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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

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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 Enlarged Editorial Committee at its meeting to be held in Geneva, on January 11, 2005

SECTION 1: INTRODUCTION

1.1 Article 7 of the 1991 Act of the UPOV Convention establishes that "a variety shall be deemed to be distinct if it is clearly distinguishable from any other variety whose existence is a matter of common knowledge at the time of filing the application."

1.2 Document TGP/3 "Varieties of Common Knowledge" states that "common knowledge" has its natural meaning and that members of the Union should take into account not only knowledge that exists in documented form, but also the knowledge of relevant communities around the world provided that this knowledge can be credibly substantiated so as to satisfy the standard of proof of the civil law courts.

1.3 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), hereinafter referred to as "the General Introduction", states, with respect to common knowledge (see document TG/1/3, section 5.2.2), that:

> "Specific aspects which should be considered to establish common knowledge include, among others:
>
> (a) commercialization of propagating or harvested material of the variety, or publishing a detailed description;
>
> (b) the filing of an application for the grant of a breeder's right or for the entering of a variety in an official register of varieties, in any country, which is deemed to render that variety a matter of common knowledge from the date of the application, provided that the application leads to the grant of a breeder's right or to the entering of the variety in the official register of varieties, as the case may be;
>
> (c) existence of living plant material in publicly accessible plant collections.
>
> Common knowledge is not restricted to national or geographical borders."

1.4 Document TGP/4 notes that "the list of varieties whose existence is a matter of common knowledge ("varieties of common knowledge") for a given species can be very large. Therefore, it may be appropriate to define a collection of varieties of common knowledge (a "variety collection") from within which:

(a) varieties which should be included in growing tests or other trials, as a part of the examination of distinctness, can be identified; and

(b) where required, the necessary material of the varieties is available for inclusion in such tests and trials."

1.5 Document TGP/4 also explains that "the variety collection may not contain all varieties of common knowledge. For example, there may be reasons (e.g. phytosanitary regulations) for which plant material, even if it exists, may not be obtainable. To address such situations the General Introduction (Chapter 5.3.1.2) states the following:

> " … certain supplementary procedures may be developed to avoid the need for a systematic individual comparison. For example, the publication of variety descriptions, inviting comment from interested parties, or cooperation between members of the

> Union, in the form of an exchange of technical information, could be considered as supplementary procedures. However, such an approach would only be possible where the supplementary procedures, in conjunction with the other procedures, provide an effective examination of distinctness overall. Such procedures may also be appropriate for consideration of varieties of common knowledge, for which living plant material is known to exist (see section 5.2.2) but where, for practical reasons, material is not readily accessible for examination. Any such procedures are set out in document TGP/9, "Examining Distinctness." "

1.6 The purpose of document TGP/9 is to provide guidance in the examination of distinctness in growing tests or other trials and on the use of supplementary procedures in the examination of distinctness.

SECTION 2: SELECTING VARIETIES FOR THE GROWING TRIAL

A key step in the examination of distinctness is the selection of varieties of common knowledge, from within the variety collection (see document TGP/4), to be included in the growing test or other trials. The factors which may be used in that process are explained below.

2.1 Type of variety

Document TGP/4 "Constitution and management of variety collections" explains that a variety collection may be limited to a type or types of varieties within a species or subspecies. More information is provided in document TGP/4 section 1.2.1.

2.2 Grouping characteristics

2.2.1 Function

2.2.1.1 The selection of varieties to be grown in the trial with the candidate varieties is aided by the use of grouping characteristics.

2.2.1.2 The General Introduction (document TG/1/3) sets out the functions of grouping characteristics (see document 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."

2.2.1.3 Function 1 above identifies the role of grouping characteristics in selecting varieties for the growing trial.

2.2.2 Criteria

2.2.2.1 The General Introduction sets out the criteria (document 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.
>
> […]"

2.2.2.2 The states of expression of the grouping characteristics for the candidate varieties need to be known before the (first) growing trial in order to be able to use that information in selecting varieties for the growing trial. For that reason, information is requested in the Technical Questionnaire (TQ). Document TGP/7, "Development of Test Guidelines" (Guidance Notes 13.4) states that:

> "(a) Grouping characteristics selected from the Table of Characteristics should, in general, receive an asterisk in the Table of Characteristics and be included in the Technical Questionnaire.
>
> (b) TQ characteristics selected from the Table of Characteristics should, in general, receive an asterisk in the Table of Characteristics and be used as grouping characteristics. TQ characteristics are not restricted to those characteristics used as grouping characteristics;
>
> (c) Asterisked characteristics are not restricted to those characteristics selected as grouping or TQ characteristics."

2.2.2.3 Where UPOV has developed Test Guidelines, these will provide useful grouping characteristics. However, grouping characteristics are provided in the Test Guidelines for two reasons, as specified in section 2.2.1.2. Therefore the use of each grouping characteristic for excluding varieties from the growing trial, as opposed to its use for organizing the growing trial so that similar varieties are grouped together (see section 3.5.1), should be considered carefully.

2.2.2.4 In the absence of UPOV Test Guidelines, the criteria set out in 2.2.2.1 should be used for identifying suitable characteristics which may be used for selecting varieties for the growing trial.

2.2.3 Use

2.2.3.1 Once an appropriate set of grouping characteristics has been selected it is possible to identify those varieties in the variety collection which can be excluded from the growing trial. The following theoretical example is presented for illustration:

Candidate variety A

Species: *Impatiens walleriana* Hook. f.
UPOV Test Guidelines: document TG/102/4
Grouping characteristics:

(a) Leaf: variegation (QL);
(b) Flower: type (QL);
(c) Flower: number of colors (eye zone excluded) (QL);
(d) Flower: main color (PQ) with the following groups:
Gr. 1: white
Gr. 2: yellow
Gr. 3: pink
Gr. 4: blue pink
Gr. 5: orange
Gr. 6: red
Gr. 7: purple
Gr. 8: violet

Example: Information for candidate variety A provided in the Technical Questionnaire

	Characteristics	Candidate variety A	Varieties in the variety collection	Exclusion from the growing trial
(7)	**Leaf: variegation**			
QL	absent		1[ ]	YES
	present	9[X]	9[ ]	NO
(15)	**Flower: type**			
QL	single	1[X]	1[ ]	NO
	double		2[ ]	YES
(17)	**Flower: number of colors (eye zone excluded)**			
QL	one	1[X]	1[ ]	NO
	two		2[ ]	YES
	more than two		3[ ]	YES
(18)	**Flower: main color**			
PQ	white		1[ ]	YES
	yellow		2[ ]	YES
	pink	3[X]	3[ ]	NO
	blue pink		4[ ]	NO
	orange		5[ ]	YES
	red		6[ ]	YES
	purple		7[ ]	YES
	violet		8[ ]	YES

2.2.3.2 Varieties with expression “pink (3)” and “blue pink (4)” can be clearly distinguished from varieties in all the other color groups but more accurate information is needed within those groups because there is a continuous variation from “pink (3)” to “blue pink (4)”. Therefore it is necessary to include both groups in the growing trial (see also section 3.5).

2.2.4 Number of Growing Cycles

2.2.4.1 In cases where there is more than one growing cycle, it may be possible after the first growing cycle to go further in the elimination of varieties from the growing trial. This may be possible using information obtained from the candidate variety and varieties in the

variety collection from the same growing trial. This is particularly relevant in the case of non-qualitative grouping characteristics which are more influenced by the environment. A second growing cycle also allows the possibility to correct the grouping of a candidate variety if the information provided in the TQ proved to be inappropriate.

2.2.4.2 Continuing with the example above, before the second growing cycle it may be possible to exclude some varieties with Flower: main color, "blue pink (4)", which have been included in the first growing trial. This may be possible because the characteristic in the Test Guidelines is recorded using the reference number of the RHS Colour Chart, thus making it possible to have more precision for the states of expression.

2.2.4.3 In cases where there is, in general, a single growing cycle, it is important to ensure that reliable grouping characteristics are used, to avoid the need to grow an exceptional second growing cycle because the candidate variety was wrongly grouped and needs to be grown in a trial with varieties which were not selected in the first growing cycle.

2.3 Phenotypic distance

2.3.1 Definition

"Phenotypic distance" methods take input data (e.g. descriptions) and derive a measure of similarity/difference between varieties under comparison. In contrast to the characteristic-by-characteristic approach, "phenotypic distance" approach calculates distances between varieties using phenotypic data in order to obtain an overall comparison of varieties.

2.3.2 Defining thresholds

2.3.2.1 When using phenotypic distance for the selection of varieties for the growing trial, the objective is to calculate the pair-wise distances between the varieties of common knowledge and the candidate variety, by using descriptive and/or other information. These distances can then be related to a threshold to decide on whether a direct comparison in a growing trial is necessary or not. The experience of crop experts will be necessary to calibrate different parameters depending on the method used, e.g. the threshold distance value or the weighting of similarity/difference computations. This calibration is aimed at avoiding the exclusion of varieties of common knowledge from the trial, which should, in fact, have been included.

2.3.3 Methods

2.3.3.1 GAIA

2.3.3.1.1 The GAIA method, developed by experts from France, calculates a phenotypic distance between two varieties, which is a sum of distances for individual characteristics. For each species, this system must be calibrated to determine the weighting given to differences in each characteristic and the threshold for the phenotypic distance used to eliminate varieties from the growing trial.

2.3.3.1.2 In the GAIA method, the word "weighting" is used to designate the contribution of a given characteristic to the total distance between a pair of varieties, and the word "distance"

to designate the global distance between a pair of varieties, as the result of the addition of the weightings of all characteristics.

2.3.3.1.3 The weighting is based upon the size of the difference and on the individual characteristic (reliability, environmental influence in the testing location and genetic regulation). The following theoretical examples are presented for clarification:

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

Shape of ear:
1 = conical
2 = conico-cylindrical
3 = cylindrical

Comparison between difference in notes and weighting		
	Different in notes	Weighting
conical (1) vs. conical (1)	0	0
conical (1) vs. conico-cylindrical (2)	1	2
conical (1) vs. cylindrical (3)	3	6
conico-cylindrical (2) vs. conico-cylindrical (2)	0	0
conico-cylindrical (2) vs. cylindrical (3)	1	2
cylindrical (3) vs. cylindrical (3)	0	0

When the crop experts compare a variety 'i' with conical ear (note 1) to a variety 'j' with cylindrical ear (note 3), they attribute a weighting of 6 etc. The weightings are summarized in the form of a weighting matrix:

Weighting matrix 'i'				
		Variety 'i'		
Variety 'j'		**1**	**2**	**3**
	1	0	2	6
	2		0	2
	3			0

Example 2: "Length of husks", observed on a 1 to 9 scale, the crop experts have defined the following 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

The weighting between a variety 'i' with very short husks (note 1) and a variety 'j' with short husks (note 3) is 0. Experts consider that a difference of 3 notes is necessary in order to recognize a non-zero distance between two varieties. Even if the difference in notes is greater than 3, the experts do not increase the distance to more than 2.

2.3.3.1.4 The threshold, called "Distinctness Plus" threshold, is determined by the crop expert at a level which is higher than the difference needed to establish distinctness, thereby ensuring that all pairs of varieties, having a distance equal or greater than the Distinctness Plus threshold, would be distinct in the growing trial. The determination of the Distinctness Plus threshold needs to be based on experience gained with the varieties of common knowledge in order to minimize the possibility of excluding from the growing trial a pair of varieties which should be further compared in the field. The following theoretical example is presented for clarification:

In the following example, the crop expert uses a Distinctness Plus threshold $\mathbf{S_{dist}}$ of 10 to decide whether to include a variety in a growing trial or not.

Matrix for a qualitative analysis for 5 characteristics for varieties A and B						
	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 this example $D_{qual} = 8 < S_{dist}$ so varieties A and B are declared "GAIA NON-distinct" and are included in the growing trial.

2.3.3.1.5 The GAIA method can be used for the selection of varieties for the growing trial as follows:

(i) Selecting varieties for the (first) growing trial: using information provided in the TQ by the applicant and the information already held on varieties in the variety collection to exclude from the growing trial those varieties for which the phenotypic distance with the candidate variety is greater than GAIA Distinctness Plus threshold.

(ii) Selecting varieties for subsequent growing trials (if appropriate): eliminating from subsequent growing cycles all pairs of varieties reaching or surpassing the GAIA Distinctness Plus threshold. After the first growing cycle, some varieties in the trial are obviously different from all candidates, and their inclusion in the second growing cycle is not necessary.

2.3.3.1.6 Details of the GAIA method are provided in section 5.4.

2.3.3.2 Other

2.4 Parent formula of hybrid varieties

2.4.1 In some Test Guidelines, e.g. Maize (document TG/2), Rape seed (document TG/36) and Sunflower (document TG/81), an optional method for selecting varieties for the growing trial is described, based on the parent lines and the formula of the hybrid.

2.4.2 The method is based on the following steps:

(i) Description of parent lines according to the Test Guidelines.

(ii) Checking the originality of those parent lines in comparison with the variety collection, based on the Table of Characteristics of the Test Guidelines, in order to identify similar parent lines.

(iii) Checking the originality of the hybrid formula in relation to the hybrids in the variety collection, taking into account the most similar parent lines.

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

2.4.3 Details on the use of the parent formula are provided in Annex III.

2.5 Photographs

2.5.1 Document TGP/7 states that the Test Guidelines may require that a representative color photograph of the variety should accompany the information provided in the Technical Questionnaire. In these cases, it is recommended that guidance be provided by the authority to enhance the usefulness of the photograph (e.g. to include a metric scale in the picture, to define what parts of the plant should be included; light conditions, background color, etc).

2.5.2 Photographs can provide additional useful information to that already provided by the characteristics described in the Technical Questionnaire under section 5 “Characteristics of the variety to be indicated”. In particular photographs may provide information on characteristics not included in the TQ. This may, for example, concern shapes and plant

structures, which are not easy for applicants to describe by means of notes in the Table of Characteristics and, therefore, might not be included as characteristics in Section 5 of the TQ. In addition the information provided in photographs on characteristics included in the TQ may be more discriminatory than that provided in section 5 of the TQ and may allow more varieties to be eliminated from the growing trial.

SECTION 3: GROWING TRIAL ORGANIZATION

3.1 Number of independent growing cycles

3.1.1 A key aspect of growing trials is to determine the appropriate number of growing cycles. In that respect, document TGP/7, Annex I: TG Template, section 4.1.2, states:

> "4.1.2 Consistent Differences
>
> 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 in at least two independent growing cycles."

3.1.2 The Test Guidelines, where available, specify the recommended number of growing cycles.

3.2. The notion of independent growing cycles

3.2.1 As indicated in section 3.1, where there is a need of more than one growing cycle, the growing cycles should be "independent".

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

3.2.3 For some perennial crops, e.g. fruit trees, grasses, etc., the same plants are examined over successive years. In such cases, the independence of growing cycles is also satisfied.

3.2.4 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.

3.2.5 The use of more than one location in order to obtain independent growing cycles in a given year would require that the variety-by-location interaction is as large as the variety-by-cycle (year) interaction in any characteristic used for distinctness.

3.3 Use of multiple locations in the examination of distinctness

Document TGP/7 "Development of Test Guidelines" (see Annex I, TG Template, section 3.2) clarifies that "Tests are normally conducted at one place". Thus, the use of more than one place is not the normal practice, and the factors below should be taken into account in such cases:

3.3.1 Purpose

It may be considered appropriate to conduct tests at more than one place for the following purposes:

3.3.1.1 Reserve trial

Authorities may designate a primary location, but organize an additional reserve trial in a separate location. Normally only the data from the primary location will be used, but in cases where this location has a major problem the reserve trial will be available to prevent the loss of one year's results.

3.3.1.2 Different agro-climatic conditions

Varieties of a different geographical origin may require different agro-climatic growing conditions. 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.

3.3.1.3 Independent growing cycles

See section 3.2.5.

3.3.2 Use of information from multiple locations

3.3.2.1 Where more than one location is used, it is necessary to establish decision rules, to cover, for example, if the two varieties need to be distinct in only one location or in all the locations.

3.3.2.2 In the same way, it is necessary to define the way in which the information obtained in the centers would be used; e.g. whether it will be averaged over centers or whether each center would be considered individually.

3.4 Type of plot for observation

The Test Guidelines specify the type/s of plot for the growing trial (e.g. spaced plants, row plot, drilled plot, etc.) in order to examine distinctness but also uniformity and stability. Guidance on trial design is provided in document TGP/8.

3.5 Similar varieties

3.5.1 Grouping characteristics

3.5.1.1 As noted in section 2.2.1.2, one of the functions of grouping characteristics is:

> "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."

3.5.1.2 In the example for a candidate variety A of the species *Impatiens walleriana* Hook. f. provided in section 2.2.4, it was seen that varieties could be excluded from the growing trial on the basis of grouping characteristics. Continuing with that example provides an illustration of how grouping characteristics can be used to organize the growing trial for varieties included in the trial.

- Varieties which have not been excluded in the previous process (see section 2.2.4) will be included in the growing trial for direct comparison with candidate variety A. On the basis of characteristic "Flower: main color", the varieties in the growing trial are grouped and candidate variety A is placed in the PINK GROUP:

PINK GROUP: Flower: main color: pink (3)

BLUE PINK GROUP: Flower: main color: blue pink (4)

BLUE PINK GROUP is also included because there is no clear cut-off between states pink (3) and blue pink (4).

3.5.2 Phenotypic distance

3.5.2.1 Phenotypic distance can be used to organize the growing trial so that similar varieties are grouped together (see section 2.3.2).

3.5.3 Photographs

3.5.3.1 Photographs, provided by the applicant in the Technical Questionnaire can provide useful information to arrange varieties in the growing trial. In particular, they can be used to identify the most similar varieties and to indicate on which characteristics the examiner should focus the observations.

SECTION 4: ASSESSING DISTINCTNESS FROM THE GROWING TRIAL

4.1 Introduction

4.1.1 The main factors for the choice of methods for the assessment of distinctness are:

(a) the type of expression of characteristics,
(b) the type of variety, and
(c) the method of observation.

4.1.2 These factors determine the type of data obtained (see document TGP/8, section 3) and the appropriate method for the assessment of distinctness. The crucial element is the variation of the characteristics in the species. The following sections provide explanations on the different factors and their relationship.

4.2 Type of expression of characteristic

Characteristics can be classified according to their types of expression or, in other words, according to their observed variation within a species. The variation includes genetic and environmental variation between and within varieties. The General Introduction defines the following types of expressions of characteristics (see document TG/1/3, section 4.4):

> "Qualitative characteristics" are those that are expressed in discontinuous states (e.g. sex of plant: dioecious female (1), dioecious male (2), monoecious unisexual (3), monoecious hermaphrodite (4)). These states are self-explanatory and independently meaningful. All states are necessary to describe the full range of the characteristic, and every form of expression can be described by a single state. The order of states is not important. As a rule, the characteristics are not influenced by environment.
>
> "Quantitative characteristics" are those where the expression covers the full range of variation from one extreme to the other. The expression can be recorded on a one-dimensional, continuous or discrete, linear scale. The range of expression is divided into a number of states for the purpose of description (e.g. length of stem: very short (1), short (3), medium (5), long (7), very long (9)). The division seeks to provide, as far as is practical, an even distribution across the scale. The Test Guidelines do not specify the difference needed for distinctness. The states of expression should, however, be meaningful for DUS assessment.
>
> In the case of "pseudo-qualitative characteristics", the range of expression is at least partly continuous, but varies in more than one dimension (e.g. shape: ovate (1), elliptic (2), circular (3), obovate (4)) and cannot be adequately described by just defining two ends of a linear range. In a similar way to qualitative (discontinuous) characteristics – hence the term "pseudo-qualitative" – each individual state of expression needs to be identified to adequately describe the range of the characteristic".

4.3 Type of variety

The decision as to whether a difference between two varieties can be considered to be clear is influenced by the variation of a characteristic between and within varieties. The features of propagation determine the level of genotypic variation within varieties.

4.3.1 Self-pollinated and vegetatively propagated varieties

Vegetatively propagated, truly self-pollinated and mainly self-pollinated varieties normally have relatively little variation within varieties.

4.3.2 Cross-pollinated and synthetic varieties

Within cross-pollinated and synthetic varieties, variation is normally greater than for self-pollinated and vegetatively propagated varieties, especially in quantitative and some pseudo-qualitative characteristics. For qualitative and some pseudo-qualitative characteristics the variation within varieties is mainly considered for uniformity assessment. If there is no, or very little, variation in a characteristic, variation within varieties does not need to be considered for the assessment of distinctness and the same principles apply as for self-pollinated varieties.

4.3.3 Hybrid varieties

The assessment of distinctness for hybrid varieties should follow the same rules as for other types of varieties. Distinctness can be tested at the level of the hybrid itself or by consideration of the parent lines (see section 5.5). The appropriate methods should be chosen according to the degree of variation within varieties.

4.4 Method of observation

4.4.1 The expression of characteristics can be observed visually (V) or by measurement (M). Both types of observation can be based on single, individual plants (S) or on groups of plants/plots as a whole (G). The four resulting possibilities are listed below.

4.4.2 The following symbols are used in Test Guidelines to indicate the recommended method of observation for the assessment of distinctness:

MG: single record for a group of plants or parts of plants based on measurement(s)
MS: records for a number of single, individual plants or parts of plants obtained by measurement
VG: single record for a group of plants or parts of plants based on visual observation(s)
VS: records for a number of single, individual plants or parts of plants obtained by visual observation.

4.4.3 The choice of the method of observation and, in particular, the determination of the appropriate number of observations, depends on whether the data will be used for the assessment of distinctness or for the assessment of uniformity or for the assessment of both. For distinctness, the “typical” expression of the varieties must be recorded, which may, after observation of the plot, be possible with only one record. The assessment of uniformity implies that it is necessary to observe single plants.

4.4.4 The symbols MG, MS, VG and VS should be used in the Test Guidelines to indicate the recommended method of observation for the assessment of distinctness. Normally the same data can be used for distinctness and for the variety description. The relationship between the data used for distinctness, uniformity and variety description is illustrated in the examples provided in section 4.6.

4.4.5 The indication of G and S refers to the number of records retained for further evaluations, if necessary. In the case of MG and VG, a single record may be based on a single observation of the plot (e.g. intensity of green color: one note is given after one observation of the plot as a whole - VG). In other situations it may be necessary to make several observations of the plot in order to establish the single record (e.g. hairiness of lower side of leaf: leaves of several plants have to be observed, but finally only one note is recorded - VG).

4.4.6 In the Test Guidelines there should be an indication of how many individual plants should be observed in the case of VS/MS (e.g. all observations should be made on {x} plants or parts taken from each of {x} plants).

4.4.7 The method of observation determines the type of data and thus the choice of methods for the assessment of distinctness (see document TGP/8, section 3). In the case of VG, i.e. one record for each variety, it is not possible or necessary to apply statistical methods. If it is intended to apply a specific statistical method the experts need to consider the data structure required.

4.4.8 The elements determining the most appropriate method of observation are discussed in section 4.5.

4.5 Relationship between the different factors

Any recommendation on the choice of the method for the assessment of distinctness should take into account the relationship between the aspects presented above.

4.5.1 Visual Observation vs. Measurement

4.5.1.1 The choice of visual observation (V) or measurement (M) will be influenced by:

(a) Type of expression of characteristic: qualitative and pseudoqualitative characteristics are, in general, observed visually. Quantitative characteristics can be measured or visually observed. Measurement is only possible in case of quantitative characteristics. If visual observation fulfills the requirements for the DUS assessment it is preferable because visual observations are, in general, quicker and cheaper.

(b) Genotypic and/or environmental variability between and within varieties influence whether it is appropriate to record quantitative characteristics by visual observation. Measurements provide a higher level of information and more precise data (objective units). For an assessment of distinctness, visual observations require sufficient variation between, and a low level of variation within varieties since they may be less precise (subjective units).

(c) Number of varieties in the collection: more precision may be necessary in order to distinguish a larger number of varieties. Measurements provide more precise data.

(d) Resources (equipment, staff): visual assessment is usually less time consuming than measurements. Measurements for some characteristics may be

partly automated (e.g. imaging). Different characteristics may be assessed simultaneously (e.g. thousand seed weight + kernel length; length + width of petals).

(e) Relation between workload and precision required.

4.5.1.2 Where there is doubt regarding the use of visual observation for a quantitative characteristic as the distinguishing characteristic in relation to another variety, it should be measured, if this is possible with reasonable effort.

4.5.2 Single observation of a group of plants (G) or observation on individual plants (S)

4.5.2.1 If there is relatively little variation within varieties (excluding off-types) compared to the variation between varieties, the expression of characteristics can be recorded by a single observation of a group of plants in order to provide sufficient data for assessment of distinctness as well as for the variety description. These conditions are fulfilled in most characteristics in self-pollinated and vegetatively propagated varieties and for most qualitative and pseudo-qualitative characteristics in cross-pollinated varieties.

4.5.2.2 If considerable genotypic and/or environmental variation occurs within varieties, it is necessary to observe individual plants in order to determine the mean expression as well as the variation within a variety. Distinctness is then assessed by comparing variety means calculated on the basis of the individual plant data, taking into account the random variation inherent in the variety means. This is the normal situation for quantitative characteristics in cross-pollinated varieties.

4.5.3 Assessing clear differences in relation to type of expression

4.5.3.1 The General Introduction provides guidance on whether a difference between two varieties can be considered to be clear according to the type of expression of the characteristics (see document TG/1/3).

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. […]"

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."

4.6 Examples

4.6.1 Examples for characteristics recorded by visual observation

4.6.1.1 Qualitative characteristics

In qualitative characteristics the states of expressions are self-explanatory and independently meaningful. Notes are provided for each of the states. Such characteristics are, in general, recorded by a single observation of a group of plants for distinctness and the off-type procedure is applied for uniformity.

Examples:

Barley (self-pollinated) — Lowest leaves: hairiness of leaf sheaths (absent, present)

⇨ Distinctness - single record based on visual observation of a number of individual plants(VG)

⇨ Uniformity - off-types, fixed population standard based on visual observation of individual plants

Field bean (cross-pollinated) — Plant: growth type (determinate, indeterminate)

⇨ Distinctness - single record based on visual observation of the plot (VG)

⇨ Uniformity - off-types, relative population standard based on visual observation of individual plants

4.6.1.2 Quantitative characteristics

Quantitative characteristics can be recorded by observation of a group of plants (mainly in self-pollinated and vegetatively propagated species) or by observations of single plants (mainly in cross-pollinated species).

Examples:

Wheat - (self-pollinated) — Ear: glaucosity (absent or very weak to very strong)

⇨ Distinctness - single record based on visual observation of the plot (excluding off-types) (VG)

⇨ Uniformity - off-types, fixed population standard based on visual observation of individual plants

Ryegrass (cross-pollinated)	Plant: growth habit (erect to prostrate)
	⇨ Distinctness - variety means calculated from records of visually observed individual plants (VS) ⇨ Uniformity - relative uniformity based on variances, using records of visually observed individual plants.

4.6.1.3 Pseudo-qualitative characteristics

Pseudo-qualitative characteristics are recorded like qualitative characteristics. Distinctness is assessed from a single record based on visual observation (VG) and the off-type procedure is applied for uniformity.

Radish (cross-pollinated)	Radish: shape (transverse elliptic, circular, elliptic, obovate, broad rectangular, rectangular, narrow rectangular, narrow obtriangular, iciclical)
	⇨ Distinctness - single record based on visual observation of individual plants (VG)
	⇨ Uniformity - off-types, relative population standard based on visual observation of individual plants.

4.6.2 Examples for characteristics recorded by measurements

4.6.2.1 The table provides an example for recording measurements in self-pollinated varieties (barley) with very little within-variety variation (single record) and in cross pollinated varieties (rye) with substantial plant to plant variation (records of individual plants).

4.6.2.2 In the case of barley, distinctness for the characteristic “Plant: length” is usually based on a single record for each variety. The replicated measurements within a plot determine the mean plot value and the replications are not considered for further evaluations. If appropriate, the replications can be used to calculate a least significant difference (LSD) for distinctness. Uniformity in this example is assessed on the basis of off-types, which are observed visually.

4.6.2.3 The data obtained from individual plant measurements in rye are used for the assessment of distinctness and uniformity.

	Single record per variety (MG)	Records of individual plants (MS)
Example	Barley, document TG/19/10, Characteristic 12: Plant: length (stem, ear and awns)	Rye, document TG/58/6, Characteristics 10 + 11: Leaf next to flag leaf: length of blade Leaf next to flag leaf: width of blade
Recording of data	Replicated measurements in the plots and calculation of the plot mean value in order to determine a representative value for the plot (1-5 measurements in the plot depending on the variability within the plot) Measurement of all plot replications of the test and calculation of the overall mean value in order to determine a representative value for the variety under the specific year x location conditions Counting of off-types	60 plants per variety are recorded according to the Test Guidelines. The leaf next to flag leaf is collected from 60 plants (20 neighboring plants from each of 3 replicates). The plants at the beginning and the end of a row should be excluded. Measurement of leaf length and width (mm) (e.g. using a ruler on the desk).
Distinctness assessment	on the basis of one record per variety (single measurements are not used for further evaluations)	on the basis of 60 single plant records per variety; same data for D & U (mean, SD)
Uniformity assessment	on the basis of off-types	
Description	mean value of variety transformed into note	mean value of variety transformed into note

4.7 Summary

The following table summarizes the normal method of observation, although there may be exceptions:

	TYPE OF CHARACTERISTIC		
Method of propagation	QL	PQ	QN
VEGETATIVELY PROPAGATED	VG	VG	VG/MG/MS
SELF-POLLINATED	VG	VG	VG/MG/MS
CROSS POLLINATED	VG/(VS*)	VG/(VS*)	VS/VG/MS/MG
HYBRIDS	VG/(VS*)	VG/(VS*)	**

* records of individual plants only necessary if segregation should be recorded

** to be considered according to the type of hybrid (see section 4.3.3).

SECTION 5: METHODS FOR THE ASSESSMENT OF DISTINCTNESS IN THE GROWING TRIAL

5.1 Introduction

The assessment of distinctness can be based on visual observation (visual assessment) or based on the analysis of 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 details on the types of characteristics, the data obtained and the methods for the assessment of distinctness, see document TGP/8.3

5.2 Visual assessment

5.2.1 Introduction

The General Introduction (document TG/1/3) states that:

> "5.4 *Interpretation of Observations for the Assessment of Distinctness Without the Application of Statistical Methods*
>
> 5.4.1 In cases where there is very little variation within varieties, the determination of distinctness is usually on the basis of a visual assessment, rather than by statistical methods.
>
> 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."
>
> 5.4.4 In the case of pseudo-qualitative characteristics, guidance for the interpretation of observations for the assessment of distinctness without the application of statistical methods, is provided in document TGP/9, "Examining Distinctness.""

5.2.2 Quantitative characteristics

5.2.2.1 As explained in section 5.2.1, the General Introduction clarifies that, for quantitative characteristics, a difference of two notes often represents a clear difference, but that is not an absolute standard for assessment of distinctness and that this depends on many factors which are explored below:

Location / Year

5.2.2.2 Document TGP/7/1 "Development of Test Guidelines" (see Annex III: GN 28) explains that example varieties are important to adjust the description of the characteristics for year and location effects, as far as possible. However, it states that "Nevertheless, because of the possibility of particular interactions between the variety genotype and location (e.g. influence of photoperiod), it should not be assumed that descriptions developed in different countries or locations using the same set of example varieties will be the same […]." Thus, in cases where descriptions of varieties have been produced in different locations or different years it is not appropriate to assume that a difference of two notes between varieties, for a quantitative characteristic, demonstrates that the varieties are necessarily distinct. The difference in notes required to establish distinctness on the basis of descriptions produced in different locations or years will need to be evaluated on a case-by-case basis.

5.2.2.3 In cases where the descriptions of varieties are produced in the same location and year, i.e. in the growing same trial, the environmental variation within the trial, together with the possibility of making suitable adjustments for such variation, will need to be considered in relation to whether two notes represents a satisfactory basis for distinctness. Furthermore, where two varieties are situated side-by-side in the trial it may be possible to establish distinctness even where the two varieties are attributed the same note.

Range of Scale

5.2.2.4 Document TGP/7/1 "Development of Test Guidelines" (see Annex III: GN 20) explains that, in the case of quantitative characteristics, it is necessary to determine the appropriate range to describe the characteristic. In general, a standard "1-9" scale is used, but a "limited" range (notes 1-5) and a "condensed" range (notes 1-3) have also been accepted. Thus, when deciding on the number of notes required to establish distinctness, the range of the scale will need to be taken into account.

5.2.3 Pseudo-qualitative characteristics

5.2.3.1 In the same way as for quantitative characteristics, the number of notes which may establish distinctness is influenced by factors such as location, year and environmental variation within the trial. Also, as with quantitative characteristics, the range of the scale (number of notes) also varies. However, an important additional factor with pseudo-qualitative characteristics is that, whilst a part of the range is continuous, there is not an even distribution across the scale and it varies in more than one dimension (e.g. shape: ovate (1), elliptic (2), circular (3), obovate (4)). This means that it is difficult to define a general rule on the number of notes to establish distinctness within a characteristic.

5.2.3.2 The following examples illustrate why deciding on the number of notes required to establish distinctness needs particular care:

Example 1:

Type of mottling: only diffuse (1); diffuse and in patches (2); diffuse, in patches and linear bands (3); diffuse and in linear bands (4).

Example 2:

Shape: broad elliptic (1), medium elliptic (2), narrow elliptic (3), ovate (4)

Example 3:

Color: green (1), yellow green (2), green yellow (3), yellow (4), orange (5), red (6)

In the case of Examples 1 and 2, it is not appropriate to say that the "difference" between varieties with states 1 and 2 is less than between varieties with states 1 and 4, although they are respectively 1 and 3 notes "different". In some cases for example, the difference between notes 2 and 3 may be greater than between notes 1 and 4. However, Example 3 demonstrates that, for some pseudo-qualitative characteristics, it might be possible to follow a similar approach to that used for quantitative characteristics in some parts of the range e.g. varieties with states 2 and 3 (1 note difference) have less difference than those with states 1 and 4 (3 notes difference).

5.3 Measurements

5.3.1 Introduction

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 DUS analysis or for description of the characteristic (see document TGP/8.3 "Types of characteristics and their scale levels").

5.3.2 Pair-wise comparisons

The General Introduction, in section 5.5.2.2.3 notes that, for measured characteristics, pair-wise comparison is the simplest way of establishing distinctness. This method is particularly recommended in cases when:

(a) There are clear differences between varieties.

(b) The differences are always of the same sign.

(c) The differences can be expected to recur in subsequent trials (e.g. variety A is consistently and sufficiently greater than B).

(d) A sufficient number of comparisons has been made.

5.3.3 The Combined Over-Years Distinctness Criterion (COYD)

5.3.3.1 To assess distinctness for varieties on the basis of a quantitative characteristic it is necessary to calculate 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. Amongst the possible ways of establishing minimum distances is the method known as the Combined-Over-Years Distinctness (COYD).

5.3.3.2 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;

– calculating 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.

5.3.3.3 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.

5.3.4 Chi-square/Fisher exact tests

In the particular case where the plants of a given variety can have different states of expression for a characteristic, i.e. there is a big variability within variety the Chi-square method can be appropriate. Such characteristics can be important for distinctness purposes if the frequency of plants expressing the different states in a variety is consistent over time. For example, in Lucerne, the frequency of plants with the different states of the "flower color" characteristic (white or yellow (1), violet (2), very dark violet (3), variegated (4)) is used to assess distinctness between varieties. In this second case, the Chi-square or the Fisher exact tests can be used to assess distinctness (Snedecor, G.W.; Cochran W. (1937); Kanji G. K. (1993)). The Chi-square or the Fisher exact tests compare the frequencies of plants expressing the different states of the characteristic in different varieties. Details about these methods are provided in document TGP/8.

5.3.5 LSD

The General Introduction states the following in relation to the use of the LSD method for the assessment of distinctness (see document TG/1/3, section 5.5.3.1):

> [...] 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 [...]

Further details are provided in document TGP/8.

5.4 Parent formula of hybrid varieties

5.4.1 In some Test Guidelines, e.g. Maize (document TG/2), Rape seed (document TG/36) and Sunflower (document TG/81), an optional method for selecting varieties for the growing trial is described, based on the parent lines and the formula of the hybrid.

5.4.2 The method is based on the following steps:

(i) Description of parent lines according to the Test Guidelines.

(ii) Checking the originality of those parent lines in comparison with the variety collection, based on the Table of Characteristics of the Test Guidelines, in order to identify similar parent lines.

(iii) Checking the originality of the hybrid formula in relation to the hybrids in the variety collection, taking into account the most similar parent lines.

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

5.4.3 Details on the use of the parent formula are provided in Annex III.

SECTION 6: SUPPLEMENTARY PROCEDURES

Supplementary procedures may be developed to avoid the need for a systematic individual comparison, where such procedures, in conjunction with the other procedures, provide an effective examination of distinctness overall. Such procedures may also be appropriate for consideration of varieties of common knowledge for which living plant material is known to exist but where, for practical reasons, material is not readily accessible for examination. Some such procedures are explored below.

6.1 Publication of variety descriptions

The General Introduction notes that the publication of variety descriptions inviting comment from interested parties may be considered as a supplementary procedure to avoid the need for a systematic individual comparison (see document TG/1/3, section 5.3.1.2). An example of the use of such a procedure can be found in document TGP/6 Section 2.2, which explains the procedure used in Australia.

6.2 Cooperation between members of the Union

The General Introduction states that cooperation between members of the Union in the form of exchange of technical information could also be used as a supplementary procedure.

6.3 Use of randomized "blind" testing

6.3.1 After or during the examination of distinctness some doubts may exist over the distinctness of a variety on the basis of the trials. In such cases, the following situations are possible:

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

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

6.3.2 In the case of visually observed characteristics one possible arrangement for the additional test is “blind” testing.

6.3.3 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 and (a) similar variety(ies) are not clearly definable. In such a case, another test during or after the examination of distinctness may provide evidence for a definitive decision by the authority.

6.3.4 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: randomized parts of plants from the varieties under examination (e.g. leaves or fruit).

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

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

if the expert and the breeder always identify the variety,

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

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

6.4 The use of panels of experts

There may be cases where the assistance of a group of experts in a given crop may be appropriate. When a panel of experts is used, it is recommended that clear rules on the tasks and responsibilities of the experts involved as well as on the management of the information submitted for the purposes of examination be established in order to maintain the transparency of the system.

[Annex I follows]

ANNEX I

ANNEX I: THE GAIA METHODOLOGY

The GAIA method has been developed by experts from France to calculate a phenotypic distance between two varieties. The principle is to compute a phenotypic distance between a pair of varieties, which is a sum of distances for individual characteristics. For each species, this system must be calibrated to determine the weighting given to the difference in each characteristic used and the threshold for the phenotypic distance used to eliminate varieties from the growing trial.

1. Weighting of characteristics

1.1 Weighting is defined as the contribution in a given characteristic to the total distance between a pair of varieties. 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. For that reason the role of the crop expert is essential.

1.2 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 and stored in the GAIA database. Experts can give zero weighting to small differences, thus, even if two varieties have different observed values in many characteristics, the overall distance might be zero. The same weighting is attributed to any pair of varieties whose absolute differences between observed values are the same for a given characteristic.

1.3 The weighting should be simple and consistent. The following three rules are given:

(i) the distances for the characteristic should be integer values, i.e. 0, 1, 2, 3, etc. where 3 is considered to be about 3 times greater than 1;

(ii) 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*;

(iii) define the values by "try-and-check" (see Diagram 1.)

1.4 The following simple example shows the computation of the distance between two varieties on the basis of a qualitative characteristic:

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

Shape of ear:
1 = conical
2 = conico-cylindrical
3 = cylindrical

Comparison between difference in notes and weighting		
	Different in notes	Weighting
conical (1) vs. conical (1)	0	0
conical (1) vs. conico-cylindrical (2)	1	2
conical (1) vs. cylindrical (3)	3	6
conico-cylindrical (2) vs. conico-cylindrical (2)	0	0
conico-cylindrical (2) vs. cylindrical (3)	1	2
cylindrical (3) vs. cylindrical (3)	0	0

When the crop experts compare a variety 'i' with conical ear (note 1) to a variety 'j' with cylindrical ear (note 3), they attribute a weighting of 6 etc. The weightings are summarized in the form of a weighting matrix:

Weighting matrix 'i'				
		Variety i		
Variety 'j'		**1**	**2**	**3**
	1	0	2	6
	2		0	2
	3			0

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

2. Determining "Distinctness Plus"

The threshold for the phenotypic distance used to eliminate varieties from the growing trial is called "Distinctness Plus" and is determined by the crop expert at a level which is higher than the difference needed to establish distinctness. This ensures that all pairs of varieties having a distance equal or greater than the threshold (Distinctness Plus) would be distinct in the growing trial. The Distinctness Plus threshold must be based on experience gained with the varieties of common knowledge and must minimize the possibility of not including in the growing trial a pair of varieties which should be further compared in the field.

3. Computing GAIA phenotypic distance

3.1 The principle is to compute a phenotypic distance between two varieties, which is the total distance between a pair of varieties as the result of the addition of the weightings of all characteristics (see section 2 of this Annex). Thus, the GAIA phenotypic distance is:

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

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 the weighting of characteristics k, which is a function of the difference observed between variety *i* and variety *j* for that characteristic k.

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

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

3.2 Varieties are compared in pairs in different combinations of pair-wise comparisons, e.g.:

- compare two varieties,
- compare a given variety to all varieties in the variety collection,
- compare all candidate varieties to all the other candidate varieties and the varieties in the variety collection,
- compare all possible combinations.

4. The GAIA software

4.1 The GAIA software allows the computation of the phenotypic distance using qualitative, quantitative or electrophoretic characteristics, which can be used alone or in combination. The user can decide on the type of data and the way it is used:

(i) select all the available characteristics, or different subsets of characteristics.

(ii) define different weighting 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 a subset of characteristics only.

(iii) the way the Distinctness Plus threshold is used:

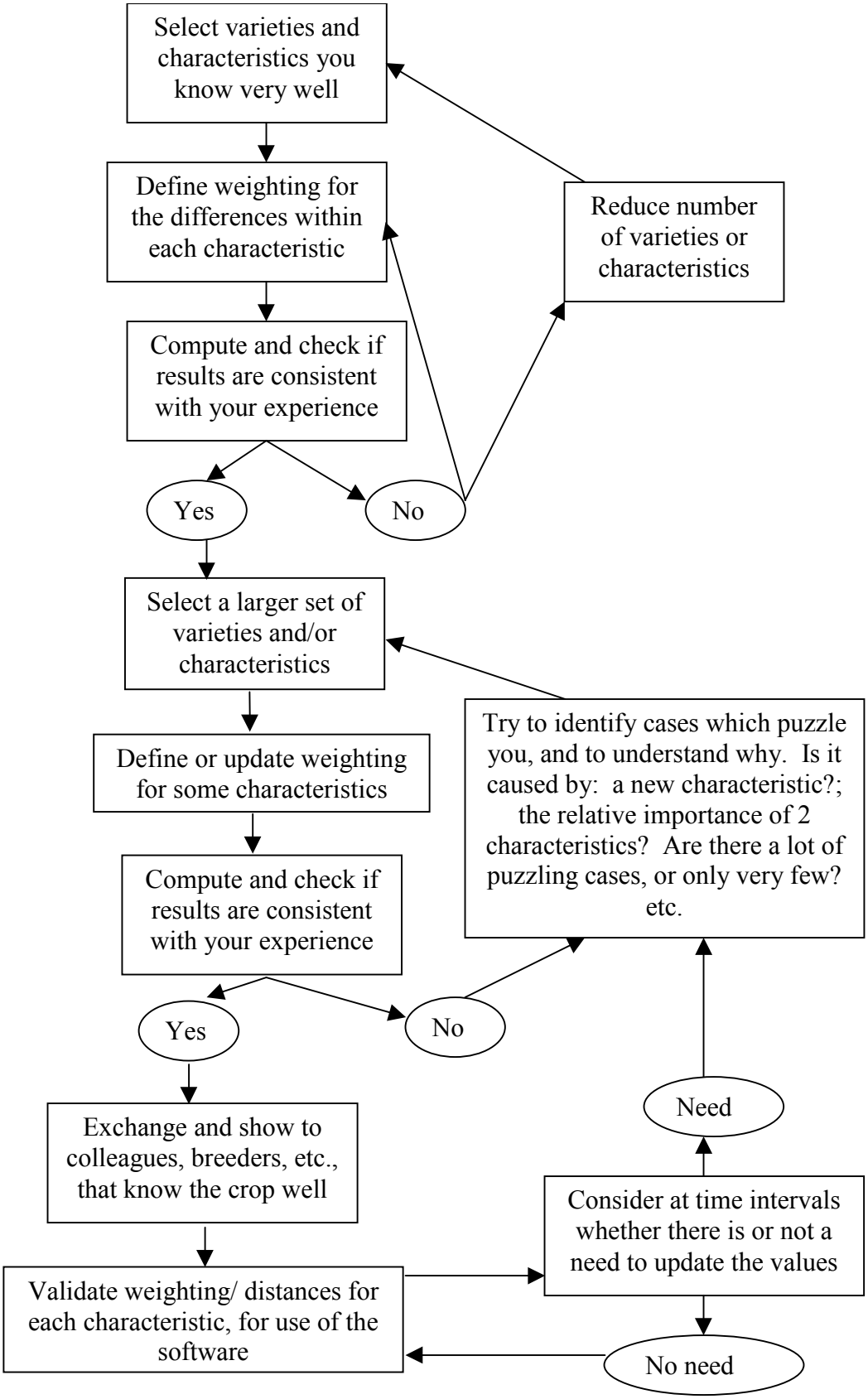
- a low Distinctness Plus threshold, which helps to find the more difficult cases (to identify similar varieties- very often used by crop experts);
- intermediate Distinctness Plus threshold (different levels according to the needs);

- a large Distinctness Plus threshold when there is a need to have a comparison which uses all the available characteristics;
- a Distinctness Plus threshold greater than the maximum distance possible on all characteristics to see all available raw data and the weightings for each characteristic

4.2 The software provides a comprehensive report for each pair-wise comparison. It computes an overall distance, but also provides all the individual absolute values and the distance contribution of each characteristic (see section 6 of this Annex).

4.3 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.

Diagram 1: "Try-and-check" process to define and revise the weightings for a crop



4.4 Section 6 of this Annex provides a screen copy of a display tree which shows how the expert can navigate and visualise the results of computations.

4.5 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 for other information systems to be used in connection with the GAIA software. ODBC allows access to the GAIA database and to other databases simultaneously.

4.6 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.

4.7 GAIA is most suitable for self-pollinated and vegetatively propagated varieties, but can also be used for other types of varieties.

5. Using the GAIA methodology

The GAIA methodology can be used:

(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).

5.1 Using phenotypic distance in the first growing cycle

5.1.1 A crop that has a large variety collection and uses only quantitative characteristics on a 1 to 9 scale; the GAIA methodology allows the selection of varieties to be included in the growing trial. This can be used to plan the first growing cycle trials as well as the subsequent growing cycles.

5.1.2 In crops with relatively few candidates and a small variety collection, which enables the crop expert to sow all candidates (e.g. an agricultural crop), and the appropriate reference varieties, in two or three successive growing cycles. The same varieties are sown in growing cycles 1, 2 and 3, in a randomized layout. 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.

5.2 Using phenotypic distance after the first growing trial

5.2.1 After one growing cycle (e.g. 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.

5.2.2 In cases where there are many candidate and reference varieties and there is a wide variability in the species (e.g. a vegetable crop); 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.

5.2.3 In crops in which there are many similar varieties, for which it is a common practice to make side-by-side comparisons, GAIA can be used to identify the similar varieties after the first cycle, in particular, when the number of varieties in a trial increases, making it less easy to identify all the problem situations. The software can help to "not miss" the less obvious cases.

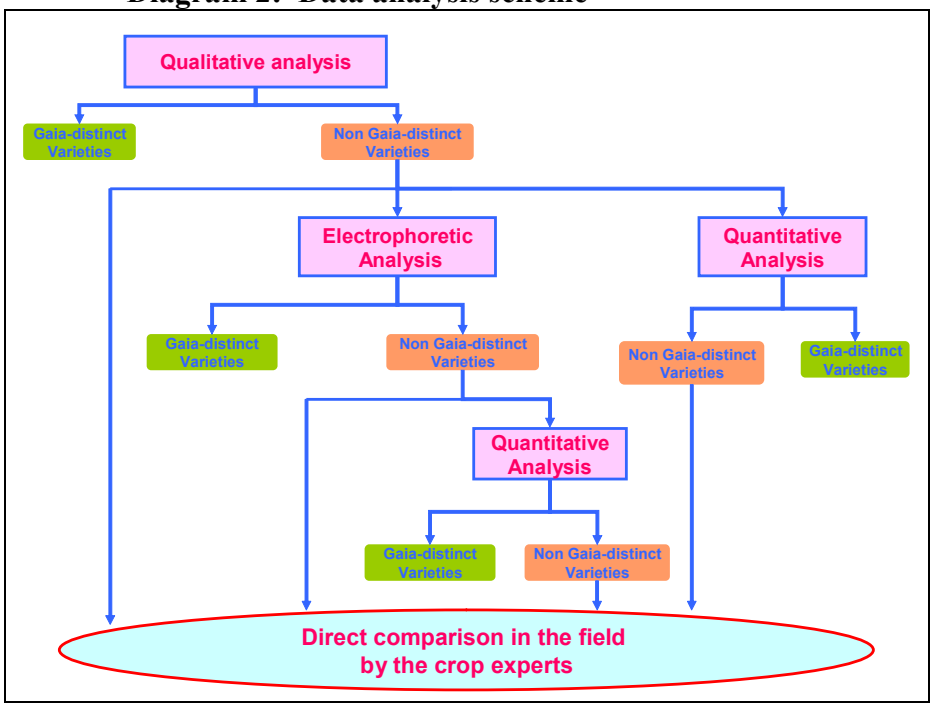
5.2.4 In vegetatively propagated ornamental varieties, the examination lasts for one or two growing cycles: after the first growing cycle, some reference varieties in the trial are obviously different from all candidates, and their inclusion in the second growing cycle is not necessary. When the number 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.

6. Example with qualitative, electrophoretic and quantitative characteristics (Zea mays data)

6.1 Introduction

6.1.1 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 2.

Diagram 2: Data analysis scheme



6.1.2 In this example, it is assumed that the crop expert has decided to use a Distinctness Plus threshold $\mathbf{S_{dist}}$ of 10 (see section 2 of this Annex).

6.2 Qualitative Analysis

6.2.1 For each characteristic, weightings according to differences between levels of expression are pre-defined in a matrix of distances.

6.2.2 "Shape of ear": observed on a 1 to 3 scale, the crop experts have attributed weightings greater than zero 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 (note 1) to a variety 'j' with cylindrical ear (note 3), they attribute a weighting of 6.

6.2.3 "Length of husks", observed on a 1 to 9 scale, the crop experts have defined the following 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

The weighting between a variety 'i' with very short husks (note 1) and a variety 'j' with short husks (note 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 greater than 3, the experts do not increase the distance more than 2.

6.2.4 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.

6.2.5 The matrix for a qualitative analysis for 5 characteristics for varieties A and B:

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

6.3 Electrophoretic analysis

6.3.1 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.

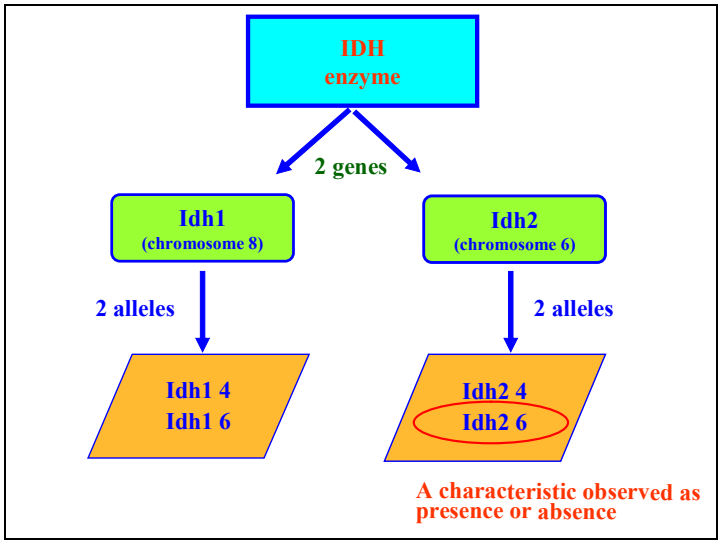


Diagram 3: The Isocitrate Deshydrogenase (IDH) enzyme has two genes (Idh1 and Idh2) located on two different chromosomes. Each of them has two alleles which are observed as 1 (presence) or 0 (absence).

6.3.2 Electrophoretic characteristics are noted as 0 or 1 (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

6.3.3 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:

$$\mathbf{D_{elec} = 2 \times 0.25 + 1 \times 1 = 1.5}$$

2 is the number of differences observed

0.25 is the weighting attributed by experts to the number of differences

1 is the number of chromosome on which differences are observed

1 is the weighting associated by experts to chromosome.

6.3.4 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.

6.3.5 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".*

6.3.6 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.

6.4 Quantitative Analysis

6.4.1 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.

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

6.4.3 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.

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

6.4.5 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

6.4.6 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} =?

6.4.7 In this 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.

6.4.8 The user must 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.

6.4.9 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 $\mathbf{D_{quan} = 3}$.

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

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

6.4.11 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".

6.4.12 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).

6.4.13 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

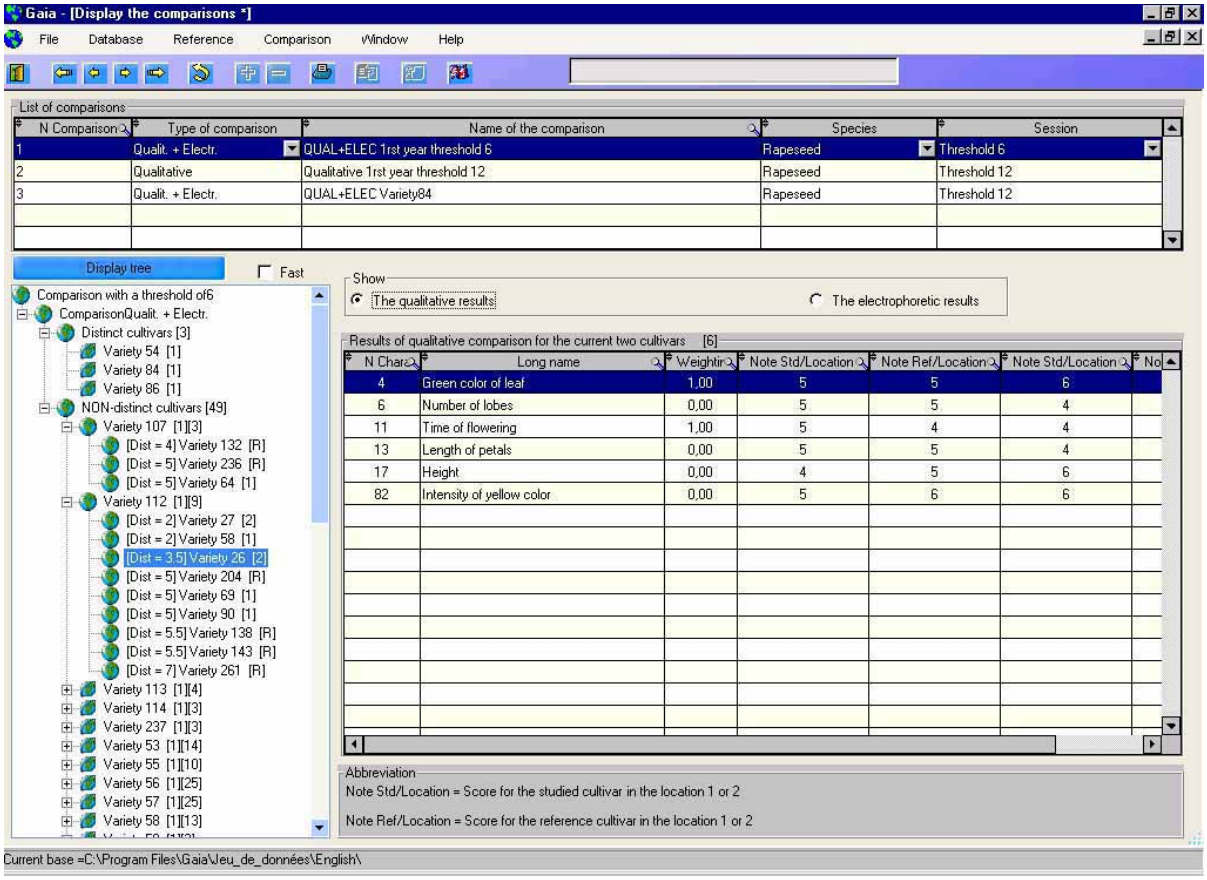
Variety i			
	1	2	3
1	0	2	6
2		0	2
3			0

6.5 Quantitative and qualitative analysis on the same characteristics

6.5.1 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.

6.5.2 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.

7. GAIA 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.).

8. Final remark

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.

[Annex II follows]

ANNEX II: THE COMBINED OVER-YEARS DISTINCTNESS CRITERION (COYD)

1. Introduction

1.1 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.

1.2 The following sections provide information on:

- 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, including when there are small numbers of varieties in trial;
- the computer software which is available to apply the procedure.

1.3 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.

1.4 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 section 7.

2. The COYD Method

2.1 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.

2.2 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.

2..3 Calculation of the COYD criterion involves analyzing the variety-by-year table of means for each characteristic to obtain 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 document 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.

2..4 Where there are small numbers of varieties in trial, the table used to calculate the COYD criterion is expanded with means from other varieties and earlier years, a different method of analysis is used to obtain a varieties-by-years mean square to estimate the varieties-by-years variation, and the resulting LSD is known as the Long-Term COYD. This is discussed in section 3.2.

2.5 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.

2.6 An example of the application of COYD to a small data set is given in Figure 1. Statistical details of the method are in section 5 of this Annex 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).

3. Adapting COYD

3.1 Differences between years in the range of expression of a characteristic

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 sections 6 and 7.

3.2 Small numbers of varieties in trials

.3.2.1 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.

3.2.2 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 analyzed 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.

3.2.3 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".

3.2.4 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.

3.2.5 Figure 2 gives an example of the application of Long-Term COYD to the Italian ryegrass (document TG/4/8(proj.1) characteristic "Growth habit in spring" (UPOV Characteristic 6). A flow diagram of the stages and DUST modules used to produce Long-Term LSDs and perform Long-Term COYD is given in Figure B2 in section 6.

3.3 Marked year-to-year changes in an individual variety's characteristic

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.

4. 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,
e-mail: sally.watson@dardni.gov.uk,
web-site: http://www.qub.ac.uk/afs/departments/bio/.

Sample outputs are given in section 6.

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

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

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

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. COYD Statistical Methods

5.1 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)

5.2 Modified joint regression analysis (MJRA)

5.2.1 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.

5.2.2 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.

5.2.3 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.

5.2.4 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.

5.2.5 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:

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

2 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.

6. The COYD Software

6.1 COYD computer program

6.1.1 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.

6.1.2 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).

6.1.3 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

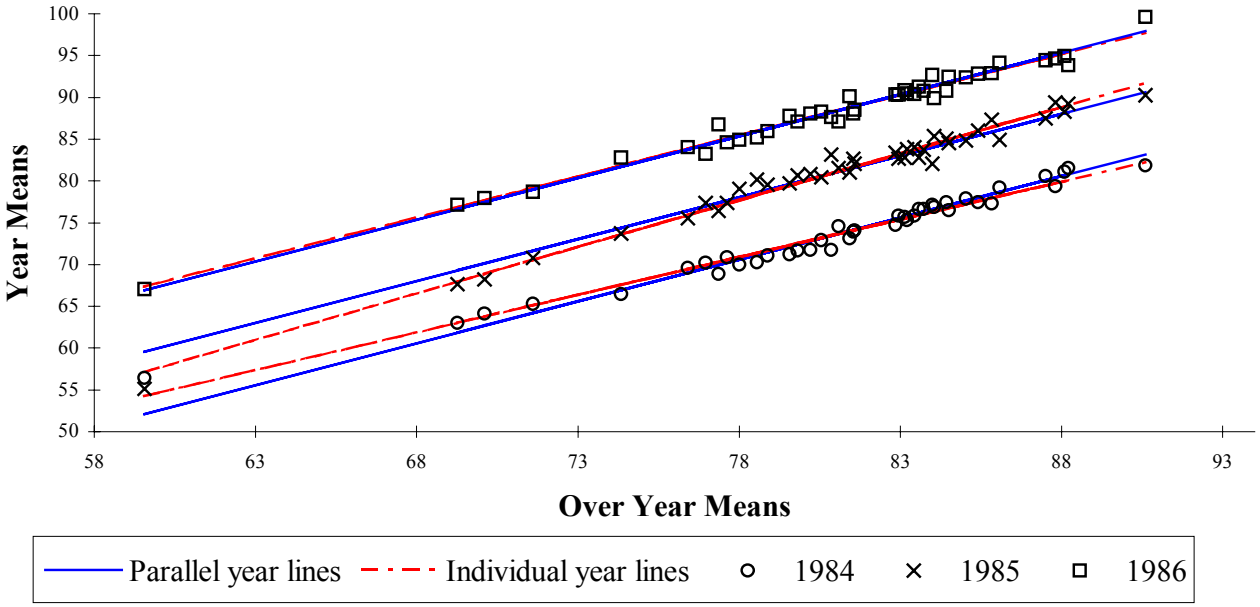
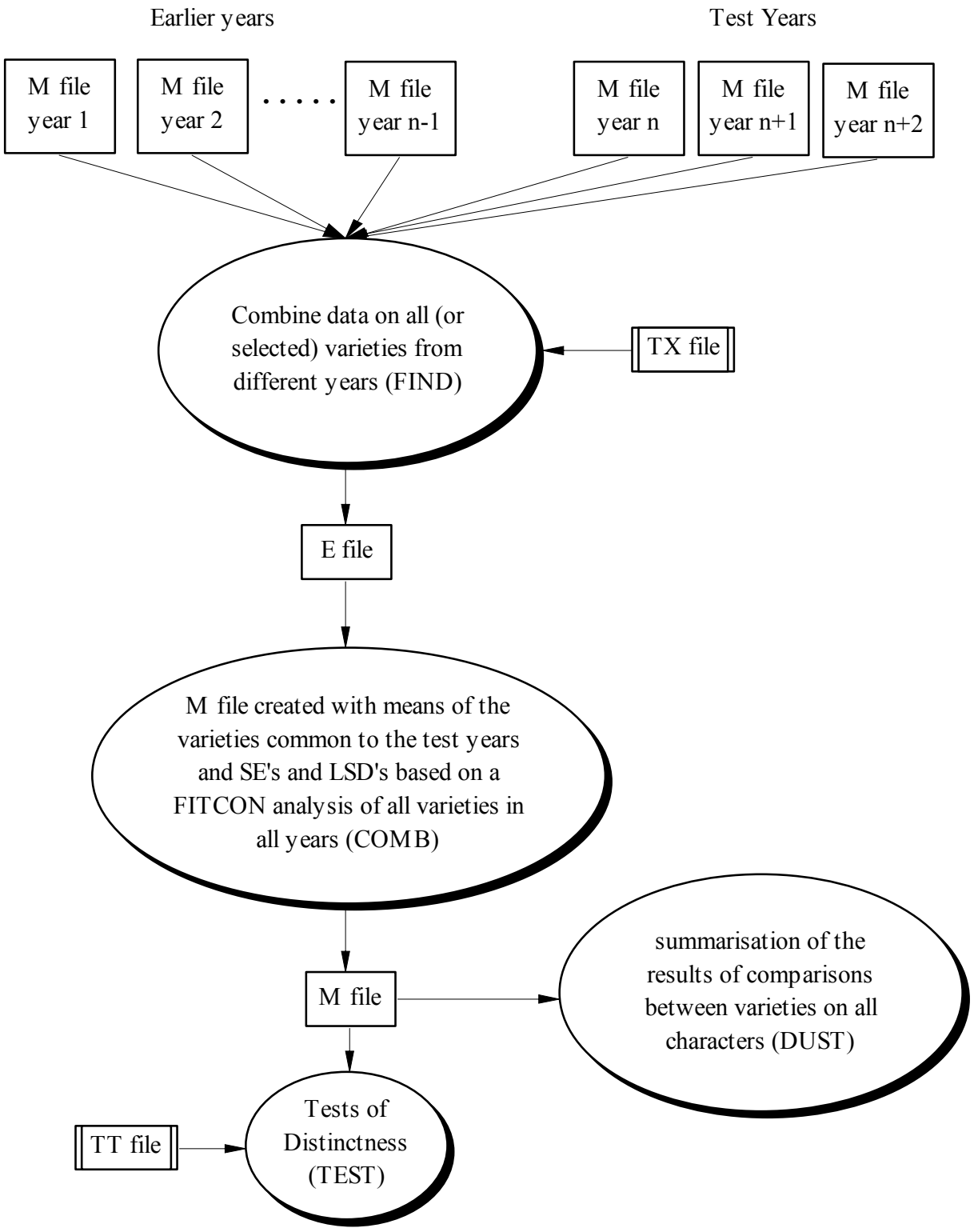


Figure B2. Flow Diagram of the stages and DUST modules used to produce long-term LSD's and perform long-term COYD



7. Distinctness testing schemes and the probability levels used for COYD

7.1 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

7.2 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.

7.3 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.

7.4 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}.

7.5 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}.

7.6 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

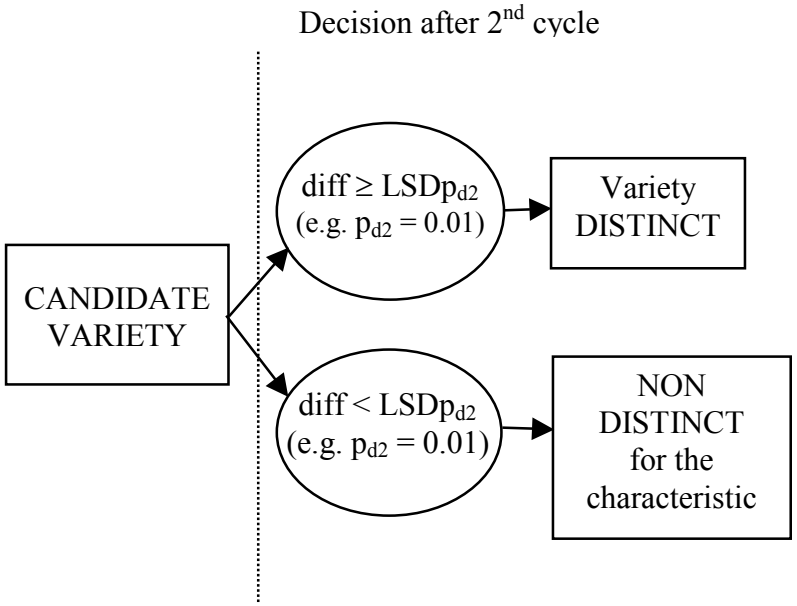
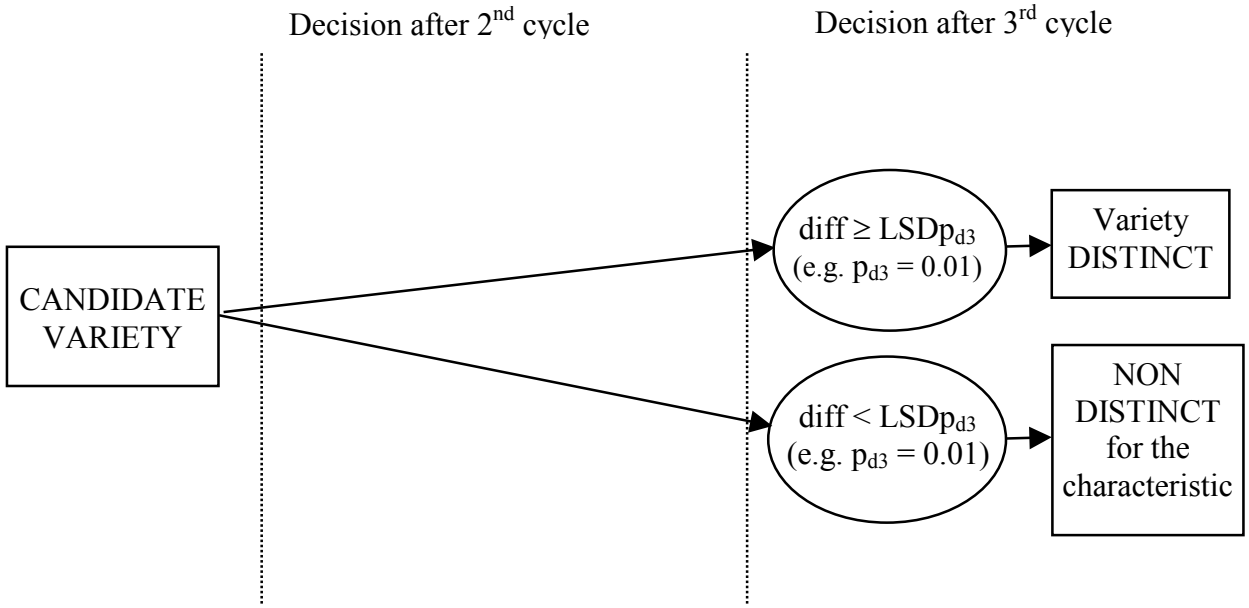


Figure 2. COYD decisions in Scheme B



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

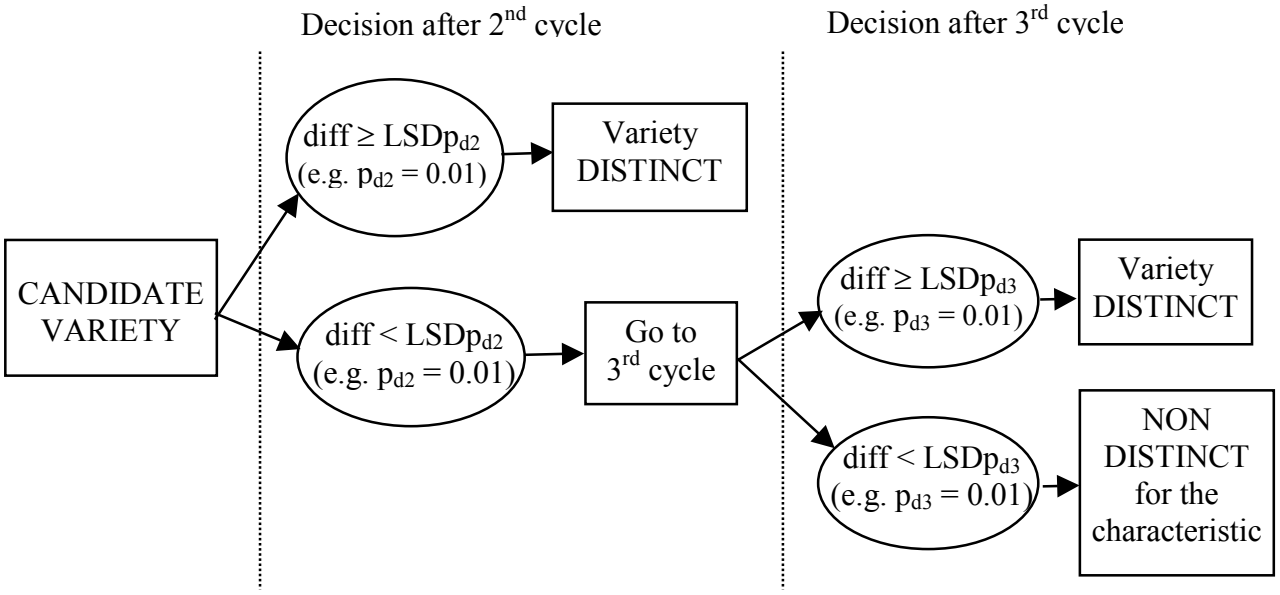
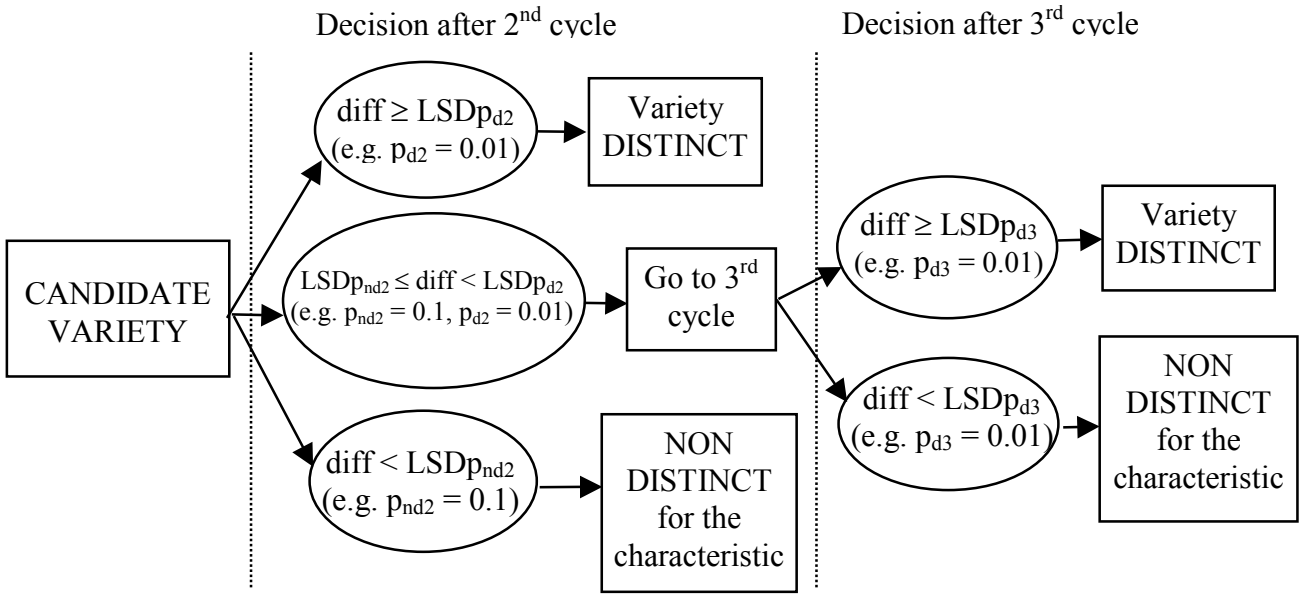


Figure 4. COYD decisions in Scheme D



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.

8. Alternative criteria

8.1 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.

8.2 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.

8.3 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.

8.4 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 $\lambda > 1.7$.

9. References

DIGBY, P.G.N. (1979). Modified joint regression analysis for incomplete variety x environment data. J. Agric. Sci. Camb. 93, 81-86.

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.

[Annex III follows]

ANNEX III

ANNEX III: PARENT FORMULA OF HYBRID VARIETIES

1. Introduction

1.1 In some Test Guidelines, e.g. Maize (document TG/2), Rape seed (document TG/36) and Sunflower (document TG/81), an optional method for selecting varieties for the growing trial is described, based on the parent lines and the formula of the hybrid.

1.2 The method is based on the following steps:

(i) Description of parent lines according to the Test Guidelines.

(ii) Checking the originality of those parent lines in comparison with the variety collection, based on the Table of Characteristics of the Test Guidelines, in order to identify similar parent lines.

(iii) Checking the originality of the hybrid formula in relation to the hybrids in the variety collection, taking into account the most similar parent lines.

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

1.3 Details on the use of the parent formula are provided in section 5.5.

2. Requirements of the method:

2.1 The application of the method has certain requirements:

(i) A declaration of the formula and submission of plant material of the parent lines of hybrid varieties.

(ii) Inclusion in the variety collection of the parent lines used as parents in the hybrid varieties of the variety collection (for guidance on the constitution of a variety collection see document TGP/4 section 1) and a list of the formulae of the hybrid varieties.

(iii) Application of the method to all varieties in the variety collection. This condition is important to obtain the full benefit.

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

3. Assessing the originality of a new parent line

3.1 The basis for establishing the originality is the list of characteristics described in the relevant Test Guidelines.

3.2 The difference between parent lines must be sufficient to be sure that the hybrids are distinct. For example:

Characteristic 1: a characteristic having two states of expression (absent/present) which are determined by two alleles of a single gene, with one dominant allele (+) for the expression "present" and one recessive allele (-) for the expression "absent".

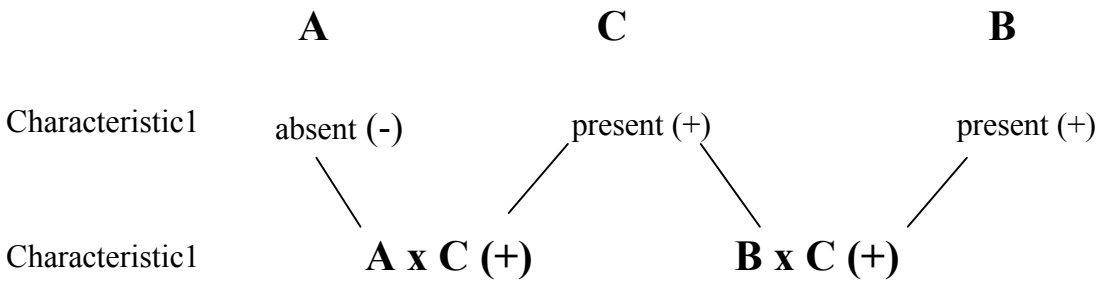
Three parent lines:

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

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

(A x C): having expression "present" for Characteristic 1
(B x C): having expression "present" for Characteristic 1

The following diagram shows the ways the two different crossings result in the same expression of Characteristic 1 (i.e. "present" in both hybrids), although parent line A(-) and parent line B(+) have different expressions.



3.3 Although the parent lines A and B are clearly different for characteristic C1, the two hybrid varieties have the same expression. Thus, a difference between A and B for Characteristic 1 is not sufficient.

3.4 With a more complex genetic control involving several genes, not precisely described, the interaction between the different alleles of each gene and between genes might also lead to similar expression at the level of the hybrid varieties. In such cases, a larger difference is appropriate to establish distinctness between two parent lines.

3.5 Determining the difference required is mainly based on a good knowledge of the species, of the characteristics and, when available, on their genetic control.

3.6 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.

4. Verification of the formula

4.1 Verification of the formula: the aim is to check if the candidate hybrid variety has been produced by crossing the parent lines declared and submitted by the applicant.

4.2 Different characteristics can be used to perform this check as soon as the genetic pattern of each parent can be identified in the hybrid. Generally, characteristics based on polymorphism of enzymes or of some storage proteins can be used.

4.3 If no suitable characteristics are available, the only possibility is to cross the parent lines using the plant material submitted by the applicant and to compare the hybrid variety seedlots (the sample submitted by the applicant and the sample harvested after the cross).

5. Uniformity and stability of parent lines

5.1 The uniformity and stability of the parent lines should be assessed according to the appropriate UPOV recommendations for the variety concerned. The uniformity and stability of the parent lines are important for the stability of the hybrid. Another requirement for the stability of the hybrid is the use of the same formula for each cycle of the hybrid seed production.

5.2 A check of the uniformity on the hybrid should also be done, even if distinctness of the hybrid has been established on the basis of the parent lines.

6. Description of the hybrid

A description of the candidate hybrid should be established, even where the distinctness of the hybrid has been established on the basis of the parent formula.

[End of Annex III and of document]