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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL COMMITTEE**Forty-Ninth Session
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REVISION OF DOCUMENT TGP/8: PART I: DUS TRIAL DESIGN AND DATA ANALYSIS:
NEW SECTION: REDUCTION OF SIZE OF TRIALS

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1. The purpose of this document is to present a proposal of a new section on the reduction of the size of the trials to be included in a future revision of document TGP/8.

2. The following abbreviations are used in this document:

TC:	Technical Committee
TC-EDC:	Enlarged Editorial Committee
TWA:	Technical Working Party for Agricultural Crops
TWC:	Technical Working Party on Automation and Computer Programs
TWF:	Technical Working Party for Fruit Crops
TWO:	Technical Working Party for Ornamental Plants and Forest Trees
TWPs:	Technical Working Parties
TWV:	Technical Working Party for Vegetables

BACKGROUND

3. The Technical Committee (TC), at its forty-eighth session, held in Geneva from March 26 to 28, 2012, considered the revision of document TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability" on the basis of document TC/48/19 Rev.. Document TC/48/19 Rev., Annex I reported that the TC had noted the comment that the Technical Working Party on Automation and Computer Programs (TWC) had received a presentation by Mrs. Sally Watson (United Kingdom) on "Cyclic Planting of Established Varieties to Reduce Trial Size" and had agreed that the text should be included in document TGP/8 Part I in a new section on the reduction of the size of the trials.

4. The TC noted that new drafts of relevant sections would need to be prepared by April 26, 2012, in order that the sections could be included in the draft to be considered by the Technical Working Parties (TWPs) at their sessions in 2012 (see document TC/48/22 "Report on Conclusions", paragraph 49).

COMMENTS BY THE TECHNICAL WORKING PARTIES IN 2012

5. At their sessions in 2012, the TWA, TWV, TWC, TWF and TWO considered documents TWA/41/21 Corr., TWV/46/21, TWC/30/21 Rev., TWF/43/21, TWO/45/21, respectively, and commented as follows:

General	The TWA considered document TWA/41/21 Corr. and received a presentation by an expert from the United Kingdom. The TWA considered the presented method useful and recommended inclusion into document TGP/8 (see document TWA/41/34 "Report", paragraphs 29 and 30).	TWA
	The TWV considered that the proposed method was useful and recommended its inclusion in document TGP/8 (see document TWA/41/34 "Report", paragraphs 29 and 30).	TWV
	The TWC agreed that the title of section 1.7 should read "Additional technical detail and example of analysis for distinctness assessment" and to add a first sentence to read "This section is of relevance to the reader interested in technical details". Section 1.7 was proposed to become 1.6 and section on References to be renumbered as 1.7 (see document TWC/30/41 "Report", paragraph 25). The TWC agreed that the document on "Cyclic Planting of Established Varieties to Reduce Trial Size" should be considered by the TC for inclusion in the proposed new section on the reduction of the size of the trials in TGP/8 Part I (see document TWC/30/41 "Report", paragraph 26).	TWC

COMMENTS BY THE ENLARGED EDITORIAL COMMITTEE IN 2013

6. The TC-EDC, at its meeting on January 9 and 10, 2013, recommended to review the Annex to document TC-EDC/Jan13/10 "Revision of document TGP/8: Part I: DUS Trial Design and Data Analysis, New Section: Reduction of Size of Trials", with regard to the term "cycle" in order to avoid confusion with the term "growing cycle".

7. The Annex to this document contains proposed text for a new section on "Reduction of Size of the Trials" to be included in document TGP/8 Part I: DUS Trial Design and Data Analysis, on the basis of the comments by the TWPs, at their sessions in 2012 and the TC-EDC, at its meeting on January 9 and 10, 2013. The amendments to the text considered by the TWPs and the TC-EDC, are indicated by highlighting and strikethrough for deletions and highlighting and underlining for additions.

8. The TC is invited to consider the proposed text for a new section on "Reduction of Size of the Trials", on the basis of the Annex of this document, for inclusion in a future revision of document TGP/8.

[Annex follows]

TGP/8: PART I: NEW SECTION: REDUCTION OF SIZE TRIALS
CYCLIC PLANTING OF ESTABLISHED VARIETIES TO REDUCE TRIAL SIZE

1.1 Summary of requirements for application of method

Cyclic planting of established varieties to reduce trial size is appropriate for use in trials where:

- distinctness is determined by COYD ;
- the number of established varieties is excessive for cost or for practical reasons;
- there should be at least 20 degrees of freedom for the MJRA-adjusted varieties-by-years mean square in the adapted COYD analysis of variance. If there are not, then cyclic planting of established varieties should not be used.

1.2 Summary

Cyclic planting of the established varieties in trial and analysis by compensated data is a system to reduce DUS trial sizes while maintaining testing stringency. It may be used in trials where distinctness is determined by COYD.

The system comprises allocating each of the established varieties in trial to one of three ~~cycles-series~~, with one ~~cycle-series~~ omitted in turn from trial each year¹. Candidate varieties are included in trial for the three years of their test period plus a fourth year. If, after DUS testing, they are granted protection, they join the established varieties in trial, are allocated to a ~~cycle series~~ and are ~~cyclically~~ omitted from trial every third year.

Distinctness is assessed by applying an adaptation of COYD to the incomplete table of variety characteristic means (candidate and established varieties) in the three year test period. Where data is missing for a variety, it is compensated for by use of two years' data from before the test period. If uniformity is determined by COYU, it may be applied to the incomplete table of variety characteristic standard deviations (candidate and established varieties) in the three year test period. Prior to its adoption, historical data should be used to compare the DUS decisions made based on the cyclic planting system with those based on the existing system.

1.3 Cyclic Planting of Established Varieties in Trial

Established varieties in trial ~~varieties~~ are allocated to one of three ~~cycle-series~~. One ~~group series~~ is omitted cyclically from trial each year (Fig. 1). Thus varieties belonging to ~~Cycle Series~~ 1 in Fig. 1 will not be planted in 2010, 2013 or 2016, whereas those in ~~Cycle Series~~ 3 will not be planted in 2012, 2015 or 2018. This will result in a smaller trial size as one third of the established varieties are omitted from the trial each year. Each candidate variety is planted in trial and has data recorded on it in each year of a three year test period (2014 to 2016 in Fig. 1 below), after which a DUS decision is taken. Because of a possible lag between final DUS testing and being granted protection, candidate varieties are kept in trial for a fourth year after the three year test period. If granted protection, they will then become an established variety in trial and will enter the cyclic planting system. Thus all newly accepted varieties are initially present in trial for four consecutive years, and all varieties entering trial in the same year follow the same cycle of omissions in future years. Hence candidate varieties that had their final year of DUS testing in 2012 in Fig. 1 are in trial for a fourth year in 2013 and so join the ~~Cycle Series~~ 2 established varieties. Candidate varieties final DUS tested in 2013, 2014 and 2015, would join ~~Cycles Series~~ 3, 1 and 2 respectively.

Established varieties are initially allocated to ~~cycles-series~~ in a manner to minimize the risk of bias. Other than the initial allocation, the choice of established varieties following each ~~cycle-series~~ is determined by the candidate varieties entered for trial in earlier years and by which established varieties the applicants choose to withdraw. Although an exactly equal number of established varieties belonging to each ~~cycle series~~ is not essential, it is likely to be beneficial to balance the numbers in each ~~cycle-series~~ in the future. This should be

¹ For the purpose of this document, "year" means a "growing cycle".

done by transferring established varieties between the cycles series by planting them in years when they should be omitted.

Figure 1. **Data patterns and usage for the test period 2014 to 2016**

TRIAL YEARS	2010	2011	2012	2013	TEST PERIOD			2017	2018
					2014	2015	2016		
Candidate Varieties					X	X	X	*	
Established Varieties									
<u>Cycle Series</u> 1		X	X		X	X		*	*
<u>Cycle Series</u> 2	O		X	X		X	X		*
<u>Cycle Series</u> 3	O	X		X	X		X	*	
New Established Varieties – Assimilation into matrix									
Final DUS tested in 2012 (<u>Cycle Series</u> 2)	O	O	X ^F	X		X	X		*
Final DUS tested in 2013 (<u>Cycle Series</u> 3)		O	X	X ^F	X		X	*	
Final DUS tested in 2014 (<u>Cycle Series</u> 1)			X	X	X ^F	X		*	*
Final DUS tested in 2015 (<u>Cycle Series</u> 2)				O	X	X ^F	X		*

X Indicates data retrieved using maximum of 4 years for distinctness testing and within the (boxed) test period for uniformity testing
O Indicates data present but not retrieved
F Indicates final DUS test year of new established varieties
* Indicates future inclusion in trial
 (within box) Indicates the data used for uniformity testing

1.3.1 The assessment of distinctness by data compensation

Conventionally, when using COYD to assess distinctness, it is applied to a complete variety (candidate and established) by test period years matrix of characteristic means. With cyclic planting, this matrix is incomplete for the established varieties. For the assessment of distinctness, where data on an established variety is missing, data held in computer files from earlier years are used to compensate for the loss of data. Due to lack of overlap years with the candidates, the value of back-data is not as high as data from the test period. In the crops to which cyclic planting has been applied to date, to maintain stringency of testing, two years of past data must be included when one year of current data is missing for an established variety. Thus for the 2014 to 2016 test period illustrated in Fig. 1, established varieties in Cycle Series 1 would have data from 2011 and 2012 retrieved, those in Cycle Series 2 data from 2012 and 2013 and those in Cycle Series 3 data from 2011 and 2013. Even where more years of past data are available (marked by an O in Fig. 1), to avoid reducing the stringency of the distinctness test, only the two most recent years are used to compensate for the missing current year. Hence, while data from 2010 and before are available for varieties in Cycles Series 2 and 3, such data are not retrieved for the 2014 to 2016 test period.

Sometimes data on an established variety will be available for a year when its cycle series suggests it would not be present in the trial. Such cases are the fourth year after the three year test period where a candidate variety has become an established variety in trial, or where an established variety is needed for a special test with a problem variety. In this case the established variety would have full data available during the test period and so no historical data would be retrieved for the distinctness testing. Thus for the test period of 2014 to 2016, successful candidate varieties final DUS tested in 2015 would have no historical data retrieved, whereas successful candidate varieties final DUS tested in 2012, 2013 and 2014 would have historical data retrieved.

1.3.2 Method of analysis for distinctness assessment

Distinctness is assessed by applying an adaptation of COYD with Modified Joint Regression Analysis (MJRA) applied to data comprising the incomplete table of variety (candidate and established) characteristic means in the three year test period together with the compensating back-data for established varieties missing during the test period. Details of the method of analysis and an example are given in section 1.7.

1.3.3 The assessment of uniformity

Conventionally, when using COYU to assess uniformity, it is applied to a complete variety (candidate and established) by test period years matrix of within variety standard deviations. With cyclic planting, as may be seen from the boxed year by variety combinations in Fig. 1, this matrix is incomplete for the established varieties. COYU is applied to this matrix and no attempt is made to compensate for the incomplete data. This is because COYU consists of pooling over years the within variety standard deviations for all available established varieties while taking into account any relationship between variety means and the standard deviations. This is done to provide a uniformity standard against which to compare the standard deviations of the candidate varieties. Consequently, it is not possible to make a correction for standard deviations from years outside the test period. As a result, only uniformity data from the established varieties within the test period are used to set the uniformity standard for the candidates.

1.4 Comparison of the cyclic planting system with the existing system

Prior to adoption of the system of cyclic planting, historical data should be used to compare the DUS decisions made based on the cyclic planting system with those based on the existing system. Providing all established varieties were planted with the existing system, the cyclic planting system can be simulated by allocating established varieties to the cycles series, replacing their data with missing data symbols in the computer files where appropriate, and including the previous years' files from which data are to be retrieved to compensate for this 'missing' data. The distinctness and uniformity decisions that would have been made based on the cyclic planting system can then be compared with those that would have been made based on the existing system. This approach also permits assessment of the number of years of back-data that should be included to compensate for when one year of data in the test period is missing for an established variety. Note: if the DUSTNT software is being used, a variety can be made to appear missing simply by removal of its AFP number from the "E file". In UK United Kingdom DUS Herbage trials, when compared with the previous system, the cyclic planting system was found to be slightly less stringent in distinctness testing and slightly more stringent in uniformity testing, with a minimal overall effect on the DUS variety pass rate.

1.5 Cyclic planting system software

The DUST program CYCL, has been developed to enable the compensated data to be retrieved, statistically analysed using MJRA, and the results presented in reports suitable for the assessment of distinctness. Uniformity assessment is based on the data within the test period and uses the DUST program COYU. Both programs are available as part of the DUST9 (MSDOS based) and DUSTNT (Windows NT and 95) versions of the DUST software.

~~1.7~~ 1.6. Method detail and example of analysis for distinctness assessment Additional technical detail and example of analysis for distinctness assessment

This section is of relevance to the reader interested in technical details. Distinctness is assessed by applying an adaptation of COYD to n data values comprising the incomplete table of variety (candidate and established) characteristic means in the three year test period together with the compensating back-data for established varieties missing during the test period. Characteristics are all analysed by Modified Joint Regression Analysis (MJRA). This scales all the variety effects in a year up or down depending on the year by multiplying the variety effects by a sensitivity for the year.

The MJRA model for the cyclic planting data with n_v varieties in n_y years is as follows:

$$c_{ij} = \mu + y_j + \beta_j v_i + \varepsilon_{ij}$$

where c_{ij} is the value on a characteristic for variety i in year j , $i = 1, \dots, n_v$ and $j = 1, \dots, n_y$

μ is the overall mean

v_i is the effect of the i th variety with $\sum v_i = 0$

y_j is the effect of the j th year with $\sum y_j = 0$

β_j is the sensitivity of year j .

ε_{ij} is a random error associated with variety i in year j

This model is an adaptation of one proposed by Digby, P (1979) where year effects are scaled for a variety by multiplying them by a variety sensitivity. As the model is non-linear, it cannot be fitted directly to the data, but must be fitted iteratively to obtain estimates of the variety means and least significant differences (LSD's), which are based on the MJRA-adjusted varieties-by-years mean square and are used to compare the variety means and determine distinctness. The LSD's and the MJRA-adjusted varieties-by-years mean square are on $(n - 1 - 2(n_y - 1) - (n_v - 1))$ degrees of freedom, which should be at least 20 degrees of freedom.

1.7.1 1.6.1 Example of distinctness assessment

Consider the following matrix of n within year variety means c_{ij} . Variety A represents candidate varieties and varieties B, C and D represent the three cycles series of established varieties. The test period is years 4 to 6.

Example data

Variety	Year					
	1	2	3	4	5	6
A	-	-	-	6	2	3
B	-	6	4	-	6	7
C	7	10	-	8	11	-
D	11	-	14	10	-	17

Model fitting provides final estimates of $\hat{\mu}, (\hat{y}_1, \dots, \hat{y}_6), (\hat{\beta}_1, \dots, \hat{\beta}_6), (\hat{v}_1, \dots, \hat{v}_4)$ as 7.862, (-2.12, 0.55, -1.20, -0.12, 1.16, 1.73), (0.91, 1.14, 1.26, 0.36, 1.39, 1.28), (-5.09, -2.12, 1.38, 5.81), from which the following table of means is derived:

Variety	Year						Means
	1	2	3	4	5	6	
A	-	-	-	6	2	3	2.78 = 7.86 + -5.09
B	-	6	4	-	6	7	5.76
C	7	10	-	8	11	-	9.24
D	11	-	14	10	-	17	13.67
Means	5.74	8.42	6.66	7.75	8.92	9.03	
Sensitivities	0.91	1.14	1.26	0.36	1.37	1.39	

The model fitting also provides standard errors for the means on 1 degree of freedom, which together with the two-tailed 1% critical t-value on 1 degree of freedom, gives the following table of 1% LSD values between all variety pairs:

Variety	A	B	C
B	15.75		
C	18.00	15.64	
D	18.39	15.64	18.83

Comparison of the 1% LSD between varieties A and D (18.39) with the difference in their means of 10.89 indicates these varieties are not significantly different at the 1% level. Further details of the analysis and the worked example are given in Camlin *et al* (2001).

Note: the above example serves to illustrate the method, but is on an artificially small dataset. It results in LSD's and the MJRA-adjusted varieties-by-years mean square on 1 degree of freedom. The recommended minimum for use of the method in practice is 20 degrees of freedom.

1.6 1.7 References

Camlin, M.S., Watson, S., Waters, B.G. and Weatherup, S.T.C. (2001). The potential for management of reference collections in herbage variety registration trials using a cyclic planting system for reference varieties. *Plant Varieties and Seeds*, 14:1-14.

Digby, P (1979) Modified joint regression for incomplete variety x environment data. *Journal of Agricultural Science* 93, Cambridge, 81-86.