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STATISTICAL METHODS FOR TESTING DISTINCTNESS

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STATISTICAL METHODS FOR TESTING DISTINCTNESS

I. INTRODUCTION

1. To receive protection under the UPOV Convention, a new variety must be distinct from all varieties whose existence is a matter of common knowledge ("varieties of common knowledge"). Distinctness is assessed by comparing the characteristics of the candidate variety with the varieties of common knowledge (varieties of common knowledge).

2. Section 5.3.3 of document TG/1/3 (General Introduction) states:

"5.3.3 The Criteria for Distinctness Using Characteristics

The UPOV Convention does not elaborate the term "clearly distinguishable." However, in order to provide some guidance on the interpretation of the term, the following basis has been developed for the use of characteristics to clearly distinguish varieties. A variety may be considered to be clearly distinguishable if the difference in characteristics is:

(a) consistent, and
(b) clear.

5.3.3.1 Consistent Differences

5.3.3.1.1 One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic on at least two independent occasions. This can be achieved in both annual and perennial varieties by observations made on plantings in two different seasons or, in the case of other perennial varieties, by observations made in two different seasons after a single planting. Guidance on the possible use of other approaches, such as two different environments in the same year, is explored in document TGP/9, "Examining Distinctness."

5.3.3.1.2 However, in some circumstances the influence of the environment is not such that a second growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. For example, if the growing conditions of the crop are controlled, such as in a greenhouse with regulated temperature and light, it may not be necessary to observe two growing cycles. In addition, the differences observed between varieties could be so clear that a second growing cycle may not be necessary. In both these circumstances, the features of propagation of the variety and the quality of the plant material will need to be taken into account.

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

3. 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. However, for measured characteristics as well as for visually assessed characteristics statistical methods can be applied.

4. For example:

(1) The Combined-Over-Years Distinctness criterion (COYD);

(2) The 2 x p% distinctness criterion;

(3) A fixed criterion.

5. The last of these is not a statistical method except to the extent that the fixed difference should be chosen to be large enough to clearly distinguish varieties in repeated trials. It may be used, for example, when a characteristic is scored using integers on a 1 to 9 scale. The variability in average scores for a variety may be much less than one point on this scale, and so a one-point difference may be considered an adequate basis for establishing distinctness.

6. COYD is a method that is often used. This method is described below. The 2 x p% criterion is also described briefly.

7. Both methods use the technique of analysis of variance and associated concepts. A number of textbooks provide an introduction to this subject; the book by Mead *et al* (1992) is a good example. Document TWC/18/10 describes the COYD method in more detail.

II. THE COMBINED-OVER-YEARS DISTINCTNESS CRITERION

Description of method

8. The COYD method is based on an over-year analysis of variety means from the two or three years of trials that a candidate is under test. Based on this analysis, a candidate is judged to be distinct from a reference variety if it is significantly different from the variety for at least one characteristic. The COYD method involves a number of steps.

9. The first stage in a COYD analysis is to calculate variety means for each trial and each characteristic. The form of analysis applied will depend on the design used for the trials: usually randomised complete block designs. For such designs, the variety estimates derived from the analysis of variance would simply be the arithmetic means over the blocks.

10. The next stage would be to apply, characteristic-by-characteristic, an analysis of variance to the variety-by-year means derived in step 1. Note that usually only varieties common to all years are included in the analysis (but see section 3). The analysis of variance table has the following form:

Source	Degrees of freedom (df)	Sum of Squares (SS)	Mean squares (MS)	F-ratio (F)
Year	y-1	SS_{year}	$MS_{year} = SS_{year}/(y-1)$	$MS_{year}/MS_{residual}$
Variety	v-1	$SS_{variety}$	$MS_{variety} = SS_{variety}/(v-1)$	$MS_{variety}/MS_{residual}$
Variety-by-year interaction	(v-1) . (y-1)	$SS_{residual}$	$MS_{residual} = SS_{residual}/((v-1).(y-1))$	
Total	vy-1			

where "y" is the number of years and "v" is the number of varieties. Note that the variety-by-year interaction is also known as the residual error. $MS_{residual}$ is also known as the variety-by-year variance.

11. A least significant difference (LSD) for comparing variety means is calculated based on this analysis. This is calculated using the following formula:

$$LSD = t_{p,df_{residual}} \sqrt{\frac{2}{y} MS_{residual}}$$

where $df_{residual}$ is the degrees of freedom for the variety-by-year interaction and $t_{p,df}$ is the critical value taken from Student t table for a two-tailed t-test with significance level p and with df degrees of freedom. The choice of p is discussed in 2.2.

12. For each characteristic, the difference between a candidate and a reference variety is compared with the LSD to assess whether the candidate is distinct from that reference variety. To be distinct overall, the candidate needs to be found distinct from all reference varieties in at least one characteristic.

13. An example to illustrate the application of COYD is given in the Appendix.

Specification of the significance level

14. Different values may be used for different types of varieties according to the particular features of their propagation (e.g. hybrid, cross-pollinated, etc…). Typical values are 0.01, 0.02 and 0.05.

Comparison with the 2 x p% criterion

15. With the 2 x p% criteria, assessments of distinctness are carried out on each of the trials during the two or three years that a candidate is under test. In each year, LSDs are based on within-trial error derived from the trial analysis of variance. A candidate is judged to be distinct from a reference variety if it is significantly different from the variety in two or more years for at least one characteristic. Note that the direction of significant differences should be consistent.

16. The two distinctness criteria may produce different decisions in practice, even if the levels of significance applied are the same. Reasons for using COYD are provided in document TWC/18/10.

Software

17. The "DUST" software system is designed to analyse DUS experiments and includes modules for COYD. Module ANAL9 should be first applied to each trial in turn to create the files of means for use in the COYD analysis. Then COYD analyses can be carried out using module TVAL9, TSUM9 or TVRP9. These modules differ in the output produced.

18. Other statistical packages with analysis of variance facilities (e.g. GenStat or SAS) may also be used to implement this method.

III. ISSUES

Trial design

19. It is important to consider the experimental design of the trials so that comparisons between candidates and reference varieties are sufficiently precise. Two components of experimental design are replication and layout.

20. Increasing replication will produce more precise trial means; however it will not necessarily greatly reduce the COYD LSD. In general, once the level of replication is set, it should be maintained since it is important to make decisions on a consistent basis.

21. Blocking is an important tool for controlling environmental variation across a trial. For trials with relatively few varieties, randomised complete block designs should be used. For trials with large numbers of varieties, there are a number of approaches that could be considered:

1) Use of grouping characteristics.

In some crops, there are "grouping" characteristics. The General Introduction (Section 4.8) defines the following functions and criteria for the selection of grouping characteristics:

"Function of grouping characteristics:

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.

Criteria for the selection of trouping characteristics:

1. (a) Qualitative characteristics or

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

These grouping characteristics, can be used to divide the reference collection and candidate varieties into groups. These groups can be kept together within the main design (a type of nested design), and, in the analysis, comparisons need only be made within the groups. Characteristics used for grouping should consistently allocate a variety to the same group.

2) Use of incomplete block designs.

This is under review by the TWC.

3) Cyclic controls.

If distinctness assessments are carried out over three years, it is possible to use a system of cyclic planting of the reference collection (document TWC/17/11). This ensures that each reference variety is tested alongside the candidates for two years in every three-year cycle. A special form of COYD analysis is required; this is implemented in the DUST software.

4) Reduction of reference collection using other information.

If information is available which can be used to identify varieties of common knowledge from which all candidates are distinct, these varieties do not need to be included in the trials.

Small numbers of varieties

22. When the number of varieties under test is small, this may mean that differences between varieties are not well estimated. For COYD, it is recommended that there should be at least 20 varieties for a trial series of two years, and 10 varieties for a trial series of three years (document TWC/18/10). If this is not possible, a long-term estimate of variety-by-years variance can be derived from an analysis including earlier trials. This estimate can then be used to derive an LSD for the current series. This is known as the long-term COYD method (document TWC/18/10). The DUST software provides a mechanism for long-term COYD.

Year-to-year inconsistencies

23. Sometimes the difference between pair of varieties in a particular characteristic may vary markedly between years. It is possible to calculate an F-ratio to check whether such variation is unusual (document TWC/18/10); this is called F_3. In DUST, the user is notified if a pair of varieties would have been distinct but F_3 indicates a consistency problem. Such cases should be investigated before reaching a decision on distinctness.

Differences between years in the range of expression of a characteristic

24. Sometimes a characteristic can show a marked difference between years in its range of expression. In such cases, it may be beneficial to use an adaptation of COYD called modified joint regression analysis (MJRA). Document TWC/18/10 provides details of this method.

Missing observations

25. If, in a three-year trial series, observations on a variety are missing in one year, it may still be valid to include that variety in distinctness decisions. However, some care will be needed in the analysis, both to ensure that over-year variety means are correctly adjusted, and to calculate an appropriate LSD for use with that particular variety. In DUST, the standard COYD modules will eliminate such varieties, but there are other modules that can be used to perform the analysis correctly.

IV. REFERENCES

Mead R., Curnow R.N. & Hasted A.M. (1993) *Statistical methods in agriculture and experimental biology*. Chapman and Hall, London.

APPENDIX

An example of application of the COYD criterion

An example is given here to illustrate the application of the COYD criterion. This is based on data from a real trial series, but with variety names and characteristic names recoded. Only two characteristics will be considered, Char01 and Char02. There are 18 reference varieties (Ref01 to Ref18*)* and two candidates (Can01 to Can02). There are trials from two years (1998 and 1999) employing a randomised complete block design with four replicates. A 2% significance level is used for COYD decisions. The analysis is carried out in 3 steps below.

Step 1 – analysis of individual trials

Apply analysis of variance to each trial separately and compute variety means. The results of this for the example are given characteristic by characteristic below. The tables show the characteristic values for each block and the means over blocks.

Char01

1998

	Block scores				Mean
Variety	1	2	3	4	
Ref01	37.2	38.5	36.9	38.1	**37.7**
Ref02	36.4	34.3	36.9	35.5	**35.8**
Ref03	38.5	34.2	38.7	36.1	**36.9**
Ref04	35.7	35.2	38.7	40.8	**37.6**
Ref05	32.8	34.7	33.7	34.9	**34.0**
Ref06	37.4	35.3	36.2	35.6	**36.1**
Ref07	36.0	36.3	38.8	36.0	**36.8**
Ref08	41.4	41.0	38.7	39.6	**40.2**
Ref09	41.2	39.8	39.9	40.5	**40.4**
Ref10	37.1	35.4	36.7	37.2	**36.6**
Ref11	35.5	36.1	35.2	37.3	**36.0**
Ref12	35.9	37.7	39.3	36.7	**37.4**
Ref13	33.9	36.6	36.3	34.3	**35.3**
Ref14	37.2	36.5	36.8	35.7	**36.5**
Ref15	34.9	34.7	35.1	34.5	**34.8**
Ref16	36.9	37.4	36.3	36.7	**36.8**
Ref17	36.3	37.2	37.5	36.9	**37.0**
Ref18	40.6	40.3	39.7	40.5	**40.3**
Can01	36.5	37.9	38.5	40.0	**38.2**
Can02	38.0	38.4	37.5	38.2	**38.0**

1999

	Block scores				Mean
Variet	1	2	3	4	
Ref01	38.1	37.9	38.4	38.0	**38.1**
Ref02	37.5	40.3	38.1	41.3	**39.3**
Ref03	38.0	39.2	38.2	40.4	**39.0**
Ref04	38.3	40.9	40.3	41.3	**40.2**
Ref05	38.7	37.9	36.9	37.1	**37.6**
Ref06	38.6	38.9	37.3	38.2	**38.2**
Ref07	37.9	38.2	37.0	37.1	**37.6**
Ref08	39.7	39.7	40.6	40.7	**40.2**
Ref09	39.4	40.6	40.5	41.9	**40.6**
Ref10	36.5	38.6	38.4	39.4	**38.2**
Ref11	37.2	38.8	38.9	38.2	**38.3**
Ref12	38.0	37.9	37.2	39.2	**38.1**
Ref13	37.8	36.6	36.7	36.5	**36.9**
Ref14	38.5	37.6	38.0	40.0	**38.5**
Ref15	36.3	35.3	36.9	37.2	**36.4**
Ref16	37.4	37.2	38.9	37.6	**37.8**
Ref17	37.9	39.5	38.9	39.3	**38.9**
Ref18	40.4	40.0	41.7	42.6	**41.2**
Can01	39.1	40.6	41.1	38.5	**39.8**
Can02	37.8	37.8	38.3	39.8	**38.4**

Char02

1998

Variety	Block scores 1	2	3	4	Mean
Ref01	157.3	145.7	151.6	140.1	**148.7**
Ref02	124.7	108.5	133.0	131.9	**124.5**
Ref03	123.3	104.9	126.9	124.6	**119.9**
Ref04	144.5	126.1	125.1	130.7	**131.6**
Ref05	119.8	118.6	138.4	153.4	**132.6**
Ref06	130.8	94.6	109.3	123.4	**114.5**
Ref07	139.0	104.5	127.1	121.8	**123.1**
Ref08	130.4	106.3	142.5	117.1	**124.1**
Ref09	136.2	116.1	132.6	137.2	**130.5**
Ref10	109.3	124.2	132.1	130.9	**124.1**
Ref11	139.3	112.3	115.5	115.6	**120.7**
Ref12	148.3	121.5	138.3	120.1	**132.1**
Ref13	116.7	127.1	143.9	128.4	**129.0**
Ref14	96.3	114.7	110.0	126.1	**111.8**
Ref15	158.5	108.7	140.4	133.5	**135.3**
Ref16	127.2	121.8	137.0	140.2	**131.6**
Ref17	123.7	126.5	143.0	146.7	**135.0**
Ref18	117.8	116.9	149.8	136.1	**130.2**
Can01	112.8	121.3	134.7	117.4	**121.6**
Can02	126.9	118.3	147.4	128.3	**130.2**

1999

Variety	Block scores 1	2	3	4	Mean
Ref01	158.6	191.9	191.9	169.7	**178.0**
Ref02	173.2	173.2	175.7	164.1	**171.6**
Ref03	169.5	161.5	175.0	160.1	**166.5**
Ref04	161.7	172.8	193.8	171.9	**175.1**
Ref05	132.2	169.7	190.2	172.1	**166.0**
Ref06	147.0	165.7	171.5	157.7	**160.5**
Ref07	133.1	165.7	168.3	153.7	**155.2**
Ref08	149.5	171.6	178.9	171.9	**168.0**
Ref09	161.9	173.7	192.1	147.6	**168.8**
Ref10	155.7	181.5	186.5	162.5	**171.5**
Ref11	134.8	195.1	168.8	168.9	**166.9**
Ref12	157.4	163.1	151.9	152.5	**156.2**
Ref13	168.2	179.4	196.8	164.7	**177.3**
Ref14	168.7	183.8	181.9	178.3	**178.2**
Ref15	166.9	169.1	173.1	155.8	**166.2**
Ref16	167.7	180.6	187.1	178.8	**178.5**
Ref17	176.3	189.0	179.3	154.5	**174.8**
Ref18	173.3	202.3	192.2	171.3	**184.8**
Can01	156.2	200.4	191.9	162.1	**177.6**
Can02	164.9	187.4	183.2	170.7	**176.5**

Step 2 – analysis over years

1) Apply an over-years analysis of variance to the variety means from each year.
   a) Compute ANOVA table
   b) Compute an over-years 2% LSD
   c) Compute over-years variety means

2) Compare the difference in these mean values between a candidate and reference variety with the LSD. Denote a distinct ("D") or not distinct ("ND).

The results of this for the example are given characteristic by characteristic below. The tables show the mean characteristic values from each trial, the over-year mean, and the COYD decisions for each candidate based on the characteristic. The ANOVA tables and 2% LSDs are given below each table.

Char01

Variety	Trials 1998	Trials 1999	Mean	Decision Can01	Decision Can02
Ref01	37.7	38.1	**37.9**	ND	ND
Ref02	35.8	39.3	**37.5**	ND	ND
Ref03	36.9	39.0	**37.9**	ND	ND
Ref04	37.6	40.2	**38.9**	ND	ND
Ref05	34.0	37.6	**35.8**	D	D
Ref06	36.1	38.2	**37.2**	D	ND
Ref07	36.8	37.6	**37.2**	D	ND
Ref08	40.2	40.2	**40.2**	ND	D
Ref09	40.4	40.6	**40.5**	ND	D
Ref10	36.6	38.2	**37.4**	ND	ND
Ref11	36.0	38.3	**37.1**	D	ND
Ref12	37.4	38.1	**37.7**	ND	ND
Ref13	35.3	36.9	**36.1**	D	D
Ref14	36.5	38.5	**37.5**	ND	ND
Ref15	34.8	36.4	**35.6**	D	D
Ref16	36.8	37.8	**37.3**	ND	ND
Ref17	37.0	38.9	**37.9**	ND	ND
Ref18	40.3	41.2	**40.7**	ND	D
Can01	38.2	39.8	**39.0**		ND
Can02	38.0	38.4	**38.2**	ND	

ANOVA

Source	**df**	**SS**	**MS**	**F-ratio**
Year	1	23.8	23.8	46.5
Variety	19	74.9	3.9	7.7
Residual	19	9.7	0.51	
Total	39	108.4		

LSD 2% 1.8

Char02

Variety	Trials 1998	Trials 1999	Mean	Decision Can01	Decision Can02
Ref01	148.7	178.0	**163.4**	ND	ND
Ref02	124.5	171.6	**148.0**	ND	ND
Ref03	119.9	166.5	**143.2**	ND	ND
Ref04	131.6	175.1	**153.4**	ND	ND
Ref05	132.6	166.0	**149.3**	ND	ND
Ref06	114.5	160.5	**137.5**	ND	ND
Ref07	123.1	155.2	**139.1**	ND	ND
Ref08	124.1	168.0	**146.0**	ND	ND
Ref09	130.5	168.8	**149.7**	ND	ND
Ref10	124.1	171.5	**147.8**	ND	ND
Ref11	120.7	166.9	**143.8**	ND	ND
Ref12	132.1	156.2	**144.1**	ND	ND
Ref13	129.0	177.3	**153.2**	ND	ND
Ref14	111.8	178.2	**145.0**	ND	ND
Ref15	135.3	166.2	**150.8**	ND	ND
Ref16	131.6	178.5	**155.0**	ND	ND
Ref17	135.0	174.8	**154.9**	ND	ND
Ref18	130.2	184.8	**157.5**	ND	ND
Can01	121.6	177.6	**149.6**		ND
Can02	130.2	176.5	**153.4**	ND	

ANOVA

Source	**df**	**SS**	**MS**	**F-ratio**
Year	1	18797	18797	374.6
Variety	19	1496	78.7	1.6
Residual	19	953	50.2	
Total	39	21246		

LSD 2% 18.0

Step 3 – collation of results across characteristics

The significance of comparisons between the candidates and reference varieties from step 2 are collated so that an overall decision can be made for each candidate.

The results of this are given candidate by candidate below. The tables show the distinctness decisions for each characteristic and the decisions over the two characteristics.

Can01

Variety	Char01	Char02	COYD 2 characteristics
Ref01	ND	ND	ND
Ref02	ND	ND	ND
Ref03	ND	ND	ND
Ref04	ND	ND	ND
Ref05	D	ND	D
Ref06	D	ND	D
Ref07	D	ND	D
Ref08	ND	ND	ND
Ref09	ND	ND	ND
Ref10	ND	ND	ND
Ref11	D	ND	D
Ref12	ND	ND	ND
Ref13	D	ND	D
Ref14	ND	ND	ND
Ref15	D	ND	D
Ref16	ND	ND	ND
Ref17	ND	ND	ND
Ref18	ND	ND	ND
Can02	ND	ND	ND

Can02

Variety	Char01	Char02	COYD 2 characteristics
Ref01	ND	ND	ND
Ref02	ND	ND	ND
Ref03	ND	ND	ND
Ref04	ND	ND	ND
Ref05	D	ND	D
Ref06	ND	ND	ND
Ref07	ND	ND	ND
Ref08	D	ND	D
Ref09	D	ND	D
Ref10	ND	ND	ND
Ref11	ND	ND	ND
Ref12	ND	ND	ND
Ref13	D	ND	D
Ref14	ND	ND	ND
Ref15	D	ND	D
Ref16	ND	ND	ND
Ref17	ND	ND	ND
Ref18	D	ND	D
Can01	ND	ND	ND

On the basis of these calculations, both candidates are distinct from 6 varieties (but different ones) but not distinct from 12 reference varieties so, overall, they would be declared not distinct. In practice, more characteristics would be available and this would give a greater chance of finding distinctness.

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