)

2.1.3.2 Using these characteristics it may be possible to create up to 54 groups within a collection of wheat varieties (see Table 1). Using the information provided by the applicant in the Technical Questionnaire, the candidate variety is allocated in the corresponding group in the DUS growing trial.

2.1.3.3 At the end of this first growing cycle, the expression of characteristics will have been recorded in the same place for all varieties in the DUS trial, making more accurate comparisons possible. Thus, in a second growing cycle the candidate variety can be placed close or even next, to those varieties which are the most similar or not distinct from the candidate variety after the first growing cycle.

Table 1 Wheat: possible groups to be formed using the grouping characteristics presented in TG/003/11

26.Seasonal type	16. Ear: color	10.Straw: pith in cross section (halfway between base of ear and stem node below)	14.Awns or scurs: presence
winter type	white	thin	both absent
alternative type			
spring type			
winter type	colored		
alternative type			
spring type			
winter type	white	medium	
alternative type			
spring type			
winter type	colored		
alternative type			
spring type			
winter type	white	thick	
alternative type			
spring type			
winter type	colored		
alternative type			
spring type			
winter type	white	thin	scurs present
alternative type			
spring type			
winter type	colored		
alternative type			
spring type			
winter type	white	medium	
alternative type			
spring type			
winter type	colored		
alternative type			
spring type			
winter type	white	thick	
alternative type			
spring type			
winter type	colored		
alternative type			
spring type			
winter type	white	thin	awns present
alternative type			
spring type			
winter type	colored		
alternative type			
spring type			
winter type	white	medium	
alternative type			
spring type			
winter type	colored		
alternative type			
spring type			
winter type	white	thick	
alternative type			
spring type			
winter type	colored		
alternative type			
spring type			

2.2 The Use of Phenotypic distance[3]

2.2.1 The notion of "Phenotypic Distance"

The notion of phenotypic distance is to make an overall comparison of varieties in a similar way to that undertaken by crop experts in the field trials. To calculate a "phenotypic distance" between two varieties, i.e. the result of the addition of the phenotypical differences in a set of individual characteristics, each difference is weighted according to its value and reliability. For each species, this system must be calibrated to determine the weight which can be given to each difference and to evaluate the reliability of each characteristic in a given environment and for the genetic variability concerned. It means that the role of the expert remains essential.

2.2.2 The notion of "Distinctness Plus"

In order to have a secure system, the notion of "Distinctness Plus" has been introduced. It means that, based on the "Phenotypic Distance" (i.e. the computation of the differences taking into account their size and the reliability of each characteristic), the threshold used to discriminate varieties is higher than the minimum distance used by the expert to establish distinctness. The threshold determined by the crop expert is at a level which ensures that all pairs of varieties having a distance equal or greater than the threshold are clearly distinct in the growing trial. Therefore, they do not require further comparison in the growing trial. As previously noted, the threshold must be based on experience gained with known varieties and must minimize the risk of taking a wrong decision. It would be a wrong decision to eliminate a pair of varieties which should be further compared in the field. With this approach, it is possible to develop a software and to automate the application (see section 2.2.4.1 The GAÏA software).

2.2.3 Using phenotypic distance[4]

The following examples are given below as a list of possible uses:

2.2.3.1 Using phenotypic distance in the first growing cycle

(a) A crop that has a large number of reference varieties and uses only quantitative characteristics on a 1 to 9 scale:

The GAÏA software allows the selection of reference varieties that is needed if it is not possible to put all the reference varieties in the growing trial. This information is already available beforehand, and can be used to plan the first growing cycle trials as well as the subsequent growing cycles.

(b) In a "small" agricultural crop:

---

[3] The information from this section is mainly taken from TGP/9.3.2, paragraphs 3 and 4, and TWC/21/4, paragraphs 14 and 15.

[4] Text of this section taken from TWC/21/4, paragraphs 18 to 26

There are relatively few candidate and reference varieties, which enables the crop expert to sow all candidates, and the appropriate reference varieties, in two or three successive growing cycles. The same varieties are sown in growing cycles 1, 2 and 3, and the layout is randomized. The software will help to identify the pairs with a small distance, to enable the expert to focus his attention on these particular cases when visiting the field.

2.2.3.2 Using phenotypic distance after the first growing trial

(a) After one growing cycle in the examination of an ornamental crop:

The absolute data and distance computations are an objective way to confirm the opinion or the decision of the expert. There might be cases where pairs of varieties have a small distance, but nevertheless the expert has clear evidence of distinctness. If more growing cycles are necessary before a decision is taken, the software helps to identify on which cases the expert will need to focus.

(b) In a vegetable crop: where there are many candidate and reference varieties:

There is wide variability in the species, so on the one hand there are already obvious differences after only one cycle, but on the other hand some varieties are very similar. In order to be more efficient in their checks, the crop experts wish to grow "similar" varieties close to each other. The raw results and distances will help to select the "similar" varieties and decide on the layout of the trial for the next growing cycle.

(c) In a difficult crop, there are varieties which are so similar that it is a common practice to make side-by-side comparisons for such varieties, identified after the first cycle:

If the number of varieties in the crop is not too large, the crop expert will easily detect the cases which should be checked. However, when the number of varieties in a trial increases, it becomes less easy to identify all the problem situations. The software can help to "not miss" the less obvious cases.

(d) In vegetatively propagated ornamental varieties, the examination lasts for one or two growing cycles:

After the first growing cycle, some reference varieties in the trial are obviously different from all candidates, and their inclusion in the second growing cycle is not necessary. When the number of varieties is large, the raw data and distance(s) can help the expert to detect reference varieties for which the second growing cycle is unnecessary.

2.2.4. Methodologies for using phenotypic distance[5]

2.2.4.1 The GAÏA software

2.2.4.1.1 With the aim of calculating phenotypic distances, experts from France developed the GAÏA software. The principle is to compute a phenotypic distance between two varieties, which is a sum of distances for individual characteristics. For the difference observed

[5] Most of the text of this section has been taken from TWC/21/4, paragraphs 1 to 17 and 27 to 45.

between two varieties, in a given characteristic, a distance/weighting is derived from the absolute value of the difference and a metric defined for the characteristic. The global distance is a sum of the distances on each characteristic:

$$dist(i,j) = \sum_{k=1,nchar} W_k(i,j)$$

where:

$dist(i,j)$ is the computed distance between variety i and variety j.

k is the k^{th} characteristic, from the *nchar* characteristics selected for computation.

$W_k(i,j)$ is a function of the difference observed between variety *i* and variety *j* for characteristic k.

OV_{ki} is the observed value on characteristic *k* for variety *i*.

$$W_k(i,j) = f\left(\left|OV_{ki} - OV_{kj}\right|\right)$$

2.2.4.1.2 For a given characteristic, a weighting is attributed to the absolute difference between two varieties. The weightings have been previously defined by the crop expert and stored in the GAIA database. The same weighting is attributed to any pair of varieties whose absolute differences between observed values are the same. If *i, j, n* and *m* are varieties.

$$\left|OV_{ki} - OV_{kj}\right| = \left|OV_{kn} - OV_{km}\right| \qquad W_k(i,j) = W_k(n,m)$$

a practical example is presented in Annex I.

2.2.4.1.3 The weighting is equivalent to a distance contribution. Crop experts prefer to use the word "weighting" when they consider the distance contribution on a given characteristic, and "distance" for the global distance on all characteristics. The word "weighting" is not correct, but nevertheless we will use it for the *distance contribution, made by each characteristic*, in order to simplify communication and exchange between experts. Weighting depends on the size of the difference and on the individual characteristic. The weightings are defined by crop experts on the basis of their expertise in the crop and on a "try-and-check" learning process. The values for the weightings defined by the experts are stored in GAIA database. Experts can give zero weighting to small differences. Thus, even if two varieties have different observed values in many characteristics, the resulting distance might be zero.

2.2.4.1.4 Varieties are compared in pairs. The crop expert can compare different combinations of pair-wise comparisons, for instance:

- compare two varieties,
- compare a given variety to all available varieties,
- compare all candidate varieties to all [candidate + reference] varieties,
- compare all possible combinations.

2.2.4.1.5 The crop expert can also select all the available characteristics, or different subsets of the characteristics.

2.2.4.1.6 The crop expert obtains a comprehensive report for each pairwise comparison. The software computes a global expert distance, but also provides all the individual absolute values and the distance contribution of each characteristic (see Annex II for an example).

2.2.4.1.7 The use of the results may differ from expert to expert. The most frequent use of the software in France is at present to fix and apply a threshold for the distance which enables the crop expert:

(i) to eliminate from subsequent growing cycles all pairs of varieties reaching or surpassing the GAIA distance threshold;

(ii) to focus on close varieties, having a GAIA distance lower than the threshold, for the next growing cycle(s).

2.2.4.1.8 The threshold determined by the crop expert is at a level which ensures that all pairs of varieties having a GAIA distance equal or greater than the threshold are clearly distinct in the field or in the greenhouse. Therefore, they do not require further comparison in the field or in the greenhouse. The threshold must be based on experience gained with known varieties and must minimize the risk of taking a wrong decision. It would be a wrong decision to eliminate a pair of varieties which should be further compared in the field.

2.2.4.1.9 In France, greater weighting values are chosen for characteristics which are known to have polygenic control and are little influenced by environmental conditions. Monogenic controlled characteristics, or characteristics for which the level of expression is dependent on environmental conditions, are considered with care and lower values, or even a zero value is given for the weighting.

2.2.4.1.10 GAIA software computes information for the crop expert. The crop expert can use this information according to his own needs (see section 2.2.3).

2.2.4.1.11 At present the software can use qualitative, quantitative and/or electrophoretic data. These types of data can be used alone or in combination, as shown in Diagram 1.

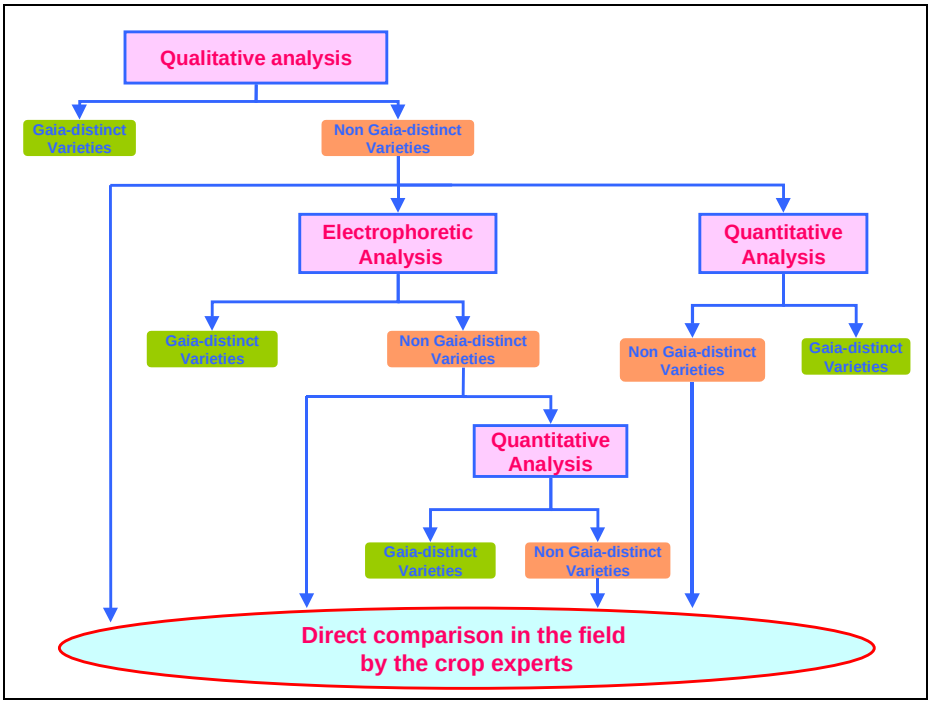


Diagram 1: Use of different types of characteristics

2.2.4.1.12 Software options change according to qualitative, electrophoretic or quantitative characteristics.

2.2.4.1.13 The user decides not only the type of data in the computation, but also the set of characteristics to use from those characteristics which are available.

2.2.4.1.14 For practical reasons, a distance threshold is used. This enables the crop expert to identify similar varieties which have a small distance (below the threshold) between them. This threshold can be used in different ways. The crop expert can use:

- a low threshold, which helps to find the more difficult cases (similar varieties);
- intermediate thresholds (different levels according to the needs);
- a large threshold when there is a need to have a comparison which uses all the available characteristics.

2.2.4.1.15 In order to minimize computation time, as soon as the threshold is achieved for a comparison between two given varieties, the software proceeds to the next pair of varieties. Remaining characteristics and their raw values will not be shown in the summary output, and will not contribute to the distance.

2.2.4.1.16 Often the crop expert looks for varieties which are similar. A low threshold is then appropriate.

2.2.4.1.17 If the crop expert wishes to see all available raw data and the different weighting for each characteristic, he must choose a threshold which is greater than the maximum distance possible on all characteristics.

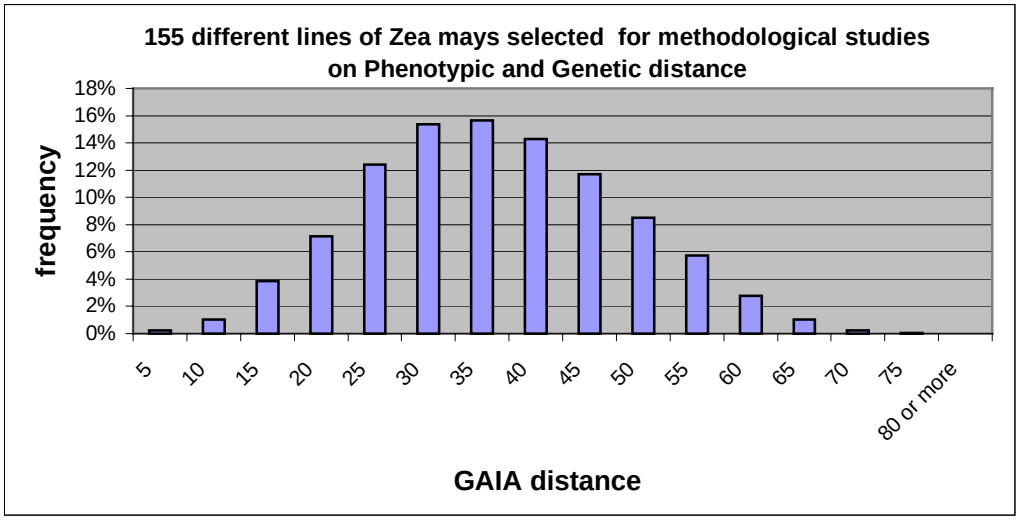
2.2.4.1.18 There is no absolute rule to decide whether a distance is "small" or "big". The crop experts themselves define the distance values.

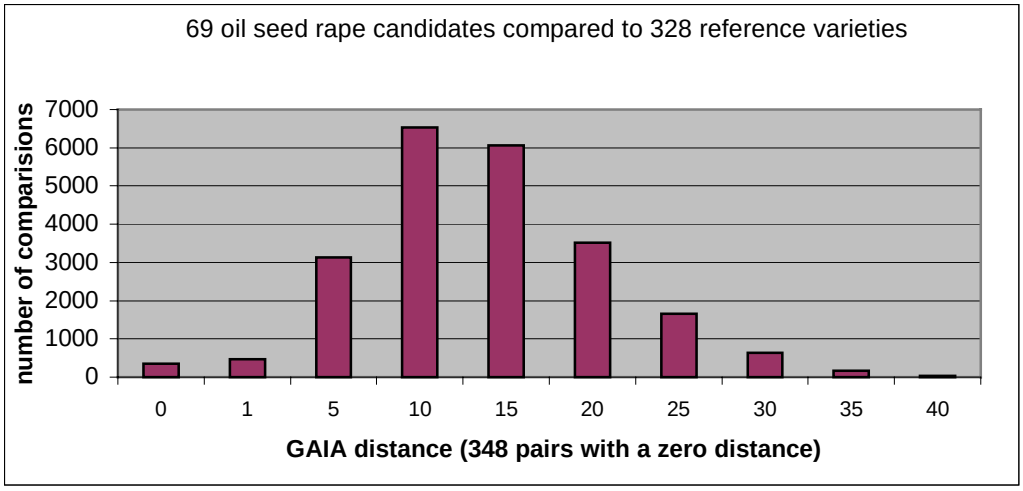
- Experts can choose different values as the weighting/distance for a characteristic (1, 2, 5, etc.).

- Some crops have more characteristics than others.

- The crop expert can use all available information, or only a subset of characteristics.

2.2.4.1.19 For these reasons the absolute values of distances vary. The same applies for the threshold.





2.2.4.1.20 The definition of the weighting "by characteristic" is necessary prior to use the software, and is important. There is no unique way to define these values; some practical considerations are described below.

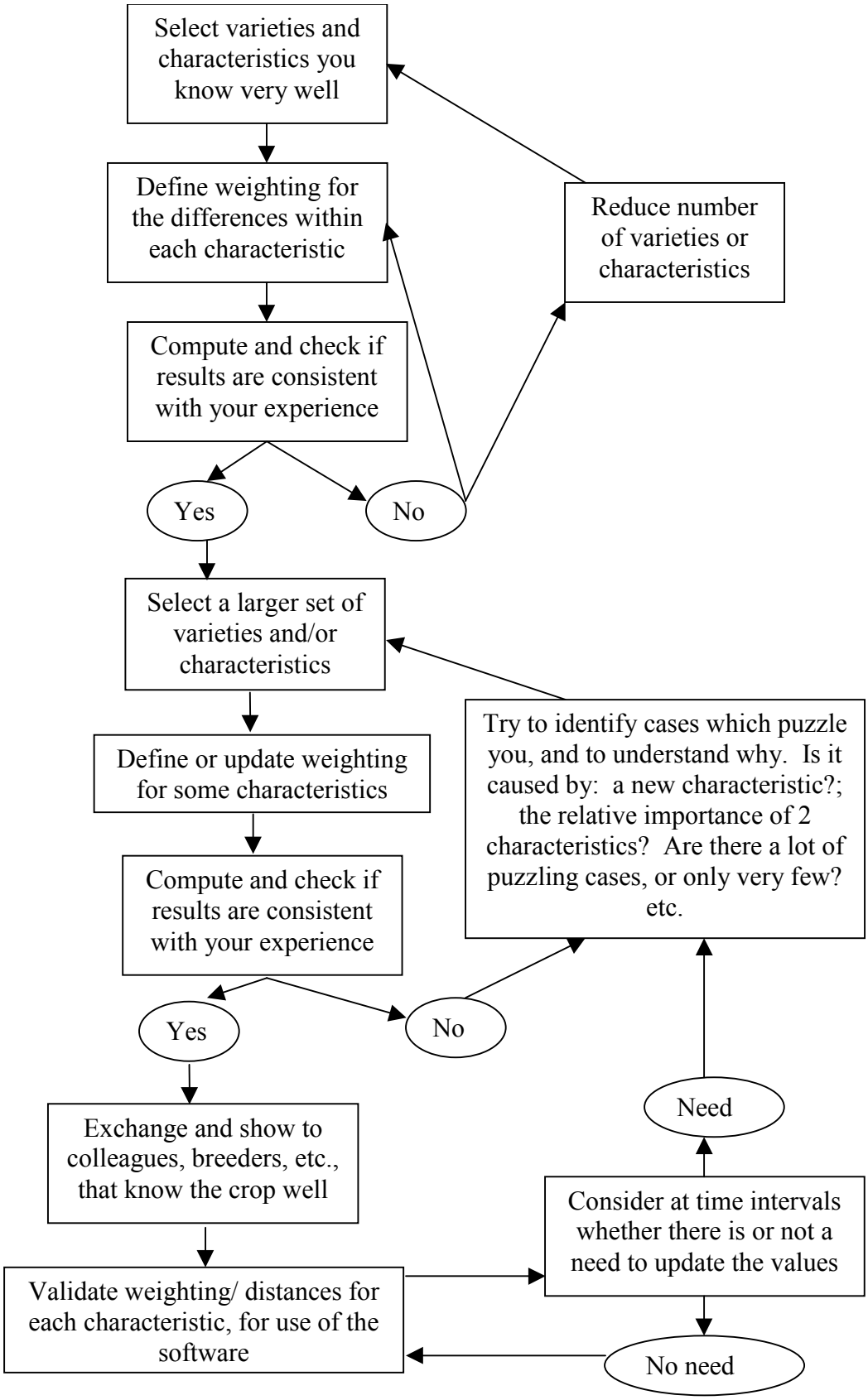
2.2.4.1.21 The two key aspects are simplicity and consistency; three simple "rules of thumb" are given here:

- the distances by characteristic should be integer values, for instance 0, 1, 2, 3, etc. where 3 is a distance or a weighting which is considered to be about 3 times greater than 1;

- if for a characteristic a given difference “expressed as an absolute value” is considered as a double distance for character *a* compared to character *b*, the distance value for this difference should be double that in character *a* than it is in character *b*;

- define the values by “try-and-check” iterations as shown in Diagram 2.

Diagram 2: "Try-and-check" process to define and revise the weightings for a crop



2.2.4.1.22 Annex I describes, a simple example of the computation of the distance between two varieties on the basis of 5 qualitative characteristics.

2.2.4.1.23 Annex II provides, in more detail, an example where successively qualitative, electrophoretic, and quantitative characteristics are used to compare two varieties.

2.2.4.1.24 Annex III provides a screen copy of a display tree which shows how the expert can navigate and visualise the results of computations.

2.2.4.1.25 GAIA software has been developed with WINDEV-7.5. The general information (species, characteristics, weighting, etc.), the data collected on the varieties and the results of computations are stored in an integrated database. Import and Export facilities allow the use of your own information system in connection with GAIA software. ODBC allows access to the GAIA database and to other databases simultaneously[6].

2.2.4.1.26 For qualitative characteristics, 1 or 2 notes per variety can be used. In general, two notes are present when there are two trial locations. For electrophoresis data, only one description can be entered per variety. For quantitative characteristics at least 2 values (different trials, repeats, etc.) are necessary and the user selects which to use in the computation.

2.2.4.1.27 GAIA is mainly used for self-pollinated and vegetatively propagated crops, but GAIA does not have special restrictions according to the crop.

2.2.4.2 Others

Methodologies for using phenotypic distances developed in future will be included in this section.

[6] A User manual and a description of the software are also available with the software (e-mail to christophe.chevalier@geves.fr). The software is freely available for members of the International Union for the Protection of New Varieties of Plants (UPOV), but it is forbidden to distribute the software to other parties.

SECTION 3: TRIAL ORGANIZATION

3.1 Number of independent growing cycles

3.1.1 Growing cycles

TGP/7 "Development of Test Guidelines", in Chapter 3.1 makes reference to the number of growing cycles and provides guidance that it may be necessary to clarify what is meant by a growing cycle. For example, in the case of fruit species, the following explanations may be used:

> *"(a) Fruit species with clearly defined dormant perio*d
>
> "…The growing cycle is considered to be the duration of a single growing season, beginning with bud burst (flowering and/or vegetative), flowering and fruit harvest and concluding when the following dormant period ends with the swelling of new season buds."
>
> *(b) Fruit species with no clearly defined dormant period*
>
> "…The growing cycle is considered to be the period ranging from the beginning of active vegetative growth or flowering, continuing through active vegetative growth or flowering and fruit development and concluding with the harvesting of fruit."

3.1.2 The notion of independent growing cycles

3.1.2.1 [7]The General Introduction states that:

> "5.3.3.1 Consistent Differences
>
> "5.3.3.1.1 One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic on at least two independent occasions. This can be achieved in both annual and perennial varieties by observations made on plantings in two different seasons or, in the case of other perennial varieties, by observations made in two different seasons after a single planting. Guidance on the possible use of other approaches, such as two different environments in the same year, is explored in document TGP/9, "Examining Distinctness."
>
> "5.3.3.1.2 However, in some circumstances the influence of the environment is not such that a second growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. For example, if the growing conditions of the crop are controlled, such as in a greenhouse with regulated temperature and light, it may not be necessary to observe two growing cycles. In addition, the differences observed between varieties could be so clear that a second growing cycle may not be necessary. In both these circumstances, the features of propagation of the variety and the quality of the plant material will need to be taken into account.

[7] These 4 paragraphs has been taken from TGP/9.6 Draft 1

"5.3.3.1.3 The individual Test Guidelines specify whether several independent growing cycles are required to show sufficient consistency, or whether, for certain species, the growing test could be made in one growing cycle."

3.1.2.2 When the varieties are grown over successive years and the layout of the plants in the trial is randomized (at least partly), the requirement for independence of the growing cycles is usually assumed to be satisfied.

3.1.2.3 For plants grown in greenhouses, provided the time between two sowings is not "too short" and the layout of the plants in the trial is randomized (at least partly), two growing cycles can overlap and still be considered as independent.

3.1.2.4 For some perennial crops, such as fruit trees, the same plants are examined over successive years. In this case, the condition of independence of growing cycles is also satisfied.

3.1.3 Basis for determining the number of independent growing cycles

3.1.2.1 TGP/7: Annex I: TG Template Section 4.1.2 states that:

"4.1.2 Consistent Differences [i]

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic by at least two independent observations."

3.1.2.2 When necessary, further guidance should be provided.

3.2 Use of Multiple Locations in the Examination of Distinctness

3.2.1 Reasons for Using More than one Location[8]

3.2.1.1 Tests are normally conducted at one place, although more than one location may be used in some cases.

3.2.1.2 The concomitance of the DUS studies for protection, and the VCU studies for inclusion in National Lists, are reasons why, in practice, the same variety is grown in more than one location. This does not fall under the scope of the work of UPOV, and results from VCU trials are usually not suitable for DUS studies.

[8] This section taken from TGP/9.6 Draft 1, paragraph 8

3.2.1.3 Varieties of a different geographical origin may require different agro-climatic growing conditions. Different locations can be used in order that the *ad hoc* growing conditions are met. The varieties are distributed to the most appropriate location or, if the choice of the appropriate location is not obvious from the information known at the reception of the samples, to more than one location."

3.2.1.4 Some offices might have a primary location, backed up by a safety location. Normally only the data from the primary location will be used, but in cases where this location has a major problem then the second one will be available to prevent the loss of one year's results.

3.2.1.5 Some offices may have more than one location for a given crop (too many varieties to grow all in one place, existence of trials in an official office and on breeders' premises, etc.). If a given variety is present in one location only and compared to all the appropriate reference varieties, there is no difference in practice, with the case of a single location. If a given variety is present in more than one location, it is common practice to consider the two locations as completely different. Each location is considered to be a separate examination. After two or more cycles, each location has a result. When both examinations result in the same conclusion (either acceptance or refusal), the result is simply more sure. Should the result be different in the two locations (for instance a lack of uniformity or distinctness in one location only), the practice is to accept the variety if the DUS study has a positive conclusion in one of the locations. Offices should not give a positive decision if in one location distinctness is established but there is a lack of uniformity, while in the other location distinctness is not established but there is sufficient uniformity.

3.2.1.6 More than one location can be used in order to obtain independent trials in a given year. In such cases, the locations must have different environments.

3.2.1.7 In order to provide a double-check for consistency, some offices systematically grow the varieties in more than one location (usually 2). In this case, the consistency over cycles for each location and the consistency between locations is checked. It is imperative that DUS studies have a positive outcome in both locations and an overall consistency.

3.2.2 Use of information from multiple locations

3.2.2.1 As described in the previous section, there are several reasons for using trials in more than one location. Before making a definitive decision about that, it is necessary to take into account some relevant aspects:

(a) To establish a decision rule:

A. If the two varieties are distinct in any of the centers,
B. If the two varieties are distinct in the only center,
C. If the two varieties are distinct in all the centers

(b) The variety-by-year interaction

(c) How to use the information obtained in these centers; whether it will be averaging over centers or each center would be considered individually.

(d) Is consistency necessary between the testing places?

(f) To set up the standard probability and the LSD year Testing Center.

SECTION 4: FACTORS IN THE CHOICE FOR THE ASSESSMENT OF DISTINCTNESS

4.1 Introduction[9]

4.1.1 The appropriate method for examining distinctness depends on the methods of recording the expression of a characteristic in a specific crop and the resulting set of data (see TGP/8). If the plant to plant variation within varieties is very small relative to the variation between varieties, the characteristic can be recorded using a single observation for a variety. In the case of greater plant to plant variation it is necessary to take records from individual plants and to calculate the mean expression of the variety in order to assess distinctness between varieties and to describe a variety.

4.1.2 The variation within varieties has both genotypic and environmental components. The level of genotypic variation is determined by the features of propagation. The recommended method of observation, based among other considerations on the level of variation within varieties, is provided in the Test Guidelines in Chapter 3 (including the number of individual plants to be observed, if applicable) and, if appropriate, also in Chapter 8 for each characteristic.

4.2 Type of Variety

The following section provides guidance on typical methods for examining distinctness according to the particular features of propagation of the variety especially in the case of measured characteristics.

4.2.1 Self-pollinated varieties

4.2.1.1 In cases where there is very little variation within varieties, the determination of distinctness is usually on the bases of visual assessment, rather than by statistical methods.

4.2.1.2 In respect to measured characteristics, the General Introduction states the following:

> "5.5.3.1 Self-Pollinated and Vegetatively Propagated Varieties
>
> "UPOV has endorsed several statistical methods for the handling of measured quantitative characteristics. One method established for self-pollinated and vegetatively propagated varieties is that varieties can be considered clearly distinguishable if the difference between two varieties equals or exceeds the Least Significant Difference (LSD) at a specified probability level with the same sign over an appropriate period, even if they are described by the same state of expression. This is a relatively simple method but is considered appropriate for self-pollinated and vegetatively propagated varieties

[9] Based upon TGP/9.4.1 Draft 2, paragraphs 1 and 2

because the level of variation within such varieties is relatively low. Further details are provided in document TGP/9, "Examining Distinctness."

4.2.1.4 Nevertheless[10] if a characteristic in self-pollinated or vegetatively propagated varieties is recorded by observation of individual plants, the same methods as for cross-pollinated varieties can be applied (see section 4.2.3). This situation might occur where there is considerable plant to plant variation within varieties due to environmental effects. However, in general, one single observation per plot for each variety is sufficient in vegetatively propagated, truly self-pollinated and mainly self-pollinated varieties.

4.2.2 Vegetatively propagated varieties

See section 4.2.2.

4.2.3 Cross-pollinated varieties

Within variety variation is normally greater for quantitative characteristics in cross-pollinated varieties, including synthetic varieties, due to genotypic variation. In this case, the expression of a variety should be recorded using observations on a number of individual plants. Distinctness can then be assessed by comparing the differences in variety means with a measure of random variation inherent in the variety means (see TGP/9.5.4 "Statistical Methods").

4.2.4 Hybrid varieties[11]

The assessment of distinctness for hybrid varieties should follow the same rules depending on the degree of within variety variation. Distinctness can be tested at the level of the hybrid itself or under consideration of the parental lines.

4.2.4.1 General

4.2.4.1.1 Breeders working on hybrid programs focus their attention on the inbred lines to get a good general and specific combining ability.

4.2.4.1.2 An inbred line with a good genetic value is generally used in many different hybrid varieties. It means that the examination of distinctness of an inbred line can be used for these different hybrid varieties.

4.2.4.1.3 For different species, the knowledge of the genetic control of some characteristics gives the possibility to verify of the formula declared by the applicant. So a clear link can be established between the hybrid variety and the parental lines.

[10] Based on TGP/9.4.1 Draft 2, paragraph 4, third sentence.
[11] Based upon the text of TGP/9.5 Draft 1

4.2.4.1.4 The examination of distinctness of inbred lines, which are generally highly homozygous and, therefore, uniform, is easier compared to, examination of distinctness on a three-way cross or double-cross hybrid which needs to be made plant-by-plant basis.

4.2.4.1.5 The management of the reference collection is facilitated and a few hybrids have to be sown each year.

4.2.4.1.6 Most of the lines are applied for plant breeders' rights and thus have to be examined for distinctness

4.2.4.2 Use of the Parental Formula

4.2.4.2.1 In the Test Guidelines for certain species (maize, sunflower, oilseed rape) an optional method is described for the examination of distinctness of hybrid varieties with a pre-screening approach based on the parental components of the hybrid and its formula.

4.2.4.2.2 The method includes four main steps, which are:

(i) Description of parental lines according to the Test Guidelines of the given species.

(ii) Check of the originality of these parental lines in comparison with the reference collection, based on the characteristics in Chapter 7 of the Test Guidelines, in order to screen the closest inbred lines.

(iii) Check of the originality of the hybrid formula in comparison with those of the hybrid varieties of common knowledge, taking into account the closest inbred lines.

(iv) Assessment of the distinctness at the hybrid level of varieties with similar formula.

4.2.4.2.3 The basic principle of the method is that two inbred lines, A and B, used in two different crosses: A x C; and B x C, will give two different hybrids according to the UPOV rules as soon as the difference between A and B is large enough and clearly qualified.

4.2.4.2.4 The aim of the paper is to describe how to use this method.

Assumptions of the method:

(i) A compulsory declaration of the formula and a compulsory submission of plant material of the components (inbred line and intermediate hybrids).

(ii) The management of a reference collection of the inbred lines used as parents in the hybrid varieties of common knowledge and a list of the formula of these varieties.

(iii) A uniform application of this method to all varieties of the given species. This condition is important to get the full benefit.

(iv) A rigorous approach established to assess the originality of any new inbred line in order to be confident on the distinctness of the hybrid variety including it.

Assessing the originality of a new inbred line

4.2.4.2.5 The basis for establishing the originality is the list of characteristics described on the Test Guidelines of the species concerned.

4.2.4.2.6 The difference between lines must be sufficient to be sure that the hybrids are distinct. [12]The following example illustrates this point:

C1: a characteristic having two states of expression (absent/present) which are determined by two alleles of one single gene, with one dominant allele (+) for the expression "present" and one recessive allele (-) for the expression "absent"

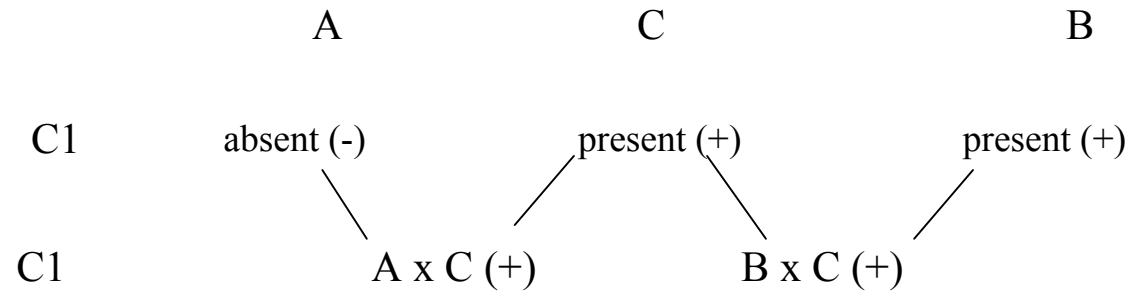
Three parental lines:

A: having the recessive allele (-) with expression "absent"
B: having the dominant allele (+) with expression "present"
C: having the dominant allele (+) with expression "present"

Crossing the above mentioned lines to obtain the following F1 hybrids:

(A x C): having characteristics C1 "present"
(B x C): having characteristics C1 "present"

The following diagram shows the ways the two different crossings results in the same expression of characteristic C1 (i.e. "present" in both hybrids) in spite of the fact that line A(-) and line B(+) have different expressions.



4.2.4.2.7 Although the lines A and B are clearly different for characteristic C1, the two hybrid varieties are not different. In this case it means that a difference between A and B on one characteristic is not enough.

4.2.4.2.8 If we consider characteristics with a more complex genetic control involving several genes not precisely described, the interaction between the different alleles of each gene and between genes can also lead to similar expression at the level of the hybrid varieties. In such a case, it is generally pertinent to consider a larger difference compared to the regular minimum distance taking into account to establish distinctness between two inbred lines.

4.2.4.2.9 The definition of the minimum distances is mainly based on a good knowledge of the species, of its characteristics and, when available, on their genetic control.

[12] Explanation added by the Office

4.2.4.2.10 Such approaches have been developed on different species in France using software with which the closest lines can be detected using combinations of characteristics with consideration of their variability within the species; their susceptibility to environmental effect and their reliability.

Other conditions have to be fulfilled

4.2.4.2.11 Check of the truthfulness of the formula: the aim is to check if the candidate hybrid variety has really been produced by crossing the parental lines declared and submitted by the applicant.

4.2.4.2.12 Different characteristics can be used to do this check as soon as the genetic pattern of each parent can be identified in the hybrid.

4.2.4.2.13 Generally characteristics based on polymorphism of enzymes or of some storage proteins can be used.

4.2.4.2.14 If no characteristic is available or pertinent enough, the only possibility is to cross the parental lines using the plant material delivered by the applicant and to compare the hybrid variety seedlots (the sample submitted by the applicant and the sample harvested after the cross),

4.2.4.2.15 Check of uniformity and stability of each parental lines.

4.2.4.2.16 These two criteria represent an important condition to get stability of the hybrid; the other one is the use of the same formula each cycle of the hybrid seed production.

4.2.4.2.17 To assess uniformity and stability of the parental lines, the UPOV recommendations on this type of variety have to be followed.

4.2.4.2.18 A check of the uniformity on the hybrid seedlot has also to be done even if there is no need to assess distinctness at the hybrid level.

Description of the hybrid

4.2.4.2.19 In case of the assessment of Distinctness only based on the parental lines, a description of the candidate hybrid has to be established.

4.2.5 Rootstocks[13]

4.2.5.1 General

4.2.5.1.1 Under specific circumstances, for the examination of distinctness, uniformity and stability, it may be appropriate to define a number of characteristics which are observed routinely and to set up an additional list of characteristics which is only used if a new variety cannot be considered clearly distinguishable using the routine characteristics. This may be appropriate, for example, when different growing trials are necessary for the observation of different groups of characteristics, but normally one group of characteristics is sufficient for

[13] Based upon the text of TGP/9.4.2 Draft 1

the examination of distinctness for new varieties. The general procedure for the examination of distinctness with two tables of characteristics will be illustrated with the example of rootstocks varieties.

4.2.5.1.2 Rootstock varieties are seed propagated or vegetatively propagated, and are grown on their own roots. This is in contrast to fruit varieties which are normally grafted on to a rootstock, i.e., xeno-vegetatively propagated.

4.2.5.1.3 Rootstock varieties used for pome fruit varieties are preferably cultivated in stoolbeds, whether they are vegetatively propagated by layers, by softwood or hardwood cuttings. The field trial management follows the cultivation method used for plant production in nurseries: the plants are cut back annually, and the rooted stool-layers are harvested in late autumn. To enable comparison, the whole trial should be designed as stoolbed plots. The plants under test are artificially held in a vegetative state and any visible organ of the plant is definitely not older than one year. Usually neither flowers nor fruits can be observed on these plants. The examination of distinctness is based upon characteristics which refer only to vegetative organs.

4.2.5.1.4 Breeding activities in rootstocks are aimed at the impact of a new variety on the varieties for fruit production which are grafted on them (e.g. ease of tree management, earliness of fruit bearing, yield, fruit quality, reduced susceptibility to soil, weather or pathogens). As a consequence, morphological variation between rootstock varieties may be rather limited and it may be that a new variety cannot be clearly distinguished on the basis of the characteristics of the vegetative organs only.

4.2.5.1.5 For such situations, it is recommended that flower and fruit characteristics be examined. Therefore, as a precaution, in addition to the stoolbeds, another five plants should be planted and managed as trees or bushes.

4.2.5.1.5 If the plants of rootstock varieties are normally grown as normal trees in the DUS trial (e.g. seed propagated varieties, vegetatively propagated stone fruit varieties), vegetative as well as generative characteristics can be observed on the same plants.

4.2.5.2 Two sets of example varieties

4.2.5.2.1 The UPOV Test Guidelines for apple rootstocks, Pyrus rootstocks and Prunus rootstocks (TG/163/3, TG/169/3, TG/187/1) do not include flower or fruit characteristics. For many varieties the table of characteristics included in the Test Guidelines for rootstocks is sufficient for the examination of distinctness. If flower, fruit or seed characteristics are necessary for the examination of distinctness, the Test Guidelines for rootstocks refer to the Test Guidelines for the respective fruit varieties.

4.2.5.2.2 If the vegetative characteristics included in the rootstock Test Guidelines are sufficient for the assessment of distinctness, only these characteristics will be examined for uniformity and stability and will be included in the variety description. If generative characteristics which are part of the fruit Test Guidelines are observed for the assessment of distinctness, these characteristics should also be uniform and stable and included in the variety description.

4.3 Type of characteristic[14]

The General Introduction (see Sections 5.3.3.2 and 5.5.2) explains that determining whether a difference between two varieties is clear depend on many factors, in particular, the type of expression of the characteristics (qualitative, quantitative or pseudo-qualitative characteristics) and states that:

"5.3.3.2.1 Qualitative Characteristics

In qualitative characteristics, the difference between two varieties may be considered clear if one or more characteristics have expressions that fall into two different states in the Test Guidelines. Varieties should not be considered distinct for a qualitative characteristic if they have the same state of expression.

"5.3.3.2.2 Quantitative Characteristics

Quantitative characteristics are considered for distinctness according to the method of observation and the features of propagation of the variety concerned. The different approaches are considered later in this Chapter.

"5.3.3.2.3 Pseudo-Qualitative Characteristics

A different state in the Test Guidelines may not be sufficient to establish distinctness (see also section 5.5.2.3). However, in certain circumstances, varieties described by the same state of expression may be clearly distinguishable.

[14] The content of this section is from TG/1/3, Sections 5.3.3.2 and 5.5.2.

SECTION 5: METHODS FOR THE ASSESSMENT OF DISTINCTNESS

5.1 Introduction

The assessment of characteristics can be made either visually or by measurements. In both cases it may be possible to observe individual plants (or parts of plants) or groups of plants (or parts or plants). Depending on the type of expression of the characteristic and the method of observation, different types of data will be obtained. For further detail in the types of characteristics, the data obtained and the methods for the assessment of distinctness see TGP/8.3

5.2 Visual Assessment

5.2.1 Introduction

The General Introductions (Section 5.4.1) explains that in cases where there is little variation within varieties, the determination of distinctness is usually on the basis of visual assessment, rather than by statistical methods.

5.2.2 Visual Assessment and types of characteristics/data[15]

5.2.2.1 In the case of visually assessed qualitative or quantitative characteristics, the General Introduction provides the following recommendation (section 5.4):

> "5.4.2 As explained in section 5.3.3.2.1, "Qualitative Characteristics," for such characteristics the difference between two varieties may be considered clear if one or more characteristics have expressions that fall into two different states in the Test Guidelines.
>
> 5.4.3 For quantitative characteristics, a difference of two Notes often represents a clear difference, but that is not an absolute standard for assessment of distinctness. Depending on factors, such as the testing place, the year, environmental variation or range of expression in the variety collection, a clear difference may be more or less than two Notes. Guidance is provided in document TGP/9, "Examining Distinctness.""

5.2.3 Use of randomized "blind" testing

5.2.3.1 After or during the examination of distinctness some doubts may exist over the possibility to consider a variety distinct on the bases of the result of the trials. In such cases the following situations are possible:

1. With no difference observed, the application is rejected.

2. With no conclusive difference observed and a claim from the applicant, the examining authority may decide to have additional tests.

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[15] Based on TG/1/3, Section 5.4

5.2.3.2 In the case of visually observed characteristics one possible arrangement for the additional test is "blind" testing.

5.2.3.3 The aim of blind testing is to assess distinctness between a pair of varieties avoiding any pre-judgement in the observation by making the samples in the trial anonymous (the expert is "blind" in respect to the identity of the variety in each plot). This kind of test plays a clarifying role when the differences between the candidate an (a) similar variety(ies) are not clear enough, and the crop expert is not sure enough to decide on distinctness. In this case another test included during or after the examination of distinctness may supply evidence for a definitive decision by the authority.

5.2.3.4 The following are some examples of "blind" testing:

Randomized variety plots: duplicates of the same variety receive individual codes and are randomly distributed in the trial.

Plots containing a mixture of varieties: plots with a mixture of material from the varieties under examination are included in the trial.[This can be useful for seed propagated varieties].

Parts of plants of varieties: randomised parts of plants under codes from the varieties under examination (e.g. leaves or fruit).

5.2.3.5 Applicants may be part of the "blind" testing process. They may also be invited to visit the "blind" test and be requested to identify the plots of their variety.

5.2.3.6 At the end of the "blind" testing the variety can be declared as distinct:

if the expert and the breeder always identify the plots of the variety,

the difference fulfilled can be considered as a clear difference for that characteristic

5.2.3.7 In all cases, the authority takes the decision on distinctness.

5.3 Measurements

5.3.1 Introduction

[16]Different types of data can be obtained from measurements. From the statistical point of view, a characteristic is only considered at the level of the recorded data, either for analysis or for description of the characteristic.

[16] Sentence from TGP/8.4 Draft 2 paragraph 35 (now changed to TGP/8.3).

5.3.2 The Combined Over-Years Distinctness Criterion (COYD)

5.3.2.1 Summary

5.3.2.1.1 To distinguish varieties on the basis of a quantitative characteristic we need to establish a minimum distance between varieties such that, when the distance calculated between a pair of varieties is greater than this minimum distance, they may be considered as "distinct" in respect of that characteristic. There are several possible ways of establishing minimum distances from Distinctness, Uniformity and Stability (DUS) trials data. Here is described what is known as the Combined-Over-Years Distinctness (COYD) method.

5.3.2.1.2 The COYD method involves:

- for each characteristic, taking the variety means from the two or three years of trials for candidates and established varieties and producing over-year means for the varieties;

- calculate a least significant difference (LSD), based on variety-by-years variation, for comparing variety means.

- if the over-years mean difference between two varieties is greater than or equal to the LSD then the varieties are said to be distinct in respect of that characteristic.

5.3.2.1.3 The main advantages of the COYD method are:

- it combines information from several seasons into a single criterion (the "COYD criterion") in a simple and straightforward way;

- it ensures that judgements about distinctness will be reproducible in other seasons; in other words, the same genetic material should give similar results, within reasonable limits, from season-to-season;

- the risks of making a wrong judgement about distinctness are constant for all characteristics.

5.3.2.2 Introduction

5.3.2.2.1 In order to decide if two varieties are distinct in respect of a measured characteristic, a criterion is needed which will determine whether the differences found in DUS trials are clear and sufficiently consistent. The Combined-Over-Years Distinctness (COYD) method provides such a criterion.

5.3.2.2.2 This paper describes:

- the principles underlying the COYD method;

- UPOV recommendations on the application of COYD to individual species;

- details of ways in which the procedure can be adapted to deal with special circumstances. This includes when there are small numbers of varieties in trial;

– the computer software which is available to apply the procedure.

5.3.2.3 The COYD Method

5.3.2.3.1 The COYD method aims to establish for each characteristic a minimum difference, or distance, which, if achieved by two varieties in trials over a period of two or three years, would indicate that those varieties are distinct with a specified degree of confidence.

5.3.2.3.2 The method uses variation in variety expression of a characteristic from year-to-year to establish the minimum distance. Thus, characteristics which show consistency in variety ranking between years will have smaller minimum distances than those with marked changes in ranking.

5.3.2.3.3 Calculation of the COYD criterion involves analysing the variety-by-year table of means for each characteristic to get an estimate of the varieties-by-years variation, which is used in the next step: to calculate an LSD. Usually data for all candidate and established varieties which appeared in trials over the two or three test years are included in the table, the analysis is by analysis of variance (see TGP 8.5 for details), the varieties-by-years mean square is used as the estimate of the varieties-by-years variation, and the resulting LSD is known as the COYD LSD. However, where there are small numbers of varieties in trial, the approach is different.

5.3.2.3.4 Where there are small numbers of varieties in trial, the table used to calculate of the COYD criterion is expanded with means from other varieties and earlier years, a different method of analysis is used to get a varieties-by-years mean square to estimate the varieties-by-years variation, and the resulting LSD is known as the Long-Term LSD. This is discussed later.

5.3.2.3.5 Equation [1]

$$LSD_p = t_p \times \sqrt{2} \times SE(\bar{x})$$

where $SE(\bar{x})$ is the standard error of a variety's over-year mean calculated as:

$$SE(\bar{x}) = \sqrt{\frac{\text{varieties - by - years mean square}}{\text{number of test years}}}$$

and t_p is the value in Student's t table appropriate for a two-tailed test with probability p and with degrees of freedom associated with the variety-by-years mean square. The probability level p that is appropriate for individual species is discussed under UPOV RECOMMENDATIONS ON COYD below.

5.3.2.3.6 An example of the application of COYD to a small data set is given in Figure 1. Statistical details of the method are in Annex IV and in document TGP/8.5, "Statistical Methods for DUS Examination." Further information about the COYD criterion can be found in Patterson and Weatherup (1984).

5.3.2.4 UPOV Recommendations on COYD

5.3.2.4.1 COYD is recommended for use in assessing the distinctness of varieties where:

– the characteristic is quantitative;

– there are some differences between plants (or plots) of a variety.

– observations are made on a plant (or plot) basis over two or more years;

5.3.2.4.2 A pair of varieties is considered to be distinct if their over-years means differ by at least the COYD LSD in one or more characteristics.

5.3.2.4.3 The UPOV recommended probability level p for the t_p value used to calculate the COYD LSD differs depending on the crop and for some crops depends on whether the test is over two or three years. The testing schemes that usually arise in distinctness testing are described in Annex VI.

5.3.2.5 Adapting COYD to special circumstances

(i) Differences between years in the range of expression of a characteristic

5.3.2.5.1 Occasionally, marked differences between years in the range of expression of a characteristic can occur. For example, in a late spring, the heading dates of grass varieties can converge. To take account of this effect it is possible to fit extra terms, one for each year, in the analysis of variance. Each term represents the linear regression of the observations for the year against the variety means over all years. The method is known as modified joint regression analysis (MJRA) and is recommended in situations where there is a statistically significant ($p \leq 1\%$) contribution from the regression terms in the analysis of variance. Statistical details, and a computer program to implement the procedure, are described in the appendices.

(ii) Small numbers of varieties in trials

5.3.2.5.2 It is recommended that there should be at least 20 degrees of freedom for the varieties-by-years mean square in the COYD analysis of variance. This is in order to ensure that the varieties-by-years mean square is based on sufficient data to be a reliable estimate of the varieties-by-years variation for the LSD. Twenty degrees of freedom corresponds to 11 varieties common in three years of trials, or 21 varieties common in two years. Trials with fewer varieties in common over years are considered to have small numbers of varieties in trial.

5.3.2.5.3 In such trials the variety-by-year tables of means can be expanded to include means for earlier years, and if necessary, other established varieties. As not all varieties are present in all years, the resulting tables of variety-by-year means are not balanced. Consequently, each table is analysed by the least squares method of fitted constants (FITCON) or by REML, which produces an alternative varieties-by-years mean square as a long-term estimate of variety-by-years variation. This estimate has more degrees of freedom as it is based on more years and varieties.

$$\text{degrees of freedom} = \left(\begin{matrix} \text{No. values in expanded} \\ \text{variety - by - year table} \end{matrix} \right) - (\text{No. varieties}) - (\text{No. years}) + 1$$

5.3.2.5.4 The alternative varieties-by-years mean square is used in equation [1] above to calculate an LSD. This LSD is known as a "Long-Term LSD" to distinguish it from COYD LSD based on just the test years and varieties. The Long-Term LSD is used in the same way as the COYD LSD is used to assess the distinctness of varieties by comparing their over-year (the test years) means. The act of comparing the means of varieties using a "Long-Term LSD" is known as "Long-Term COYD".

5.3.2.5.5 Long-Term COYD should only be applied to those characteristics lacking the recommended minimum degrees of freedom. However, when there is evidence that a characteristic's LSD fluctuates markedly across years, it may be necessary to base the LSD for that characteristic on the current two or three-years of data, even though it has few degrees of freedom.

5.3.2.5.6 Figure 2 gives an example of the application of Long-Term COYD to the italian ryegrass characteristic "Growth habit in spring" (UPOV Char 6). A flow diagram of the stages and DUST modules used to produce Long-Term LSD's and perform Long-Term COYD is given in Figure B2 in Annex V.

(iii) Marked year-to-year changes in an individual variety's characteristic

5.3.2.5.7 Occasionally, a pair of varieties may be declared distinct on the basis of a t-test which is significant solely due to a very large difference between the varieties in a single year. To monitor such situations a check statistic is calculated, called F_3, which is the variety-by years mean square for the particular variety pair expressed as a ratio of the overall variety-by-years mean square. This statistic should be compared with F-distribution tables with 1 and g, or 2 and g, degrees of freedom, for tests with two or three years of data respectively where g is the degrees of freedom for the variety-by-years mean square. If the calculated F_3 value exceeds the tabulated F value at the 1% level then an explanation for the unusual result should be sought before making a decision on distinctness.

5.3.2.6 Implementing COYD

The COYD method can be applied using the DUST package for the statistical analysis of DUS data, which is available from Dr. Sally Watson, Biometrics Division, Department of Agriculture for Northern Ireland (DANI), Newforge Lane, Belfast BT9 5PX, United Kingdom. Sample outputs are given in Annex V.

5.3.2.7 References

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PATTERSON, H.D. & WEATHERUP, S.T.C. (1984). Statistical criteria for distinctness between varieties of herbage crops. J. Agric. Sci. Camb. 102, 59-68.

TALBOT, M. (1990). Statistical aspects of minimum distances between varieties. UPOV TWC Paper TWC/VIII/9, UPOV, Geneva.

Figure 1: Illustrating the calculation of the COYD criterion

Characteristic: Days to ear emergence in perennial ryegrass varieties

Varieties	Years 1	2	3	Over Year Means	*Difference (Varieties compared to C2)*	
Reference		Means				
R1	38	41	35	38	*35*	*D*
R2	63	68	61	64	*9*	*D*
R3	69	71	64	68	*5*	*D*
R4	71	75	67	71	*2*	
R5	69	78	69	72	*1*	
R6	74	77	71	74	*-1*	
R7	76	79	70	75	*-2*	
R8	75	80	73	76	*-3*	
R9	78	81	75	78	*-5*	*D*
R10	79	80	75	78	*-5*	*D*
R11	76	85	79	80	*-7*	*D*
Candidate						
C1	52	56	48	52	*21*	*D*
C2	72	79	68	73	*0*	-
C3	85	88	85	86	*-13*	*D*

ANALYSIS OF VARIANCE

Source	df	Mean square
Years	2	174.93
Variety	13	452.59
Variety-by-years	26	2.54

$LSD_p = t_p * \sqrt{2} * SE(\overline{X})$

$LSD_{0.01} = 2.779 * 1.414 * \sqrt{(2.54/3)} = 3.6$

Where t_p is taken from Student's t table with $p = 0.01$ (two-tailed) and 26 degrees of freedom.

To assess the distinctness of a candidate, the difference in the means between the candidate and all other varieties is computed. In practice a column of differences is calculated for each candidate. In this case, varieties with mean differences greater than, or equal to, 3.6 are regarded as distinct (marked *D* above).

Figure 2: Illustrating the application of Long-Term COYD

Characteristic: Growth habit in spring in italian ryegrass varieties

Varieties	Years 1	2	3*	4*	5*	Mean over test years	*Difference (Varieties compared to C2)*	
Reference			Means					
R1	43	42	41	44				
R2		39	45					
R3	43	38	41	45	40	42	*6*	*D*
R4	44	40	42	48	44	44.7	*3.3*	*D*
R5	46	43	48	49	45	47.3	*0.7*	
R6	51	48	52	53	51	52	*-4*	*D*
Candidate								
C1			43	45	44	44	*4*	*D*
C2			49	50	45	48	*0*	
C3			48	53	47	49.3	*-1.3*	

* indicates a test year

The aim is to assess the distinctness of the candidate varieties C1, C2 & C3 grown in the test years 3, 4 & 5.

The trial has a small number of varieties in trial because there are just seven varieties in common over the test years 3, 4 & 5 (data marked by a black border).

FITCON analysis of the variety-by-years table of means expanded to nine varieties in five years gives: varieties-by-years mean square = 1.924, on 22 degrees of freedom

Long-term $LSD_p = t_p * \sqrt{2} * SE(\overline{X})$

Long-term $LSD_{0.01} = 2.819 * 1.414 * \sqrt{(1.924/3)} = 3.19$

Where t_p is taken from Student's t table with $p = 0.01$ (two-tailed) and 22 degrees of freedom

To assess the distinctness of a candidate, the difference in the means between the candidate and all other varieties is computed. In practice a column of differences is calculated for each candidate. In the case of variety C2, varieties with mean differences greater than, or equal to 3.19 are regarded as distinct (marked *D* above).

5.3.3 Long term LSD

Included into Section 9.5.4.2.5, under "(ii) Adapting COYD to special circumstances", prepared by Mrs. Sally Watson

5.3.4 Others

SECTION 6: EXAMPLES OF DISTINCTNESS ASSESSMENT

6.1 Australia

Process for establishing distinctness under the implementation of the Australian's breeder's testing system

6.1.1 In granting of Plant Breeder's Rights (PBR), an examination process is essential in confirming that a new variety meets the technical criteria of Distinctness, Uniformity and Stability (DUS). In most UPOV member states, DUS testing is predominantly done by the relevant official testing authorities at some centralised testing facilities. However, Article 7(1) of the 1978 revision of the UPOV Convention (UPOV 78) and the Article 12 of the 1991 revision of the UPOV Convention (UPOV 91) do not strictly require that the testing should be conducted by the official testing authorities but anticipate that other testing methods could be used.

6.1.2 One such method is the so-called "breeder testing" system where the breeder (or applicant or contractor to the breeder) becomes involved in or undertakes the DUS trial. The level of involvement of the breeder in a breeder testing system varies depending on national circumstances.

6.1.3 The process of establishing distinctness under the implementation of Australian breeder testing system is outlined in the following table:

Process for Establishing Distinctness under the implementation of the Australian Breeder Testing System

MAIN STEPS	DESCRIPTION	OBJECTIVES AND ACTION
Examination of the Part 1 Application [1]	A brief description and a photograph of the variety are supplied. Claim of the main difference (s) of the new variety from the other most similar varieties of common knowledge. Full information on the origin and breeding of the variety is outlined. Indication of the main difference (s) from the parental material if the parents are varieties of common knowledge.	To establish a preliminary (*prima facie*) case that the variety is distinct from all other varieties of common knowledge. PBR offices reviews the Part 1 application. Check the claims against existing data/information. Once the *prima facie* case is established the application is accepted in the PBR scheme and the variety is protected under provisional protection for 12 months. The applicant nominates whether they wish to have the examination based on a comparative trial in Australia or on data provided by another contracting party. In both cases the data has to be verified by a PBR accredited Qualified Person (QP) [2]. *Prima facie* case not established → Application refused.

MAIN STEPS	DESCRIPTION	OBJECTIVES AND ACTION
Comparative Growing Trial in Australia **Applicant obtains UPOV Test Report** **Provisional Protection**	The location of the trial could be in a breeder's or applicant's field or in a PBR accredited Centralised Testing Centre (CTC). The QP to plan and supervise the comparative growing trial. For application based on overseas UPOV test reports, the QP is advised on the need to verify the variety description under local conditions. Upon request and at discretion of the Registrar the 12 months provisional protection period is extendable to allow the establishment of the comparative trial and record observations or to obtain the test report.	The QP reviews the Part 1 application and the UPOV Technical Guideline for the species (if available). By elimination process, The QP selects the most similar varieties of common knowledge for the comparative trial based on the following factors: 1) UPOV grouping characteristics. 2) List of PBR varieties. 3) List of other existing varieties. 4) Suggestions from the PBR office. 5) Parental/source material. 6) Personal experience with the species. 7) From other published information. The QP conducts the comparative growing trial using scientific methodologies. Record data and assessment methods. Confirm the relevant characteristics of the candidate and the comparator varieties with their states of expression. The QP is encouraged to use morphological characteristics; especially those least affected by environmental factors are preferred. Other characteristics, e.g. Phenological, physiological or biochemical are also acceptable if these characteristics meet the requirements of TG/1/3. DNA data is not accepted for establishing distinctness. Quantitative differences are established based on statistical methods. Qualitative differences are established based on visual observation. Comparative photograph is taken to show the differences between the varieties in distinctive characteristics. On the basis of comparative trial, data and photograph, the QP submits the detailed description of the variety for publication in Part 2 application form.

MAIN STEPS	DESCRIPTION	OBJECTIVES AND ACTION
Examination of the Part 2 Application[3]	The QP certifies the authenticity of the data and the scientific methodologies used in conducting the trial. There are severe penalties under the PBR Act for falsifying information or submitting misleading data.	Where necessary, an independent examination of the comparative trial by the PBR examiner at a time when the distinctive characteristics are visible. This ensures that the technical rigor is maintained in the trial and the QP's data is consistent and repeatable. PBR Examiner also checks the trial details and scientific methodologies and reserves the right to order another trial growing by an independent institution.
Examination of the Comparative trial	PBR office examines the Part 2 application and determines the need to independently examine the trial. If necessary, an independent examination is carried out by the PBR examiner. If the PBR office does not examine a trial then the decision is made from information provided that the candidate variety is clearly distinct from other varieties of common knowledge that no further examination is warranted.	PBR Examiner determines the distinctness from own observations in the form of a Field Examination Report. The Examiner's report and the Part 2 data must be consistent for a positive decision on distinctness. If the examiner's report is positive on the decision of distinctness but not consistent with QP's data, then further examination is necessary, or additional data is supplied by the QP. Where the examiner's report is negative the QP is advised and if appropriate, a further trial is conducted, otherwise the applicant is advised to withdraw the application The PBR examiner's decision, whether positive or negative, is reviewed by the Registrar. Distinctness (or U or S) not confirmed → Possible re-trial or withdrawal of the application

MAIN STEPS	DESCRIPTION	OBJECTIVES AND ACTION
Publication of the detailed description of the variety for public review **Public review process**	A public notice is published in the *Plant Varieties Journal*, which includes a detailed description of the variety including its distinctive features along with photograph showing the comparative differences. There is a six-moth waiting period after the publication of the detailed description in the *Plant Varieties Journal* to allow reasonable time for the public or industry to comment or object against a published description.	The 6-month public and peer review process is mandatory. When there is no objection or comments received within this public exposure period then the variety will proceed to a final examination for the grant of PBR. This public and peer review and transparency ensures the rigor of the breeder testing system. If an objection or comment on Distinctness (or U or S) is received within this public exposure period, the PBR office will review the objection and will give opportunity to the applicant to rebut the objection. If the issues are not resolved then a re-trial may be necessary including to re-publish (where necessary) the detailed description of the variety Where an objection is upheld and no further evidence in support of Distinctness (or U or S) is supplied → Rejection of Application.
Deposition of propagating material in a Genetic Resource Centre (GRC)	The applicant must deposit a sufficient quantity of the propagating material of the variety to an approved GRC.	Lodgement of the propagating material in GRC ensure the easy availability of the variety for any future comparative testing purposes and also the reasonable public access of the variety for any other reasons.
Final Grant Examination	Final examination checks that all the formal and technical requirements have been met, including DUS has been established and all objections have been resolved.	DUS is established → Final Grant of PBR DUS not established → Rejection of PBR

Part 1 Application: Australian PBR application comes in two parts, Part 1 and Part 2. The Part 1 Application is similar to the UPOV Technical Questionnaire and has general information about the variety, along with its origin and breeding history and other technical information. The Part 1 application is used to establish a *prima facie* case for the distinctness of the candidate variety.
Qualified Person: A qualified person, or 'QP', acts as a PBR applicant's technical consultant. They accept responsibility for overseeing the comparative trial and for providing evidence that a variety is distinct, uniform and stable. This role may involve the QP consulting on choice of comparative varieties, experimental design, management regime, collection of data, statistical analysis, photography and preparation of the harmonised description of the variety.

Part 2 Application: The Part 2 Application is submitted after the comparative trial has been completed. It contains the harmonised description of the variety including its distinctness, uniformity and stability. The QP certifies the authenticity of the description as well as the data and the scientific methodologies on which it is based.

6.2 France

6.2.1 Centralized official testing system

6.2.1.1 Background

6.2.1.1.1 In France, for most of the crops DUS testing can be characterized to be a centralized official testing system.

6.2.1.1.2 DUS testing is entrusted to an independent staff working for the Ministry of Agriculture (around 90 permanent civil servants). Most of them are employed at G.E.V.E.S. (Groupe d'études et de contrôle des variétés et des semences) which is the official agency settled by the French authorities to conduct the tests for national listing and plant breeders rights.

6.2.1.1.3 The Centralization of the tests is implemented in order to provide a common environmental basis for the technical examination of varieties and to facilitate the control of the interaction between varieties and environmental conditions.

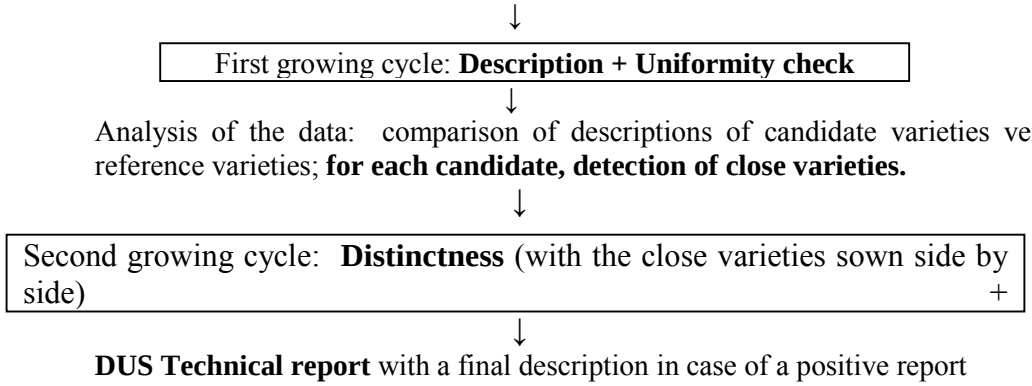
6.2.1.2 French Approach

6.2.1.2.1 Under the centralized system, all new varieties and reference varieties are described and compared in the same environment. The DUS testing procedures under this system is highlighted below in the case of an annual species:

(a) General DUS procedure for annual species

(b) Reception of an application with

- Description of the variety by the breeder
  (= technical questionnaire + additional characteristics)
- plant material



6.2.1.2.2 The management of reference collections requires careful consideration. Reference collections are composed of varieties listed and/or protected in France and in the countries with similar environmental conditions. The reference collection is up-dated each year: for each new variety, the breeder is asked to provide a seed sample and variety description. Reference seed samples are stored in cold chamber (at 5°C and at 30% relative humidity). Currently, seed samples are stored for example:

for 1200 wheat varieties
for 2000 sunflower varieties
for 3800 maize varieties
for 300 rape seed varieties.

6.2.1.2.3 The new entries in the reference collection are described under the French conditions during 2 or 3 years. After this period, these varieties are included in the trials only if necessary, depending upon the characteristics of the candidate varieties. Example varieties are systematically included in the trials.

6.2.1.2.4 The degree of involvement of the breeder in the conduct of the trials is quite low: the test is entirely done with GEVES facilities. Nevertheless, a close contact is kept with the breeder during each step of the process in order to inform him of any problem encountered and to invite him to submit complementary information if necessary. The DUS reports are established by GEVES.

6.2.2 DUS procedure on maize with the participation of the applicant

6.2.2.1 Summary for the specific conditions and rules for decision

AIM : To speed up the official studies

To get a better involvement of the applicant in the variety description work.

To limit the workload.

CONDITIONS :

OFFICIAL AGREEMENT OF THE APPLICANT BASED ON:

- The presence, for at least 5 years on French territory, of a nursery containing inbred lines, with observations on candidate and examples varieties

- The presence of technical staff able to make the description

Regular training courses and an examination to check this ability.

The Technical Committee for National Listing is in charge of the application of this official agreement on behalf of the Ministry of Agriculture.

APPLICATION FILES

- YEAR 1*: Declaration of the application

* Year 1: year during which the applicant produces the description on his own premises;

Year 2: year during which the official service produces the description and conducts the DUS study.

- YEAR 2*: Submission of all the information as requested for an application without the participation of the applicant.

  Additional information on the parental lines (if not already known)

  - Genetic origin : compulsory, possibly submitted in a separate document.
  - A set of characteristics in addition to those already mentioned in the UPOV Technical Questionnaire (16 additional characteristics)
  - Description of 11 electrophoretic characteristics

Recommendations are made on how to establish descriptions:

- Qualitative characteristics : at least 10 individual observations
- Quantitative characteristics : average value of 10 measurements and indication of the value of the closest example varieties ;
- Electrophoretic characteristics: electrophoretic pattern established on at least 4 grains plus 16 grains if there is any heterogeneity. The recommended method is described in the Handbook published by GEVES.

PLANT MATERIAL SUBMISSION

- YEAR 1: Submission of a small sample (200 kernels) of each parental line
- YEAR 2: Submission of the different categories (hybrid, - components as for any application without participation of the applicant)

  Submission of 6 non threshed ears of each parental line - - - (if not already known) with at least 100 kernels (70 for flint parental lines)

VISIT TO THE BREEDER'S PREMISES

DECISION RULES:

OFFICIAL AGREEMENT

- The agreement can be cancelled if any of the conditions are no longer fulfilled
- The agreement can also be cancelled if any applicant does not respect the general rules or if too many discrepancies appear between the descriptions submitted by the applicant in year 1 and those produced by the official service in year 2

DUS REPORT

- General rules are applicable as soon the description submitted by the applicant is officially validated.

- Validation of the description :

  If there is any discrepancy between the description submitted by the applicant and the one established by GEVES, the description made by the applicant is rejected and a third year must be undertaken.

  Discrepancies :

  • A discrepancy exists if, for any characteristic, the difference between the 2 notes for a given characteristic is higher than the minimum distance considered in the automatic comparison procedure (minimum distance = distance which is used in the software to take into account a difference).

  • For electrophoretic characteristics, no discrepancy is accepted.

- Distinctness

If it is not a problem to establish a clear distinctness based on the automatic comparison procedure on the direct observations in the trial conducted by GEVES, the inbred line is declared distinct.

If not, a third year is requested.

- Uniformity and Stability

If the uniformity of the reference seed-lot fulfils the UPOV requirement and if no more than 1 ear-row is different from the others and the reference seed-lot, the inbred line is declared uniform and stable.

If there is a lack of uniformity on either the reference seed-lot or the ear rows, a third year is requested.

If both lack uniformity, the inbred line is declared not uniform and stable.

- Description : in the case of a positive DUS report, the description is established using the description submitted by the applicant and the two descriptions (two locations) made by GEVES.

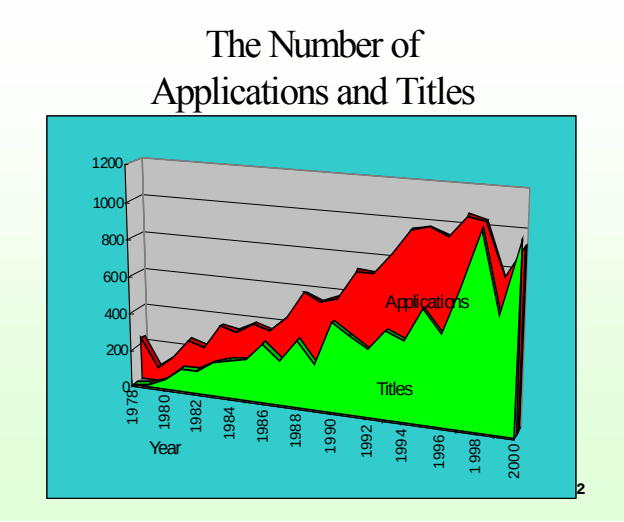
As soon as an inbred line has a positive report using this procedure, the general rules for conducting the DUS test on a hybrid including this inbred line can be applied.

6.3 Japan

6.3.1 Background

The number of applications and PVP titles granted is illustrated in Illustration 6. Applications have been filed for 575 species and genera. Since the introduction of the plant variety protection system in Japan in 1979, a total of 14,531 applications have been filed. Rose (1566), Chrysanthemum (1496), Carnation (1244), Cymbidium (834) and Rice (492) are the five top crop species, representing 38.8% of the total applications

Illustration 5



6.3.2 Japanese Approach

6.3.2.1 All PVP applications are addressed to the Minister for Agriculture, Forestry and Fisheries. The administration of the plant variety protection is the responsibility of the Seeds and Seedlings Division of the Ministry of Agriculture, Forestry and Fisheries (MAFF). An application filed with the Seeds and Seedlings Division first undergoes a formal examination and then a technical examination known as DUS testing. An examination of the proposed variety denomination is also conducted. At this stage the application is published for public comments.

The DUS testing is conducted in the following three forms:

(1) Government Growing Test
(2) On-site Inspection by Government Officials
(3) Documentary Examination

6.3.2.2 For each application the examiner should decide on how the DUS test should be conducted. The National Center for Seeds and Seedlings (NCSS) has been designated to undertake Government Growing Test. (As a result of the recent reorganization of the MAFF, the NCSS has been separated from the MAFF and has received the status of an “Independent Administrative Institution.”) Government Growing Test may also be conducted by public research stations or other appropriate institutions with necessary expertise on the crop in question, under the instruction of the examiner and in accordance to national test guidelines. The key features of the three forms are summarized below:

(1) Government Growing Test

– Conducted mainly by the National Center for Seeds and Seedlings (NCSS)
– Also conducted by a local government research institute (e.g. for rice)
– Used for vegetables, ornamental plants
– NCSS establishes the final DUS test report and variety description

(2) On-site Inspection by Government Officials

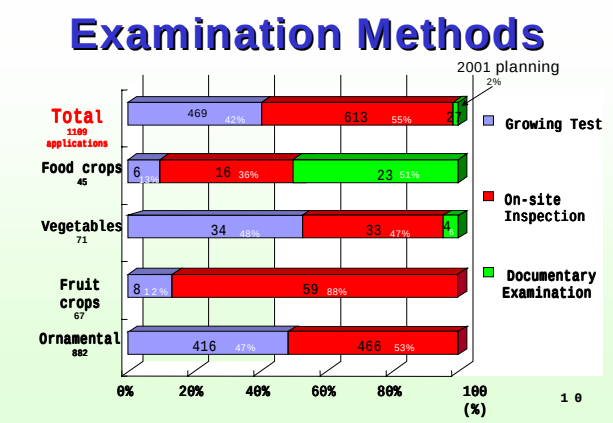
– Examiner to judge the qualification of the applicant for the setting of DUS testing on his own premises. National test guidelines are used to provide guidance.
– Used mainly for ornamental plants (orchids, rose) and fruit trees)
– Examiner visits the site of testing to verify the conformity of the test design with the instructions given in the National test guidelines and collect data for DUS test report
– Examiner establishes the final DUS test report and variety description

(3) Documentary Examination

– If a candidate variety has been tested by a public research institute for more than one year and the data provided can be considered to be reliable, the examiner may base his decision exclusively on the technical data prepared by that research institute
– The examiner can ask the research institute to submit additional data if thought necessary

6.3.2.3 The examiner takes a decision on the grant of a protection title on the basis of the test report. The examiner establishes a final description of the candidate variety. Unless any reason to reject the application has been found, or any objection or other relevant comment that might be influential on the fate of the application has been received from the public, the candidate variety should be granted a protection title.

Illustration 6 shows the how DUS test is arranged for different categories of crops.



6.3.3 Procedure of DUS Testing in Rice in Japan

6.3.3.1 Most of rice breeding activities in Japan are conducted by public breeding stations (either of the central Government or of local governments). In the formal rice breeding conducted by public breeding stations, official trials on the Value for Cultivation and Use (VCU) should be conducted before the release of any new rice varieties. Only those varieties which are officially recognized as being superior to the existing varieties will be commercialized. Normally, DUS data are also collected to ensure the reliability of the VCU trials. It is felt that in the case of rice varieties bred by Governmental breeding centers where

all technical information is collected systematically with a high level of technical reliability, the PVP examiner can safely use the technical data provided by the breeders (researchers working at governmental research institutes). Technical data provided by prefectures were also thought to be as reliable, if the PVP examiner of the MAFF retains the possibility of undergoing an inspection in the field from where the DUS data have been collected.

6.3.3.2 In the case of rice varieties bred by farmers or seed companies, which are not necessarily considered to have adequate ability of conducting DUS testing and preparing a DUS test report, a mechanism is provided to complement the DUS test results prepared by the breeders through additional trials conducted under the guidance of the PVP examiner. Because of the wide range of different environmental conditions under which rice varieties are bred in Japan (certain characteristics can be expressed only under specific environmental conditions), additional DUS testing is conducted by different regional (prefectural or governmental) rice breeding stations, which are thought to be the best location for the expression of characteristics of candidate varieties.

ANNEX I: A SIMPLE EXAMPLE OF DISTANCE COMPUTATION ON 5 QUALITATIVE CHARACTERISTICS

1. The software examines differences for each characteristic and attributes the appropriate weighting. The weighting (stored in matrices in the database) is defined by the crop experts for each characteristic before the computation.

2. Weighting matrices are established by the crop experts on the basis of their expertise.

3. For a given difference in absolute values, the weighting can change according to the characteristic.

	Ear shape	Husk length	Type of grain	Number of rows of grain	Ear diameter	
Notes for variety A (1 to 9 scale)	1	1	4	6	5	
Notes for variety B (1 to 9 scale)	3	3	4	4	6	
Difference observed	2	2	0	2	1	
Weighting, according to the crop expert	*6*	*0*	*0*	*2*	*0*	*8*

Sum of weighting = Estimation of the phenotypic distance between A and B

4. In this crop, a difference of 2 notes in the absolute value is attributed:

- a weighting/distance of 6 for the characteristic Ear shape,
- a weighting/distance of 0 for the characteristic Husk length,
- a weighting/distance of 2 for the characteristic Number of rows of grain,

5. The crop experts, therefore, consider that the difference of 2 notes on “Ear shape” indicates a greater distance between two varieties than it does on “Number of rows of grain.”

6. The crop experts also consider that, for characteristic “Husk length”, note 1 for one variety and note 3 for another variety is not sufficient to indicate a distance between two varieties.

ANNEX II: EXAMPLE WITH QUALITATIVE, ELECTROPHORETIC AND QUANTITATIVE CHARACTERISTICS (*Zea mays* DATA)

Qualitative characteristics are observed on a 1 to 9 scale. For each characteristic, weighting according to differences between levels of expression are pre-defined in a matrix of distances.

Example

For the characteristic "Shape of ear", observed on a 1 to 3 scale, the crop experts have attributed weighting to differences which they consider significant:

1 = conical
2 = conico-cylindrical
3 = cylindrical

		Variety i		
		1	2	3
Variety j	1	0	2	6
	2		0	2
	3			0

When the crop experts compare a variety i with conical ear (noted 1) to a variety j with cylindrical ear (noted 3), they attribute a weighting of 6.

For the characteristic "Length of husks", observed on a 1 to 9 scale, the crop experts have defined the weighting matrix:

1 = very short
2 = very short to short
3 = short
4 = short to medium
5 = medium
6 = medium to long
7 = long
8 = long to very long
9 = very long

		Variety i								
		1	2	3	4	5	6	7	8	9
Variety j	1	0	0	0	2	2	2	2	2	2
	2		0	0	0	2	2	2	2	2
	3			0	0	0	2	2	2	2
	4				0	0	0	2	2	2
	5					0	0	0	2	2
	6						0	0	0	2
	7							0	0	0
	8								0	0
	9									0

For this characteristic, the weighting between a variety i with very short husks (noted 1) and a variety j with short husks (noted 3) is 0.

Experts consider a difference of 3 notes is necessary in order to recognise a non-zero distance between two varieties.

Even if the difference in notes is bigger than 3, the experts do not increase the distance more than 2.

The reason for using a lower weighting for some characteristics compared to others can be that they are less "reliable" or "consistent" (e.g. more subject to the effect of the environment); and/or they are considered to indicate a lower distance between varieties.

A weighting matrix must be defined for each qualitative characteristic.

In this example, we will assume the crop expert has decided to use a distance threshold $\mathbf{S_{dist}}$ of 10 as an indicator of whether two varieties are close or not.

Let us take the first example with A and B observed for 5 qualitative characteristics:

	Ear shape	Husk length	Type of grain	Number of rows of grain	Ear diameter	
Notes for variety A (1 to 9 scale)	1	1	4	6	5	
Notes for variety B (1 to 9 scale)	3	3	4	4	6	
Difference observed	2	2	0	2	1	
Weighting according to the crop expert	*6*	*0*	*0*	*2*	*0*	$D_{qual} = 8$

In our example $\mathbf{D_{qual} = 8 < S_{dist}}$ so varieties A and B are declared "GAIA NON-distinct" and can be passed on to electrophoretic analysis.

Electrophoretic analysis

The electrophoretic characteristic is a homozygous allele in the UPOV Test Guidelines (see Diagram 3). The software does not allow the use of heterozygous alleles.

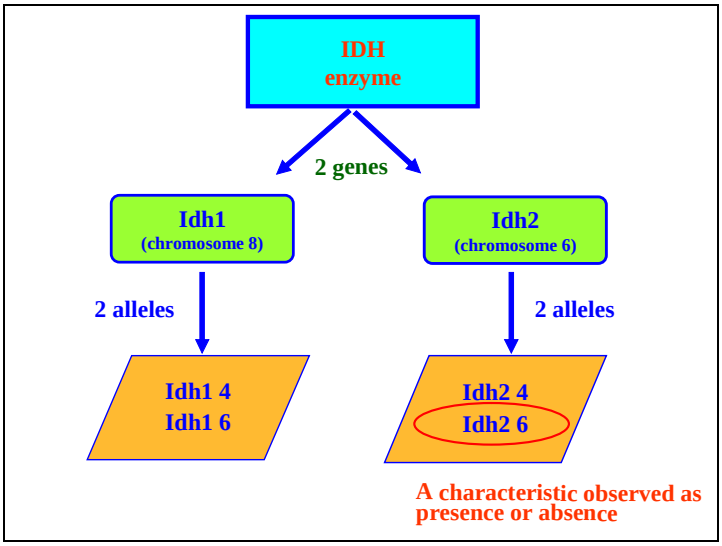
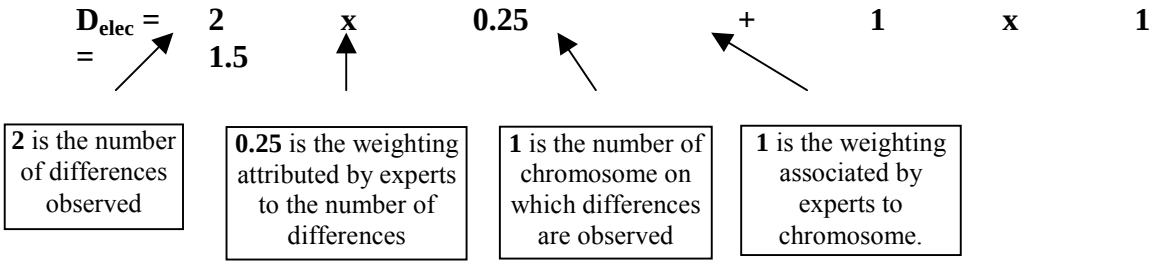


Diagram 3: The Isocitrate Deshydrogenase (IDH) enzyme has two genes (Idh1 and Idh2) located on two different chromosomes. Each of them has two alleles which are observed as 1 (presence) or 0 (absence).

Electrophoretic characteristics are noted 0 or 1 as absence or presence. The decision rule, used to give a weighting to two varieties, is the addition of the weighting number of differences observed and the weighting number of chromosomes related to these differences (see example below).

	Chromosome 8		Chromosome 6	
	Idh1 4	Idh1 6	Idh2 4	Idh2 6
Variety A	0	1	1	0
Variety B	0	1	0	1
Difference	0	0	1	1

In this example, varieties A and B are described for 4 electrophoretic characteristics: Idh1 4, Idh1 6, Idh2 4 and Idh2 6. The software looks at differences and gives the phenotypic distance using the following computation:



This formula, which might be difficult to understand, was established by the crop experts in collaboration with biochemical experts. Both the *number of differences* and the *number of chromosomes on which differences are observed* are used. Thus, less importance is attached to differences when these occur on the same chromosome, than when they occur on different chromosomes.

After qualitative and electrophoretic analysis, the phenotypic distance between varieties A and B is equal to:

$$\mathbf{D = D_{qual} + D_{elec} = 8 + 1.5 = 9.5}$$

The phenotypic distance is *lower than* $\mathbf{S_{dist}}$, *therefore varieties A and B are considered "GAIA NON-distinct"*.

Note: It is not possible to establish distinctness solely on the basis of electrophoretic analysis. It is necessary to have a minimal phenotypic distance in qualitative analysis in order to take into account the electrophoresis results. This minimal phenotypic distance must also be defined by crop experts. (For example, in France this value is 3 for rapeseed and 1 for maize with a distinction threshold equal to 6.)

Quantitative Analysis

For each quantitative characteristic, the comparison of two varieties is made by looking for consistent differences in at least two different experimental units. Experimental units are defined by the user depending on data present in the database.
It can, for example, be the data from two geographic locations of the first growing cycle, or 2 or 3 replications in the case of a single geographical location.

For a comparison to be made, the two varieties must be present in the same experimental units.

Differences observed must be greater than one of the two threshold values (or minimal distances), fixed by the crop experts.

- $\mathbf{D_{min\text{-}inf}}$ is the lower value from which a weighting is attributed,

- $\mathbf{D_{min\text{-}sup}}$ is the higher minimal distance. These values could be chosen arbitrarily or calculated (15% and 20% of the mean for the trial, or LSD at 1% and 5%, etc.)

For each minimal distance a weighting is attributed:

- $\mathbf{D_{min\text{-}inf}}$ a weighting P_{min} is attributed;

- $\mathbf{D_{min\text{-}sup}}$ a weighting P_{max} is attributed;

- the observed difference is lower than $\mathbf{D_{min\text{-}inf}}$ a zero weighting is associated.

Varieties A and B have been measured for characteristics "Width of blade" and "Length of plant" in two trials.

For each trial, and each characteristic, the crop experts have decided to define $\mathbf{D_{min\text{-}inf}}$ and $\mathbf{D_{min\text{-}sup}}$ by calculating respectively the 15% and 20% of the mean for the trial:

	Width of blade		Length of plant	
	Trial 1	Trial 2	Trial 1	Trial 2
$\mathbf{D_{min\text{-}inf}}$ = 15% of the mean	1.2 cm	1.4 cm	28 cm	24 cm
$\mathbf{D_{min\text{-}sup}}$ = 20% of the mean	1.6 cm	1.9 cm	37 cm	32 cm

For each characteristic: the crop experts have attributed the following weighting:

A weighting $\mathbf{P_{min}}$ = 3 is attributed when the difference is greater than $\mathbf{D_{min\text{-}inf}}$.

A weighting $\mathbf{P_{max}}$ = 6 is attributed when the difference is greater than $\mathbf{D_{min\text{-}sup}}$.

	Width of blade		Length of plant		
	Trial 1	Trial 2	Trial 1	Trial 2	
Variety A	9.9 cm	9.8 cm	176 cm	190 cm	
Variety B	9.6 cm	8.7cm	140 cm	152 cm	
Difference	0.3 cm	1.1 cm	36 cm	38 cm	
Weighting according to the crop expert	0	0	3	6	D_{quan} =?

In our example, for the characteristic "Width of blade", the differences observed are lower than $\mathbf{D_{min\text{-}inf}}$, so no weighting is associated.

On the other hand, for the characteristic "Length of plant" one difference is greater than the $\mathbf{D_{min\text{-}inf}}$ value and the other is greater than the $\mathbf{D_{min\text{-}sup}}$ value. These two differences are attributed different weightings.

The user must, therefore, decide which weighting will be used for the analysis:

- minimalist option: the weighting chosen is that attributed to the lowest difference;
- maximalist option: the weighting chosen is that attributed to the highest difference;
- mean option: the weighting chosen is the mean of the others.

In this example, the crop experts have decided to choose the lowest of the two weightings, so the phenotypic distance based on quantitative characteristics is $\mathbf{D_{quan} = 3}$.

In summary, at the end of all analysis, the phenotypic distance between varieties A and B is:

$$\mathbf{D = D_{qual} + D_{elec} + D_{quan} = 8 + 1.5 + 3 = 12.5 > S_{dist}}$$

The phenotypic distance is greater than the distinction threshold $\mathbf{S_{dist}}$, fixed by the crop experts at 10, so varieties A and B are declared "GAIA-distinct".

In this example, the use of electrophoresis data "confirms" a distance between the two varieties; but on the basis of qualitative and quantitative data alone, the threshold is exceeded (8 + 3 = 11 is greater than 10).

If the threshold had been set at 6, the difference on the characteristic ear shape would have been sufficient, as variety A is conical and variety B is cylindrical, which is already a clear difference.

1 = conical
2 = conico-cylindrical
3 = cylindrical

Variety i			
	1	2	3
1	0	2	6
2		0	2
3			0

Quantitative and qualitative analysis on the same characteristics

For some crops, it is common practice to produce notes on a 1 to 9 scale for quantitative characteristics. Sometimes the transformation process is very simple, sometimes it is a complex process where all available data are used, but with a special manipulation of example varieties to adjust the raw values to the notes on the scale.

GAIA can include both as two separate characteristics: the original quantitative scale; and the "transformed into qualitative notes" scale. They are associated in the description of the characteristics.
Using the knowledge of this association, when quantitative and qualitative characteristics are both present, only one characteristic is kept, in order to avoid the information being used twice.

Conclusion of Annex II

The above example was described in order to explain how GAIA uses different types of characteristics in a practical case.

The efficiency of the use of GAIA depends on the species. The following extract from the Powerpoint presentation shown at the TWC in Mexico in 2002 illustrates the potential in a crop where many years of experience are available.

Results obtained in 2000

Zea Mays

2420 inbred lines in the reference collection
307 new inbred lines in the first year of study

836 882 comparisons to be done

GAÏA

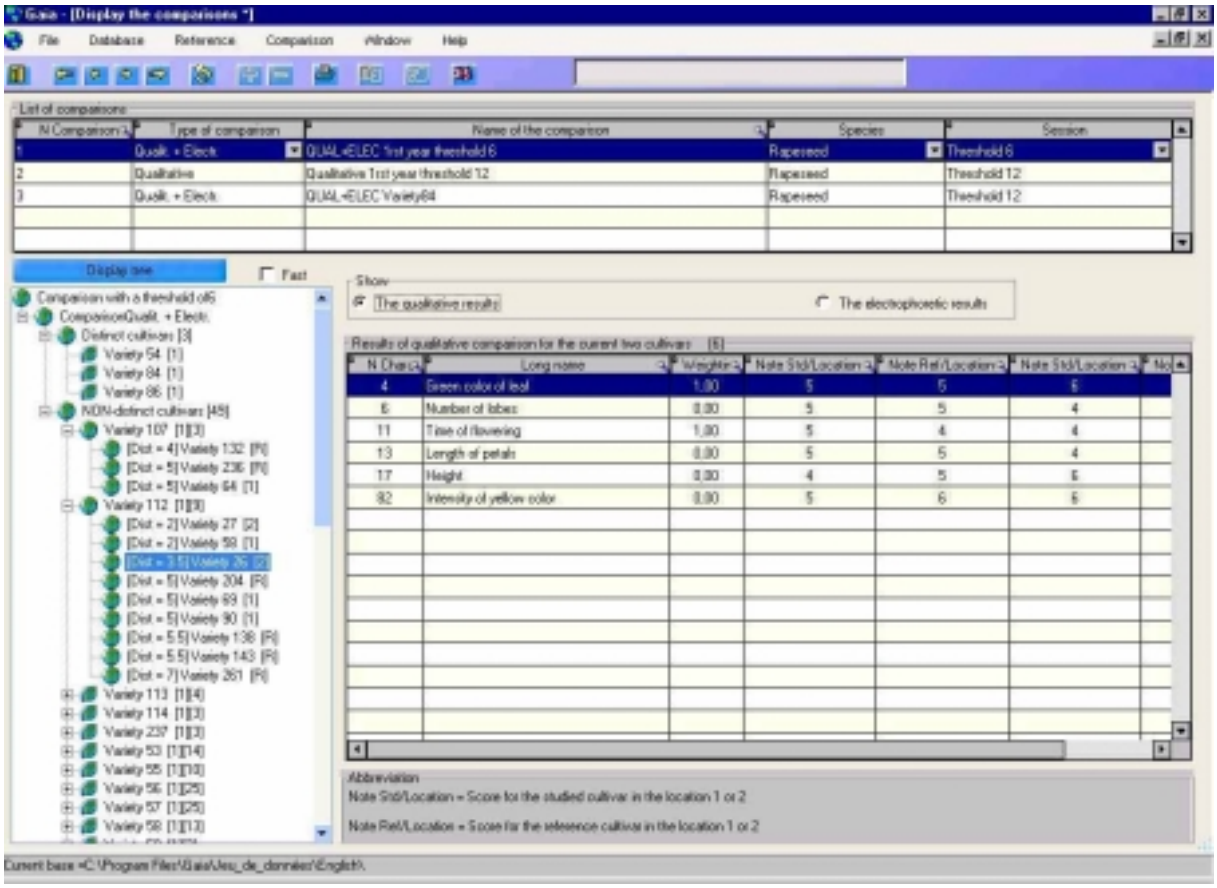
- 142 candidate varieties are distinct + (43 without electrophoresis)
- 165 candidate varieties are not distinct +

A candidate variety has on average 5.25 non-distinct varieties (17.7 without electrophoresis)

==> 864 comparisons must be done in the field in the second year of study

ANNEX III

ANNEX III: SCREEN COPY



The upper part shows 3 different computations which have been kept in the database.

The display tree on the left shows results for a [qualitative + electrophoresis at threshold of 6] computation.

Distinct cultivars [3] demonstrates that 3 varieties were found distinct from all others. There was a total of 52 (49 + 3) cultivars in the computation.

The display tree is used to navigate through all possible pairs.

The user can expand or reduce the branches of the tree according to his needs.

NON-distinct cultivars [49]. Forty-nine cultivars were found "not distinct from all others" with a threshold of 6.

The first variety, *Variety 107*, has only 3 close varieties, whereas the second, *Variety 112*, has 9 close varieties, the third, *Variety 113*, 4 close varieties, etc.

The raw data for *Variety 112* and *Variety 26* are visible for the 6 qualitative characteristics observed on both varieties.

Variety 112 [1][9] indicates variety 112 is in the first year of examination *[1]*; and has 9 close varieties according to the threshold of 6 *[9]*.

[dist=3.5]Variety 26 [2] indicates variety 26 has a GAIA distance of 3.5 from variety 112, which is in second year of examination.

The third column is the weighting according to the pre-defined matrices. The notes for both varieties are displayed for the two available locations (Std stands for "studied" which are the candidate varieties).

In this screen copy the varieties have been numbered for sake of confidentiality, the crop experts can name the varieties according to their need (lot or application number, name, etc.).

ANNEX IV: COYD STATISTICAL METHODS

ANALYSIS OF VARIANCE

The standard errors used in the COYD criterion are based on an analysis of variance of the variety-by-years table of a characteristic's means. For *m* years and *n* varieties this analysis of variance breaks down the available degrees of freedom as follows:

Source	Df
Years	*m*-1
Varieties	*n*-1
Varieties-by-years	(*m*-1)(*n*-1)

MODIFIED JOINT REGRESSION ANALYSIS (MJRA)

As noted above, the COYD criterion bases the SE of a variety mean on the varieties-by-years variation as estimated by the varieties-by-years mean square. Systematic variation can sometimes be identified as well as non-systematic variation. This systematic effect causes the occurrence of different slopes of the regression lines relating variety means in individual years to the average variety means over all years. Such an effect can be noted for the heading date characteristic in a year with a late spring: the range of heading dates can be compressed compared with the normal. This leads to a reduction in the slope of the regression line for variety means in that year relative to average variety means. Non-systematic variation is represented by the variation about these regression lines. Where only non-systematic varieties-by-years variation occurs, the slope of the regression lines have the constant value 1.0 in all years. However, when systematic variation is present, slopes differing from 1.0 occur but with an average of 1.0. When MJRA is used, the SE of a variety mean is based on the non-systematic part of the varieties-by-year variation.

The difference between the total varieties-by-years variation and the varieties-by-years variation adjusted by MJRA is illustrated in Figure B1, where variety means in each of three years are plotted against average variety means over all years. The variation about three parallel lines fitted to the data, one for each year, provides the total varieties-by-years variation as used in the COYD criterion described above. These regression lines have the common slope 1.0. This variation may be reduced by fitting separate regression lines to the data, one for each year. The resultant residual variation about the individual regression lines provides the MJRA-adjusted varieties-by-years mean square, on which the SE for a variety mean may be based. It can be seen that the MJRA adjustment is only effective where the slopes of the variety regression lines differ between years, such as can occur in heading dates.

The use of this technique in assessing distinctness has been included as an option in the computer program which applies the COYD criterion in the DUST package. It is recommended that it is only applied where the slopes of the variety regression lines are significantly different between years at the 1% significance level. This level can be specified in the computer program.

To calculate the adjusted variety means and regression line slopes the following model is assumed.

$$y_{ij} = u_j + b_j v_i + e_{ij}$$

where y_{ij} is the value for the i^{th} variety in the j^{th} year.

u_j is the mean of year j (j = 1, ..., m)

b_j is the regression slope for year j

v_i is the effect of variety i (i = 1, ..., n)

e_{ij} is an error term.

From equations (6) and (7) of Digby (1979), with the meaning of years and varieties reversed, the following equations relating these terms are derived for the situation where data are complete:

$$\sum_{i=j}^{n} v_i y_{ij} = b_j \sum_{i=1}^{n} v_i^2$$

$$\sum_{j=1}^{m} b_j y_{ij} = v_i \sum_{j=1}^{m} b_j^2$$

1. These equations are solved iteratively. All b_j values are taken to be 1.0 as a starting point in order to provide values for the v_i's. The MJRA residual sum of squares is then calculated as:

2. $$\sum_{j=1}^{m} \sum_{i-1}^{n} \left(y_{ij} - u_j - b_j v_i\right)^2$$

3. This sum of squares is used to calculate the MJRA-adjusted varieties-by-years mean square on $(m-1)(n-1) - m + 1$ degrees of freedom.

ALTERNATIVE CRITERIA

An earlier UPOV distinctness criterion is known as the 2x1% criterion. This criterion is still used in some crops, where COYD has been found not to work satisfactorily.

For two varieties to be distinct using the 2x1% criterion, the varieties must be significantly different in the same direction at the 1% level in at least two out of three years in one or more measured characteristics. The tests in each year are based on Student's two-tailed t-test of the variety means with standard errors estimated using the plot residual mean square.

The main problems with the 2x1% criterion are that:

– Information is lost because the criterion is based on the accumulated decisions arising from the results of t-tests made in each of the test years. Thus, a difference

which is not quite significant at the 1% level contributes no more to the separation of a variety pair than a zero difference or a difference in the opposite direction. For example, three differences in the same direction, one of which is significant at the 1% level and the others at the 5% level would not be regarded as significant evidence for distinctness.

– Variety measurements on some characteristics are less consistent over years than on others. However, beyond requiring differences to be in the same direction in order to count towards distinctness, the 2x1% criterion takes no account of consistency in the size of the differences from year to year.

It can be shown that, for a three-year test, the COYD criterion applied at the 1% probability level is of approximately the same stringency as the 2x1% criterion for a characteristic where the square root of the ratio of the variety-by-years mean square to the variety-by-replicates-within-trials mean square (λ) has a value of 1.7. The COYD criterion applied at the 1% level is less stringent than the 2x1% criterion if $\lambda < 1.7$, and more stringent if $\lambda > 1.7$.

[Annex V follows]

ANNEX V: COYD SOFTWARE

COYD COMPUTER PROGRAM

An example of the output from the computer program in the DUST package which applies the COYD criterion is given in Tables B 1 to 3. It is taken from a perennial ryegrass (diploid) trial involving 40 reference varieties (R1 to R40) and 9 candidate varieties (C1 to C9) in 6 replicates on which 8 characteristics were measured over the years 1988, 1989 and 1990.

Each of the 8 characteristics is analysed by analysis of variance. As this analysis is of the variety-by-year-by-replicate data, the mean squares are 6 (= number of replicates) times the size of the mean squares of the analysis of variance of the variety-by-year data referred to in the main body of this paper. The results are given in Table B 1. Apart from the over-year variety means there are also presented:

YEAR MS:	the mean square term for years
VARIETY MS:	the mean square term for varieties
VAR.YEAR MS:	the mean square for varieties-by-years interaction
F1 RATIO:	ratio of VARIETY MS to VAR.YEAR MS (a measure of the discriminating power of the characteristic - large values indicate high discriminating power)
VAR.REP MS:	average of the variety-by-replicate mean squares from each year
LAMBDA VALUE (λ):	square root of the ratio of VAR.YEAR MS to VAR.REP MS
BETWEEN SE:	standard error of variety means over trials on a plot basis i.e. the square root of the VAR.YEAR MS divided by 18 (3 years x 6 replicates)
WITHIN SE:	the standard error of variety means within a trial on a plot basis i.e. the square root of the VAR.REP MS divided by 18
DF:	the degrees of freedom for varieties-by-years
MJRA SLOPE:	the slope of the regression of a single year's variety means on the means over the three years
REGR F VALUE:	the mean square due to MJRA regression as a ratio of the mean square about regression
REGR PROB:	the statistical significance of the REGR F VALUE
TEST:	indicates whether MJRA adjustment was applied (REG) or not (COY).

Each candidate variety is compared with every other candidate and reference variety. The mean differences between pairs of varieties are compared with the LSD for the characteristic. The results for the variety pair R1 and C1 are given in Table B 2. The individual within year t-values are listed to provide information on the separate years. Varieties R1 and C1 are considered distinct since, for at least one characteristic, a mean difference is COYD significant at the 1% level. If the F_3 ratio for characteristic 8 had been significant at the 1% level rather than the 5% level, the data for characteristic 8 would have been investigated, and because the differences in the three years are not all in the same direction, the COYD significance for characteristic 8 would not have counted towards distinctness.

The outcome in terms of the tests for distinctness of each candidate variety from all other varieties is given in Table B 3, where D indicates “distinct” and ND denotes “not distinct.”

Table B 1: An example of the output from the COYD program showing variety means and analysis of variance of characteristics

PRG (DIPLOID) EARLY N.I. UPOV 1988-90

	VARIETY MEANS OVER YEARS							
	5	60	8	10	11	14	15	24
	SP.HT	NSPHT	DEEE	H.EE	WEE	LFL	WFL	LEAR
1 R	45.27	34.60	67.87	45.20	70.05	20.39	6.85	24.54
2 R2	42.63	31.84	73.85	41.96	74.98	19.68	6.67	24.44
3 R3	41.57	27.40	38.47	27.14	57.60	17.12	6.85	22.57
4 R4	33.35	21.80	77.78	30.77	78.04	18.25	6.40	21.09
5 R5	37.81	25.86	50.14	27.24	62.64	16.41	6.41	16.97
6 R6	33.90	21.07	78.73	32.84	79.15	19.44	6.46	21.79
7 R7	41.30	31.37	73.19	41.35	71.87	20.98	6.92	24.31
8 R8	24.48	19.94	74.83	32.10	62.38	15.22	6.36	19.46
9 R9	46.68	36.69	63.99	44.84	68.62	18.11	7.02	22.58
10 R10	25.60	20.96	75.64	32.31	57.20	14.68	5.51	20.13
11 R11	41.70	30.31	74.60	40.17	76.15	19.45	6.79	22.72
12 R12	28.95	21.56	66.12	27.96	59.56	14.83	5.53	20.55
13 R13	40.67	29.47	70.63	36.81	74.12	19.97	7.04	24.05
14 R14	26.68	20.53	75.84	34.14	63.29	15.21	6.37	20.37
15 R15	26.78	20.18	75.54	30.39	66.41	16.34	6.01	20.94
16 R16	42.44	27.01	59.03	30.39	72.71	17.29	6.47	22.48
17 R17	27.94	21.58	76.13	32.53	68.37	16.72	6.11	22.03
18 R18	41.34	30.85	69.80	37.28	69.52	20.68	7.09	25.40
19 R19	33.54	23.43	73.65	30.35	75.54	18.97	6.37	22.43
20 R20	44.14	34.48	68.74	42.60	64.17	18.63	6.56	22.02
21 R21	27.77	21.53	80.52	31.59	69.41	16.81	5.81	22.35
22 R22	38.90	27.83	75.68	43.25	75.08	19.63	7.46	23.99
23 R23	42.43	31.80	72.40	42.07	74.77	20.99	6.78	23.57
24 R24	38.50	27.73	73.19	37.12	75.76	19.28	6.91	22.77
25 R25	43.84	29.60	68.82	39.79	74.83	20.63	7.08	22.65
26 R26	49.48	36.53	63.45	42.01	70.46	22.14	7.84	25.91
27 R27	25.61	19.25	78.78	29.81	56.81	15.81	5.07	18.94
28 R28	26.70	20.31	79.41	32.75	66.54	16.92	6.00	21.91
29 R29	27.90	20.94	72.66	29.85	67.14	16.85	6.28	21.79
30 R30	43.07	30.34	70.53	40.51	73.23	19.49	7.28	23.70
31 R31	38.18	25.47	74.23	36.88	80.23	20.40	7.09	25.21
32 R32	35.15	27.56	71.49	37.26	63.10	18.18	6.80	23.13
33 R33	42.71	31.09	67.58	39.14	70.36	19.85	7.12	23.35
34 R34	23.14	18.05	72.09	24.29	59.37	13.98	5.63	18.91
35 R35	32.75	25.41	77.22	38.90	67.07	17.16	6.42	21.49
36 R36	41.71	31.94	77.98	44.33	73.00	19.72	7.09	23.45
37 R37	44.06	32.99	74.38	45.77	71.59	20.88	7.40	24.06
38 R38	42.65	32.97	74.76	44.42	74.13	20.29	7.38	24.32
39 R39	28.79	22.41	76.83	35.91	64.52	16.85	6.34	22.24
40 R40	44.31	31.38	72.24	43.83	74.73	21.53	7.60	25.46
41 C1	42.42	31.68	64.03	40.22	67.02	20.73	6.90	26.16
42 C2	41.77	32.35	86.11	46.03	75.35	20.40	6.96	22.99
43 C3	41.94	31.09	82.04	43.17	74.04	19.06	6.26	23.44
44 C4	39.03	28.71	78.63	45.97	70.49	21.27	6.67	23.37
45 C5	43.97	30.95	72.99	39.14	77.89	19.88	6.68	25.44
46 C6	37.56	27.14	83.29	39.16	81.18	19.47	6.97	25.25
47 C7	38.41	28.58	83.90	42.53	76.44	19.28	6.00	23.47
48 C8	40.08	27.25	83.50	43.33	80.16	22.77	7.92	26.81
49 C9	46.77	34.87	51.89	37.68	61.16	19.25	6.92	24.82
YEAR MS	1279.09	3398.82	3026.80	2278.15	8449.20	672.15	3.36	51.32
VARIETY MS	909.21	476.72	1376.10	635.27	762.41	80.21	6.44	74.17
VAR.YEAR MS	23.16	18.86	14.12	23.16	46.58	4.76	0.28	2.73
F1 RATIO	39.26	25.27	97.43	27.43	16.37	16.84	22.83	27.16
VAR.REP MS	8.83	8.19	4.59	11.95	23.23	1.52	0.15	1.70
LAMBDA VALUE	1.62	1.52	1.75	1.39	1.42	1.77	1.37	1.27
BETWEEN SE	1.13	1.02	0.89	1.13	1.61	0.51	0.13	0.39
WITHIN SE	0.70	0.67	0.50	0.81	1.14	0.29	0.09	0.31
DF	96	94	96	96	96	96	96	96
MJRA SLOPE 88	0.90	0.86	0.99	0.91	0.99	1.09	0.97	0.95
MJRA SLOPE 89	1.05	1.08	1.01	0.99	1.06	0.97	1.02	0.98
MJRA SLOPE 90	1.05	1.06	1.00	1.10	0.95	0.94	1.01	1.07
REGR F VAL	4.66	6.17	0.06	4.48	0.76	1.62	0.29	1.91
REGR PROB	1.17	0.30	93.82	1.39	47.08	20.27	74.68	15.38
TEST	COY	REG	COY	COY	COY	COY	COY	COY

Table B 2: An example of the output from the COYD program showing a comparison of varieties R1 and C1

PRG (DIPLOID) EARLY N.I. UPOV 1988-90

41 C1 VERSUS 1 R1 *** USING REGR
WHERE SIG ***

(T VALUES + VE IF 41 C1 > 1 R1)

		SIG LEVELS YEARS				COYD			T VALUES YEARS				F3	
		88	89	90		T	PROB%	SIG	88	89	90	TSCORE		
5	SP.HGHT	-	-	-1	ND	-1.78	7.88	NS	-1.05	-1.34	-2.64	-2.64	0.23	NS
60	NATSPHT	-	-1	-	ND	-2.02	4.61	*	-1.58	-2.61	-1.17	-2.61	0.22	NS
8	DATEEE	-1	-1	+	D	-3.06	0.29	**	-4.14	-6.33	0.80	-6.74	3.99	*
10	HGHT.EE	-1	-1	-5	D	-3.11	0.25	**	-2.79	-2.69	-2.06	-7.55	0.06	NS
11	WIDTHEE	-	-	-	ND	-1.33	18.58	NS	-1.47	-1.80	-0.21	0.00	0.32	NS
14	LGTHFL	+	+	-	ND	0.47	63.61	NS	0.17	1.83	-0.67	0.00	0.56	NS
15	WIDTHFL	+	-	+	ND	0.27	78.83	NS	0.31	-0.41	0.67	0.00	0.17	NS
24	EARLGTH	5	1	+	ND	2.93	0.42	**	2.10	3.33	1.01	5.43	0.84	NS

Notes

The three “COYD” columns headed, T PROB% SIG give the COYD T value, its significance probability and significance level. The T value is the test statistic formed by dividing the mean difference between two varieties by the standard error of that difference. The T value can be tested for significance by comparing it with appropriate values from Students t-table. Calculating and testing a T value in this manner is equivalent to deriving an LSD and checking to see if the mean difference between the two varieties is greater than the LSD.

The two right-hand “F3” columns give the F_3 ratio and its significance level.

The sections in boxes refer to earlier distinctness criteria. The three “T VALUES, YEARS” columns headed 88, 89 and 90 are the individual within year t-test values, and the three “SIG LEVELS, YEARS” columns headed 88, 89 and 90 give their direction and significance levels. The column containing D and ND gives the distinctness status of the two varieties by the 2 x 1% criterion. The column headed T SCORE gives the obsolete T Score statistic.

Table B 3: An example of the output from the COYD program showing the distinctness status of the candidate varieties

PRG (DIPLOID) EARLY N.I. UPOV 1988-90

SUMMARY FOR COYD AT 1.0% LEVEL *** USING REGR ADJ WHEN SIG ***

CANDIDATE VARIETIES		C1	C2	C3	C4	C5	C6	C7	C8	C9
1	R1	D	D	D	D	D	D	D	D	D
2	R2	D	D	D	D	ND	D	D	D	D
3	R3	D	D	D	D	D	D	D	D	D
4	R4	D	D	D	D	D	D	D	D	D
5	R5	D	D	D	D	D	D	D	D	D
6	R6	D	D	D	D	D	D	D	D	D
7	R7	D	D	D	D	D	D	D	D	D
8	R8	D	D	D	D	D	D	D	D	D
9	R9	D	D	D	D	D	D	D	D	D
10	R10	D	D	D	D	D	D	D	D	D
11	R11	D	D	D	D	D	D	D	D	D
12	R1	D	D	D	D	D	D	D	D	D
13	R13	D	D	D	D	ND	D	D	D	D
14	R14	D	D	D	D	D	D	D	D	D
15	R15	D	D	D	D	D	D	D	D	D
16	R16	D	D	D	D	D	D	D	D	D
17	R17	D	D	D	D	D	D	D	D	D
18	R18	D	D	D	D	D	D	D	D	D
19	R19	D	D	D	D	D	D	D	D	D
20	R20	D	D	D	D	D	D	D	D	D
21	R21	D	D	D	D	D	D	D	D	D
22	R22	D	D	D	D	D	D	D	D	D
23	R23	D	D	D	D	D	D	D	D	D
24	R24	D	D	D	D	D	D	D	D	D
25	R25	D	D	D	D	D	D	D	D	D
26	R26	D	D	D	D	D	D	D	D	D
27	R27	D	D	D	D	D	D	D	D	D
28	R28	D	D	D	D	D	D	D	D	D
29	R29	D	D	D	D	D	D	D	D	D
30	R30	D	D	D	D	D	D	D	D	D
31	R31	D	D	D	D	D	D	D	D	D
32	R32	D	D	D	D	D	D	D	D	D
33	R33	D	D	D	D	D	D	D	D	D
34	R34	D	D	D	D	D	D	D	D	D
35	R35	D	D	D	D	D	D	D	D	D
36	R36	D	D	D	ND	D	D	D	D	D
37	R37	D	D	D	D	D	D	D	D	D
38	R38	D	D	D	D	D	D	D	D	D
39	R39	D	D	D	D	D	D	D	D	D
40	R40	D	D	D	D	D	D	D	D	D
41	C1	-	D	D	D	D	D	D	D	D
42	C2	D	-	D	D	D	D	D	D	D
43	C3	D	D	-	D	D	D	ND	D	D
44	C4	D	D	D	-	D	D	D	D	D
45	C5	D	D	D	D	-	D	D	D	D
46	C6	D	D	D	D	D	-	D	D	D
47	C7	D	D	ND	D	D	D	-	D	D
48	C8	D	D	D	D	D	D	D	-	D
49	C9	D	D	D	D	D	D	D	D	-
NO OF ND VARS		0	0	1	1	2	0	1	0	0
DISTINCTNESS		D	D	ND	ND	ND	D	ND	D	D
CANDIDATE VAR		C1	C2	C3	C4	C5	C6	C7	C8	C9

Figure B1. Heading date yearly variety means against over-year variety means

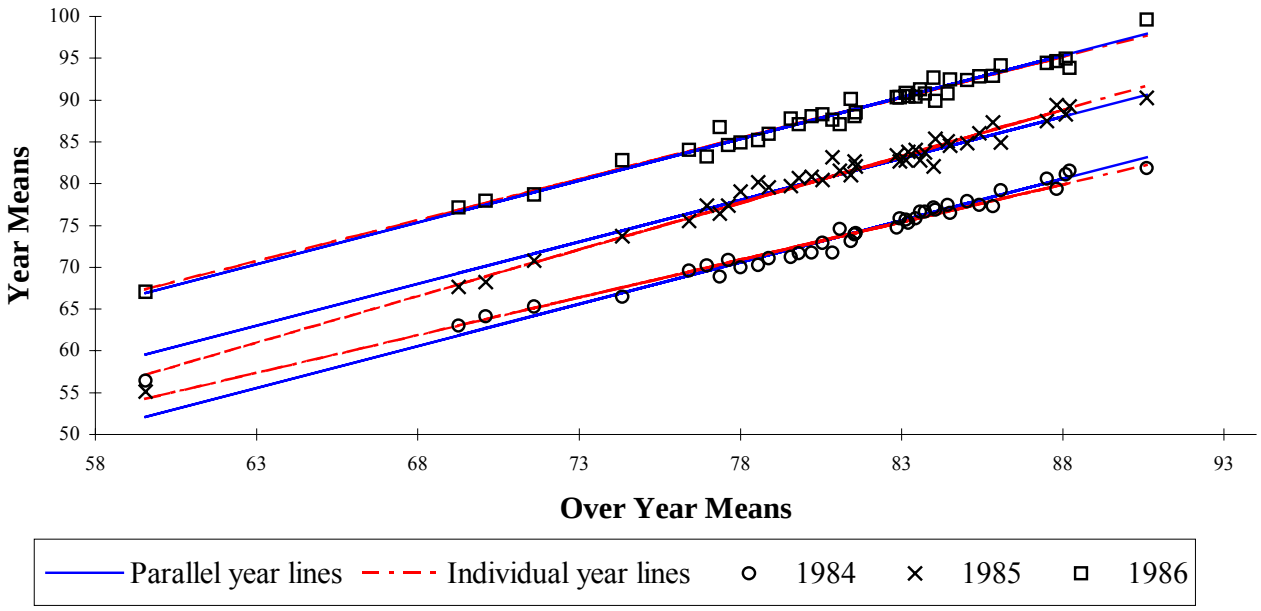
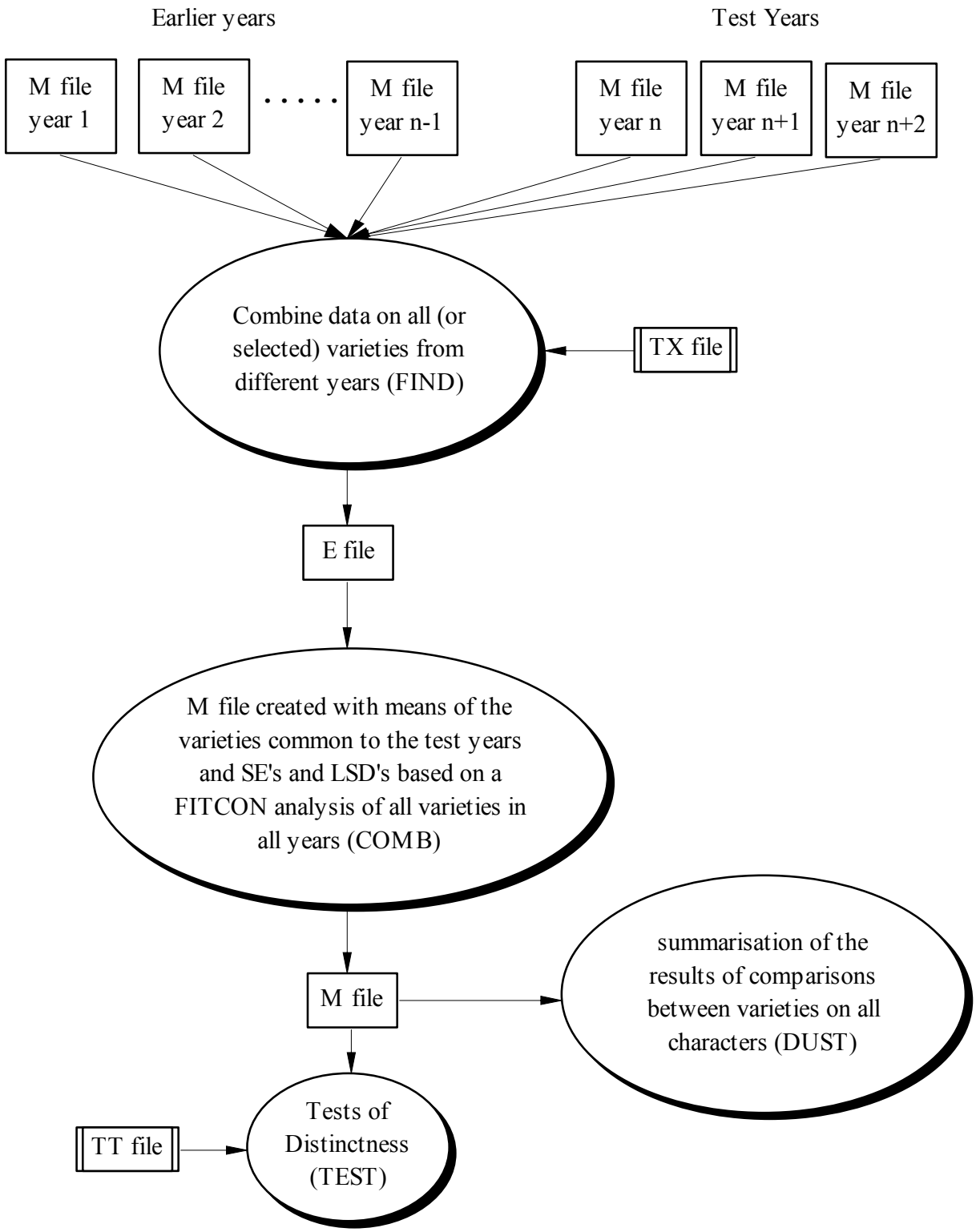


Figure B2. Flow Diagram of the stages and DUST modules used to produce long-term LSD's and perform long-term COYD



[Annex VI follows]

ANNEX VI

DISTINCTNESS TESTING SCHEMES AND THE PROBABILITY LEVELS USED FOR COYD

The distinctness test usually belongs to one of four schemes:-

Scheme A. Test is conducted over 2 independent cycles (e.g. years) and decisions are made after 2 cycles

Scheme B. Test is conducted over 3 independent cycles and decisions are made after 3 cycles

Scheme C. Test is conducted over 3 independent cycles and decisions are made after 3 cycles, but a variety may also be accepted after 2 cycles

Scheme D. Test is conducted over 3 independent cycles and decisions are made after 3 cycles, but a variety may also be accepted or rejected after 2 cycles

In schemes A and B a single decision is made, and so a single probability level *p* for the t_p value used to calculate the COYD LSD is required for each decision. These are denoted by p_{d2} and p_{d3}, and are used to decide whether a variety is distinct after 2 cycles and 3 cycles respectively.

In Scheme C decisions are made after each of two and three cycles and, as COYD LSD's must be calculated at each of these stages, the two probability levels p_{d2} and p_{d3} are needed for the t_p values used to calculate these COYD LSD's.

Scheme D is like Scheme C, except that a further decision and hence a further COYD LSD is required after 2 cycles. This decision is whether to reject a variety as not distinct, and the probability level needed for the t_p value used to calculate this COYD LSD is denoted by p_{nd2}.
In a 3 cycle test with decisions after 2 cycles (Schemes C & D) the probability level used to decide distinctness after 2 cycles, i.e. p_{d2}, may be chosen to be more stringent than the probability level used to decide distinctness after 3 cycles, i.e. p_{d3}.

The four schemes A, B, C & D are illustrated in Figures 1 to 4. In these the term "diff" represents the difference between the means of a candidate variety and another variety for a characteristic, and LSDp is the COYD LSD criterion calculated at probability level *p*.

Figure 1. COYD decisions in Scheme A

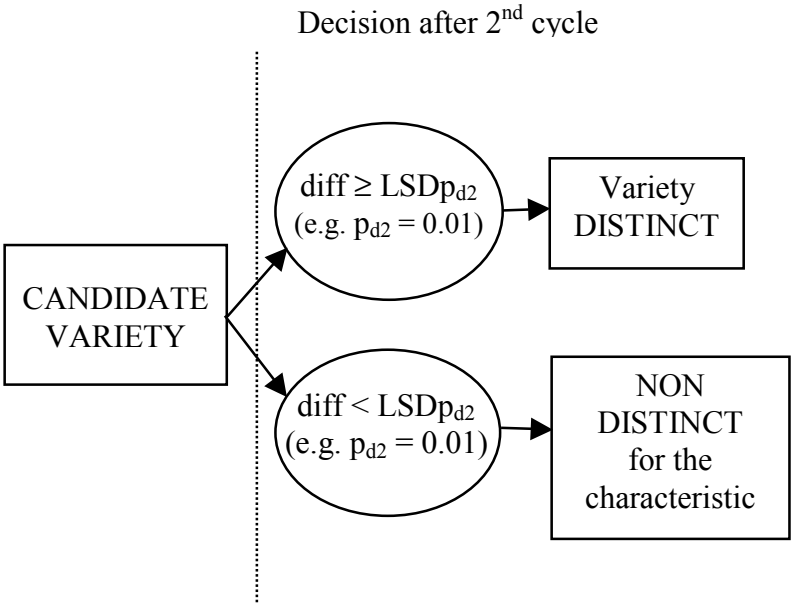
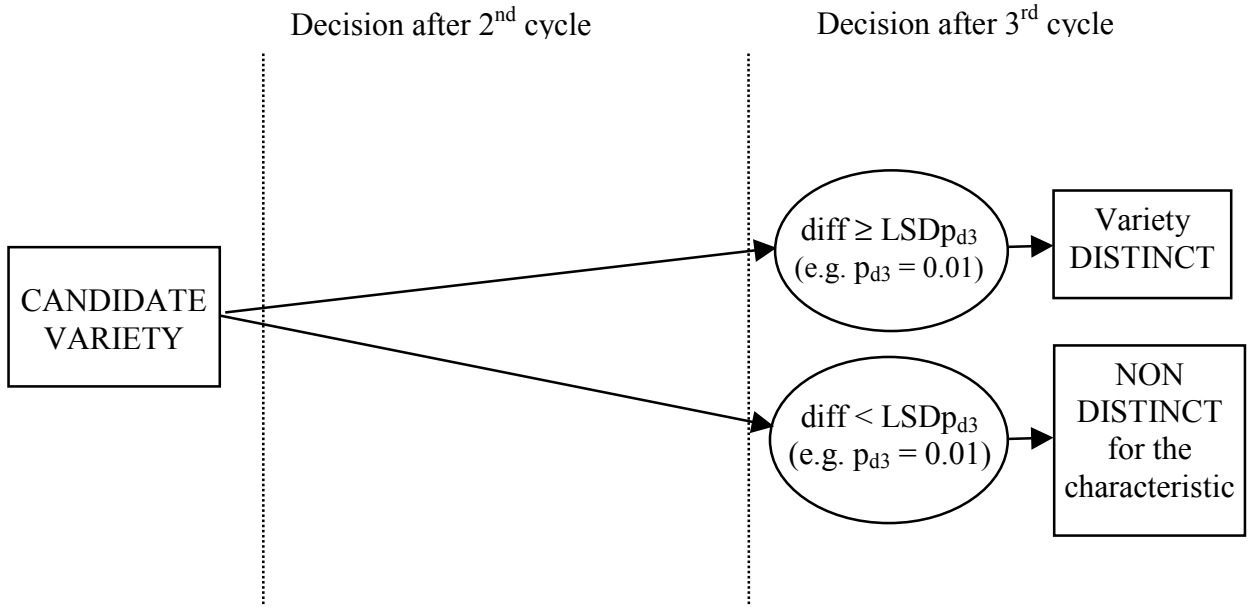


Figure 2. COYD decisions in Scheme B



NOTE:-

"diff" is the difference between the means of the candidate variety and another variety for the characteristic

LSDp is the COYD LSD criterion calculated at probability level p.

Figure 3. COYD decisions in Scheme C

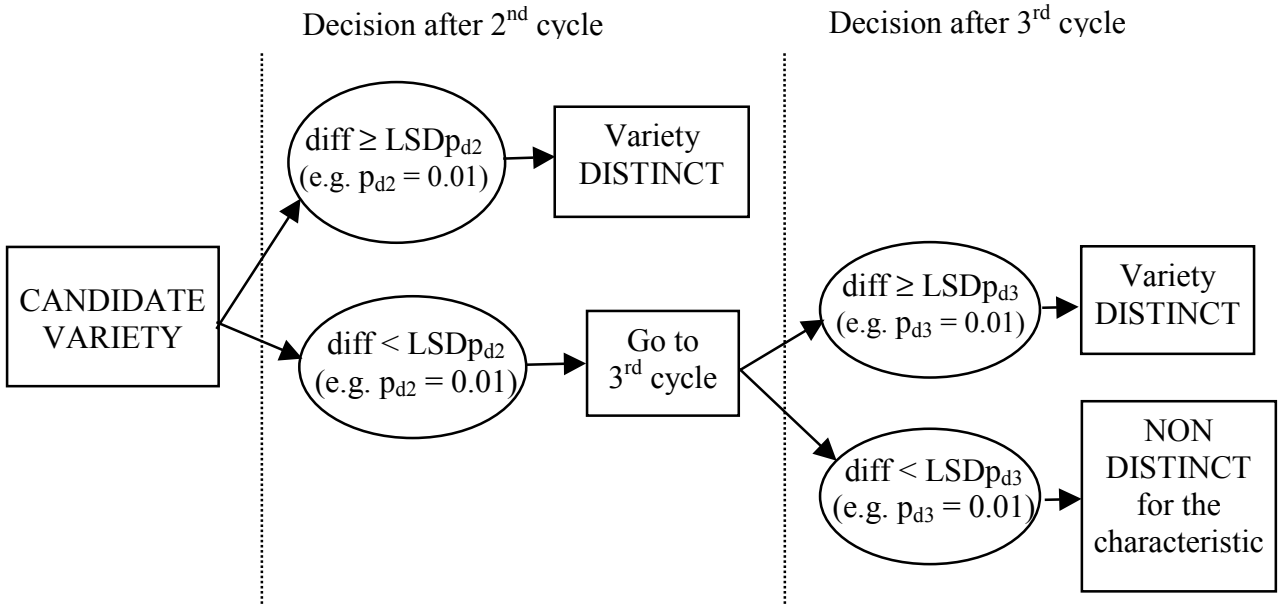
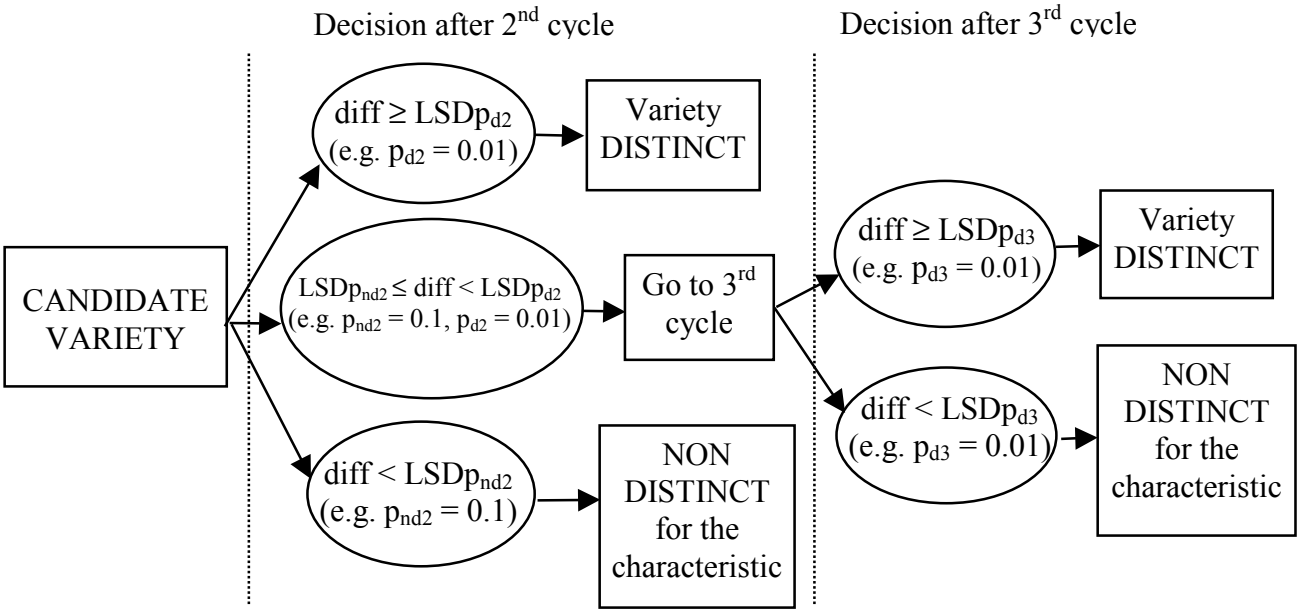


Figure 4. COYD decisions in Scheme D



NOTE:-

"diff" is the difference between the means of the candidate variety and another variety for the characteristic

LSDp is the COYD LSD criterion calculated at probability level p.

[End of Annexes and of document]