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**INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS**  
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**TECHNICAL WORKING PARTY FOR AGRICULTURAL CROPS**

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CORRIGENDUM TO

REVISION OF DOCUMENT TGP/8:  
PART I: TECHNIQUES USED IN DUS EXAMINATION  
New Section – Reduction of Size of the Trials

*Document prepared by an expert from United Kingdom*

BACKGROUND

1. The Technical Working Party on Automation and Computer Programs (TWC), at its twenty-ninth session held in Geneva, Switzerland, from June 7 to June 10, 2011, The TWC received a presentation by Mrs. Sally Watson (United Kingdom) on "Cyclic Planting of Established Varieties to Reduce Trial Size" (see documents TWC/29/26 and TWC/29/26 Add.). The TWC agreed that the text should be included in TGP/8 Part I in a new section on the reduction of the size of the trials (see document TWC/29/31 "Report" paragraph 63).
2. The Technical Committee, 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. 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).
3. The Annex to this document contains the text of a presentation on "Cyclic Planting of Established Varieties to Reduce Trial Size", prepared by Mrs. Sally Watson (United Kingdom), proposed as a text for a New Section on the Reduction of Size of the Trials.

[Annex follows]

## ANNEX

## 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, with one cycle omitted in turn from trial each year. 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 and are omitted from trial every third year accordingly.

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 groups. One group is omitted cyclically from trial each year (Fig. 1). Thus varieties belonging to Cycle 1 in Fig. 1 will not be planted in 2010, 2013 or 2016, whereas those in Cycle 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 2 established varieties. Candidate varieties final DUS tested in 2013, 2014 and 2015, would join Cycles 3, 1 and 2 respectively.

Established varieties are initially allocated to cycles in a manner to minimise the risk of bias. Other than the initial allocation, the choice of established varieties following each cycle 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 is not essential, it is likely to be beneficial to balance the numbers in each cycle in the future. This should be done by transferring established varieties between the cycles 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	*	
<b>Established Varieties</b>									
Cycle 1		X	X		X	X		*	*
Cycle 2	O		X	X		X	X		*
Cycle 3	O	X		X	X		X	*	
<b>New Established Varieties – Assimilation into matrix</b>									
Final DUS tested in 2012 (Cycle 2)	O	O	X <sup>F</sup>	X		X	X		*
Final DUS tested in 2013 (Cycle 3)		O	X	X <sup>F</sup>	X		X	*	
Final DUS tested in 2014 (Cycle 1)			X	X	X <sup>F</sup>	X		*	*
Final DUS tested in 2015 (Cycle 2)				O	X	X <sup>F</sup>	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

<sup>F</sup> 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 1 would have data from 2011 and 2012 retrieved, those in Cycle 2 data from 2012 and 2013 and those in Cycle 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 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 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, 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 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.

### 1.6 *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.

### 1.7 *Method detail and example of analysis for distinctness assessment*

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  $j = 1, \dots, n_y$

$\mu$  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  $\sum y_j = 0$

$\beta_j$  is the sensitivity of year  $j$ .

$\varepsilon_{ij}$  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 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 of established varieties. The test period is years 4 to 6.

		Example data					
		Year					
Variety		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:

		Year						
Variety		1	2	3	4	5	6	Means
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.