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**INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS**

GENEVA

# TECHNICAL WORKING PARTY ON AUTOMATION AND COMPUTER PROGRAMS

## Sixteenth Session
## Melle, Belgium, June 16 to 19, 1998

REPORT

*adopted by the Technical Working Party on Automation and Computer Programs*

Opening of the session

1. The Technical Working Party on Automation and Computer Programs (hereinafter referred to as "the Working Party") held its sixteenth session in Melle, Belgium, from June 16 to 19, 1998. The list of participants is reproduced in Annex I to this report.

2. Mr. L. Keymeulen, Director of the Center for Agricultural Research (CLO), welcomed the participants to Melle and gave a short introduction to the CLO followed by a video presentation of the different tasks. Mr. Erik van Bockstaele, Head of the Department for Plant Genetics and Plant Breeding (DVP), welcomed the participants to his department. The session was opened by Mr. John Law (United Kingdom), Chairman of the Working Party.

Adoption of the Agenda

3. The Working Party adopted the agenda as reproduced in document TWC/16/1, after having agreed to change its order, as proposed by the Chairman.

Report on Subjects of Special Interest to the Working Party Raised During the Thirty-Fourth Session of the Technical Committee, Revision of TG/1/2

4. Dr. Thiele-Wittig presented a brief report on the main items discussed during the previous session of the Technical Committee and referred participants needing further details to the full report reproduced in document TC/34/10 Rev.

5. The Chairman referred to Circular U 2685 of April 23, 1998, containing brief notes by the Chairman with particular reference to points of interest to the TWC and action points for the TWC.

6. Chairmanship of the Technical Committee: The Working Party noted that the Technical Committee, in view of the expiration of the chairmanship of Mr. Joël Guiard (France) of the Technical Committee with the closing of the forthcoming ordinary session of the Council in October 1998, had proposed to the Council that it elect Mrs. Elise Buitendag (South Africa) as new Chairman and Mr. Raimundo Lavignolle (Argentina) as new Vice-Chairman of the Technical Committee.

7. Revision of the General Introduction to Test Guidelines, Harmonization of States of Expression and their Notes: The Working Party noted that the Technical Committee had approved a report on the results of a meeting of the Editorial Committee, the Chairmen of the various Technical Working Parties and the Chairman and Vice-Chairman of the Committee, in which a general discussion on the revision of the General Introduction to Test Guidelines and on the harmonization of the states of expression and the Notes in the Test Guidelines had taken place. The Editorial Committee and the Chairmen considered that the main purpose of the General Introduction was to lay down the basic principles according to which the Test Guidelines were established and should be applied and which should themselves be applied together with the individual Test Guidelines. In addition, the document should provide new experts with information on the basic principles for the testing of varieties. The document should not be too long. Details which might change more frequently should be included in another document which would contain a collection of detailed rules, such as the methods of COYD and COYU analysis or the document on the testing of uniformity in vegetatively propagated and self-propagated varieties (documents TC/33/7 and TC/34/5), as well as lists of definitions of certain statistical terms (e.g. population standard) to facilitate understanding by crop experts and of certain botanical terms (e.g. epiphyte) to facilitate understanding by TWC experts when they were approached for statistical help. The Editorial Committee then went through document TG/1/2 and discussed and decided where changes in the present text were needed and who would have to draft the new wording. It entrusted parts for revision to the various Technical Working Parties or to individual experts and the statistical parts to the TWC.

8. The points of specific interest to the TWC for review and revision as necessary were Part (section), BI (15-16), BII (21-26), BIII (27-34), CIII 42, 46-47, 51. With respect to COY (a) two paragraph summaries had to be written on each of COYD and COYU to be incorporated into the revised TG/1/2; (b) full documents had to be incorporated into a UPOV statistical document; (c) user friendly pieces of TWC/14/4 had to be retained; and (d) two additional features for COYD were requested: (i) computer generated demonstration of COYD with data cells being filled for a single characteristic, year by year for a number of varieties; then over-year means being computed; standard errors calculated and finally the

distinctness rules being applied; to show the MJRA in action (this could be in the form of say a MS Powerpoint presentation) and (ii) to have a screen based input module that would prompt users for input and then compute the COYD analysis.

9. The Working Party agreed to the above proposal. It suggested including a part defining good statistical practice and listing the desirable properties of data before statistics could be applied. It then went through specific paragraphs of document TG/1/2 and made the following main changes:

Paragraph

15 To mention the different types of scales, nominal, ordinal and interval; it was reconfirmed that combined characteristics could only be used for distinctness if the uniformity test on the combined characteristic itself, and not only on the components, had been successful.

16 It was mentioned it might be better for this paragraph to be deleted.

21 To be split into two paragraphs according to the two sentences. Examples should be given for each case to avoid any doubt as to what was understood by true qualitative characteristic and by not true qualitative characteristic, but only handled as a qualitative characteristic.

22 To mention the COYD method in the first instance but also to keep the existing rule in the case where only data of one year were available, as all possible situations of measurements had to be covered by that paragraph

26 It should be made clear that only real combinations for two or three characteristics were meant as, for example, the length/width ratio and not multivariate components or a linear combination of characteristics.

27 To refer to the 1991 Act of the Convention and to the new definition of off-type.

28 To be split into vegetatively propagated and truly self-pollinated varieties with two separate paragraphs, but with otherwise the same wording introducing the use of the method as described in document TC/34/5 and stressing the need for a harmonized sample size in the Test Guidelines to guarantee the same probability of acceptance and/or rejection.

29 To have the population standard doubled and not the tolerated number of off-types.

31 To explain and refer to COYU; if only data from one year were available other methods should be proposed, which would have to be discussed during the coming session. Different methods were actually used in those cases or had been used before the acceptance of COYU, the validity of which was seen differently in different States (e.g. 1.6 times the average of the variance of varieties used for comparison; within year approach and combination of several within year decisions; variation between standard deviations of varieties, etc.)

10. Lack of time did not permit more detailed discussions. On the basis of the above remarks and those to be made in other Technical Working Parties, a first draft would be prepared by the Office of UPOV in cooperation with the Chairman for circulation for further comments.

11. Improvement of Document TWC/11/16 on the Testing of Uniformity of Self-fertilized and Vegetatively Propagated Species: The Working Party noted that the Technical Committee finally approved document TC/34/5, which would replace the former document TWC/11/16 for the testing of uniformity of self-fertilized and vegetatively propagated species, subject to a few changes and corrections. The Technical Committee had also referred to the existence of an older document TWC/14/4, which would provide additional explanations on the use of the former document TWC/11/16 that would themselves be applicable in the same way to document TC/34/5.

Questions Raised by Other Technical Working Parties

12. Mr. M.-H. Thiele-Wittig gave a short report on the discussions held in the Subgroup on Electrophoresis of the Technical Working Party for Agricultural Crops (TWA). The TWA Subgroup on Electrophoresis had asked that the TWC should deal with the questions of sample size, the best statistical method for establishing distinctness and whether, and how much greater, minimum distances could be preset to discourage plagiarism.

13. Sample size for tetraploid varieties: The Subgroup of the TWA noted that one still unresolved item with respect to the use of electrophoresis for ryegrass varieties was the required sample size for tetraploid varieties. The answer to this, and to the question whether the chi-squared test or the AMOVA were the right statistical method for the testing, had to be awaited from the TWC. The problem at present was that mainly bulk samples had been tested. This would not allow the testing of uniformity, which can only be judged by testing single plants.

14. The Working Party noted that a set of 60 data had been distributed to study what was an appropriate analysis and what was the right sample size to apply to those electrophoresis data. After the discussions it agreed that the set of data covering existing varieties might need to be supplemented by some very close varieties or some not distinct populations to evaluate the minimum distance. Some experts already stated that the chi-squared test may be a bad method as it over emphasized rare bands and therefore required a much too high number of plants. A good method would be a method that matched the present experts' knowledge. As at that time it was recommended to use results from electrophoresis only as supporting information, they would mainly be used in cases of very similar varieties. Therefore this fact should be taken into consideration in the selection of study data. The Working Party would therefore amend its study data set and try to obtain results for its next session.

15. Fixing a difference below the level of significance and supporting evidence: The Subgroup of the TWA finally came to the conclusion that electrophoretic characteristics should not be sufficient to establish distinctness. They should only have a supporting function and only be used in addition to another difference in a morphological characteristic. The question of how large that difference and that different requirement had to be was left open, however. Some experts of the Subgroup were of the opinion that it might be possible to

consider using electrophoretic characteristics in combination with other characteristics, and requiring clear differences in at least two or more characteristics. Others imagined them being used in the case of differences in morphological characteristics below the significance level. Some experts, however, wondered how to determine the difference in a morphological characteristic below its significance level. Another possibility could be a difference in a characteristic not used so far, like yield, but that raised the question of distance and the means of checking uniformity or stability of yield. In general, it should only be used if the crop expert was convinced that the candidate variety was a different variety, in which case the characteristic would only support what had been observed in other traditional characteristics, but at a level that alone might not have been sufficient to establish distinctness.

16. The Working Party considered that first the question of the appropriate method and sample size would have to be solved before the question of a difference, which was supported by evidence through electrophoresis, could be handled. In addition, that question was not a statistical but a political one. It was, for example, not possible to combine statistical evidence from COY results below the required level with evidence from electrophoresis characteristics. For a statistical comparison the weight of each characteristic had to be known as well as whether they were independent or correlated.

Report on New Developments in Member States

17. The Working Party received from some of its experts short reports on recent developments in their countries. The experts from Denmark, Finland, France, Germany, Spain, the Netherlands and the United Kingdom reported that they would use COYD and COYU for grasses and, with the exception of the Netherlands, also for a few other cross-fertilized species. The experts from the Czech Republic, Hungary, Poland and the Ukraine reported that they would either only use COY occasionally, or that they had only started last year using COY, or that they planned to start using it soon. The expert from Japan reported that DUSTW had been tested and found good but would not yet been used as most tests lasted only for one year.

18. The expert from France reported on the introduction of a system for which the same database structure with the same data existed in the different testing stations and which would be updated every night. In Spain, the central database would be copied weekly to the testing center. In the Netherlands, the central database could be accessed directly from the testing stations, but the administrative database was separate. The Ukraine would combine the Microsoft Office and Lotus database to combine the administrative databases with the data and description of varieties. In Poland, a centralized database combined administrative and VCU data and would in future also cover DUS data and variety descriptions. In Germany, the installation of 200 PCs in the different stations and the headquarters offices had been finished, working with Windows NT and Office 97. The building up of an e-mail system based on Microsoft still posed some problems for the trial stations. All data were stored centrally in the headquarters in Hanover. Japan used a database for the local network. It is a closed network, mainly because of the use of Japanese.

UPOV-ROM Plant Variety Database

19. The Working Party noted updated information supplied by the Office of UPOV on the UPOV-ROM Plant Variety Database. In 1997 six issues of the UPOV-ROM had been issued at two-month intervals. In 1998 the first two UPOV-ROMs had already been distributed and the third would be distributed in the current week. The software used by the French firm was the same as that developed for the WIPO ROMARIN CD-ROM. As new improvements in the latter's software had been made, the UPOV-ROM would also contain several improvements in the near future, the main one being the possibility of using it in networks. The UPOV-ROM already contained the 1997 OECD List of Cultivars eligible for certification and, although at present available only in pdf format, the list of varieties protected through the European Union Community Plant Variety Office (CPVO). Discussions were also under way to include the varieties contained in the European Union Catalogue. The UPOV-ROM has also been offered to the private sector since the beginning of the year at an annual subscription price of CHF 750 plus postage.

20. Dr. M.-H. Thiele-Wittig gave a short demonstration of the content of the UPOV-ROM with its three parts, the combined database with the taxon information, the text part in pdf (portable document file) format with information from the member States on their data, all texts of the various Acts of the UPOV Convention, the Recommendations on Variety Denominations, the General Information Brochure, the lists of addresses of National Plant Variety Protection Offices, the list of UPOV publications and various other information, and the original data from the member States.

21. At the request of the Office of UPOV, the Working Party discussed various details of the production disc. Several experts replied that the main use was in the end to replace the copying information from the national gazettes. The checking of the variety denominations was the main use. Some countries needed to incorporate the data into their own national database. For that purpose some experts would, however, need the finalization of the UPOV Code for genera and species and a more frequent (monthly) production. Without these two requirements the information could only be used as a counter check on whether data had been correctly copied from the individual gazettes. Furthermore the possibility was needed to use UPOV-ROM in the national network and more user-friendly routines to extract data. Also were mentioned the time between the supply of data and the distribution of UPOV-ROM which should also be reduced. As further subjects for consideration it was suggested to include variety descriptions and to consider offering the information on Internet.

22. The Working Party also discussed whether to request always a full data set or only updates. It was agreed to maintain requesting each second month a new full data set. The Office of UPOV should, however, prepare a circular requesting that records should be earmarked and the status of the record should be stated as already foreseen in the format to enable a separation of unchanged records from new or amended records (1 = new record, 2 = modified record, 3 = unchanged record). An unknown status of record (0) should as far as possible be avoided as soon as the adaptation to that new requirement was completed. The Working Party was, however, aware of the difficulties that requirement might create for some countries, especially those with relational databases where it was not so easy to earmark all changes (e.g. a change of telephone number of a breeder with 50 varieties which was done automatically at once for all 50 records). Some declared that they would only introduce those changes when the UPOV Code was ready and the UPOV-ROM could really allow

downloading of data into the national computer systems and therefore avoid the copying of data form the gazettes.

Image Analysis

23. The Working Party noted document TWC/16/10 on VISOR–a plant variety image database system, prepared by experts from the United Kingdom and a few further pages distributed during the session. The document reports on the progress in the development of a database system for storing and viewing photographic records of plant varieties. The VISOR system is the product of a collaborative project involving Biomathematics & Statistics Scotland (BioSS) and the Scottish Agricultural Science Agency (SASA). The main purpose of the system is to support investigations into methods for variety identification using digital image analysis. VISOR uses worldwide web browsers to view images. The system allows the user to (a) browse an image collection; (b) view all images for a variety in outline; select one image for detailed inspection; and compare all images for a pair of varieties. Several views of the same specimen can be held and viewed. Images can be stored of samples of a variety drawn from more than one plot, trial or season. Photographs of plant specimens are taken in the usual way. Samples are photographed under controlled artificial lighting conditions and the photographic images are captured on slides. The slide images are digitized and converted to the JPEG image format for storage on the computer. The VISOR system has been created from freely-available computing tools including standard file management structures for organizing the image files and WWW browsers for viewing the images. The use of the WWW also allows wide access to the photographic records.

24. VISOR can operate on a stand-alone PC or on a networked server and can be accessed across the Internet. A JAVA/JAVASCRIPT-enabled browser is required for access, preferably Netscape 4 or Internet Explorer 4. At BioSS/SASA the image database system has been used to hold three years of photographic data on carrot varieties. It includes views of the plants as whole roots, as sliced roots, and samples of the leaf foliage. A total of 160 variety sowings are currently held and these occupy approximately 10 megabytes of computer disk storage. The system can handle more than one species. The next step is to see whether it is possible to match varieties using a combination of the shape and color information contained in the photographic images. The aim is to see whether, given a photograph of a variety from a new season, the same variety from an earlier season can be found in the database. Preliminary results from three seasons of data on sliced roots of carrots have been encouraging. Work will continue on developing these approaches.

25. The Working Party noted document TWC/16/11 on digital images in plant variety testing prepared by experts from the Netherlands and a few further pages supplementing that document. The document explains that digital images can be used in variety testing for measuring characteristics described in the UPOV Test Guidelines automatically by the computer (image analysis), with image analysis characteristics being measured accurately and fast. Besides it offers possibilities to measure characteristics quantitatively which could previously only be scored visually. The characteristics obtained with image analysis can easily be analyzed with the powerful statistical tools for DUS-testing to assess distinctness and uniformity. Furthermore, it may also be possible to let the computer automatically generate part of the variety description in a fast, accurate and efficient way using the information automatically retrieved from a set of images. Another use of digital pictures is a

visual comparison of varieties, e.g. for comparing a candidate variety with the reference collection. This can be done by searching through the image database by manual browsing of images: a screen of thumbnail images is presented to the user, and the user can scroll through the list of images. A step further is to find similar varieties automatically by computer. Can the computer find similar pictures or even tell the variety identity of a plant held in front of a camera? This would be a valuable method for finding a set of reference varieties for comparison in the field (greenhouse) (pre-screening) or to compare a candidate variety in the trial with the images of the total reference collection (post-screening). It may even be used as a tool for variety identification to check for possible cases of infringement. Image matching requires that the image interpretation is done by the computer, whereas in common relational databases the feature selection and comparison is left to the human expert. For reliable and efficient matching good protocols with highly standardized images are required.

26. The document shows different image analysis tools based on size, shape, color and texture to give an idea of the possibilities of image analysis for variety testing and identification. The idea is to develop a system to find similar images of flowers in a database. The first step in the system is that the user can either give an example image himself, or extract an example image from the database. The next step is that the image is segmented and the object of interest (the flower in this case) is extracted. Several characteristics are calculated for this object, at the moment only size and color features. From these characteristics, similarity measures are calculated for each pair of flowers. Similarity or distance between images can be calculated using some kind of distance measure, like weighted Euclidean distance for each specified characteristics for each group. The weighting functions can be altered to take into account the importance of each characteristics and the correlations between the characteristics. It should be possible for the user to apply different weighting functions, depending on his interest, for example with emphasis on size, shape, color or texture. With the use of the weighting function, overall distances between images are calculated and based on the distances, similar images (varieties) are retrieved from the database. The images will be displayed in thumb-nail format to the user in decreasing order of similarity.

27. The expert from France reported on a study on automatic assessment of seed purity by artificial vision undertaken by postgraduate and Ph.D. students at GEVES, Angers, France. It was aimed at differentiating seeds of foreign species in a given seed lot. In the study 103 characteristics were measured covering size, shape, grey level distribution and texture and the three-colored channel red, green and blue. Several approaches had been studied: (a) linear approaches as K-nearest neighbors, linear discriminant analysis and fuzzy C-means clustering algorithm or (b) non-linear approaches as multi-layer perceptron network, hybrid neural network or propalistic neural network. The last mentioned method had been considered the most interesting one. Image analysis had been considered a good tool for automatic seed identification and it was planned to continue the study to develop a complete vision system for on-line discrimination and to cover more species. The same system could also be applied to flowers or leaves.

28. The experts referred to work done on the same subject some years ago in Denmark and in the United Kingdom and for grain check systems in Sweden.

Testing of Distinctness

Reference Variety Selection

29. The Working Party noted document TWC/16/13 on most similar variety: comparisons based on morphology, pedigree and molecular methods, prepared by experts from the United Kingdom. The document studies the construction of similar variety sets based on morphological data and molecular methods. Data sets from maize have been studied in detail together with pedigree information where available. The study comprised data from 50 morphology characteristics, 347 AFLPs, 258 APPCRs, 951 RFLPs and 63 SSRs. As similarity scoring system were used either the Euclidean method, the Jaccard method, or the City Block method. The study identified for each method the most similar variety for each individual variety, with the observed level of similarity recorded. Such an approach sought to quantify the agreement between molecular methods and then to compare with both the pedigree and morphological methods. A secondary approach, less prone to influences due to "near-misses", identified the most similar variety by morphological data and then determined the ranked position of that variety in the similarity set as expressed through molecular methods. This method can become cumbersome if the molecular method has multiple tied "most similar varieties" or if the pedigree matrix contained many identical low similarities.

30. The results of the study were reproduced in several tables and figures. The document highlights the interest, before establishing similar statistics for the DNA methods, of noting the ranked position of the morphologically most similar variety in the set of similarities from the molecular methods. With perfect agreement across all methods they would each rank first. Results from the application of each DNA method in establishing the most similar variety show agreements across the methods although at variance with the results based on morphology. The absolute levels of maximum similarity for the DNA methods is also of interest. The summary statistics for comparisons with the morphological results show that the maximum relationship is remarkably consistent across all DNA methods and pedigree. The median of the varietal correlations for SSRs is noticeably lower than for the other methods.

31. The document concludes that while the numbers of varieties/lines available in this project was relatively low, the amount of morphological, DNA and pedigree information utilized was very large. The DNA analysis methods have shown a measure of internal agreement when compared to the variety selected as the most similar by morphology. However, it should also be noted that for certain target varieties very consistent *but different* conclusions can be drawn. The scatter plots have shown that the range of morphological similarities is relatively low (c.0.2) compared with those for AFLP and RFLP data at 0.6 and pedigree data at over 0.9. Overall, the DNA methods appear to give better correlations between each other when identifying a most similar variety, and also correlate better with pedigree data, than does morphology. Hence the DNA methods, used singly or in combination, are well able to identify a minimum set of close varieties that are highly likely to contain the truly "most similar" variety.

32. The document will be presented with slight amendments to the coming session of the Working Group on Biochemical and Molecular Techniques and DNA Profiling in Particular (BMT). The use of the Euclidean method will be checked to avoid one or two characteristics influencing the results too much. Also another table may be added giving more than only one single most similar variety.

The Use of AFLP Markers for DUS Testing in Perennial Ryegrass

33. The Working Party noted a report on a study on the use of AFLP markers for DUS testing in perennial ryegrass made by experts from Belgium, France, the Netherlands and the United Kingdom and presented by the expert from France. In that study the usefulness of AFLP markers for DUS testing in perennial diploid ryegrass (*Lolium perenne* L.) was investigated from a set of 11 cultivars assayed for DNA polymorphism using two primer combinations. The discriminatory power provided by AFLPs was analyzed and various statistical approaches for testing distinctness were compared. Special attention was paid to (a) redundancy among markers, and (b) optimal sample sizes of both individuals and markers that were required to minimize the variance of the molecular distances between cultivars. To this end, bootstrap sampling strategies were carried out. Statistical procedures aimed for DUS testing included (a) estimation of population predictors, (b) analysis of Molecular Variance (AMOVA), (c) stepwise regression procedures, and (d) partial least squares regression procedures. The results proved that AFLP markers were discriminant enough to distinguish between the closest cultivars although a large redundancy was observed. They also suggested that a relatively small sample of individuals per cultivar (20-30) might suffice for testing distinctness. The evolution of the sampling variance of the distances between cultivars (when varying numbers of individuals and markers were surveyed) showed that it was better to examine a large number of markers rather than a large number of individuals to improve the accuracy of the distance estimate.

34. The Working Party considered this study a useful contribution for the next session of the Working Group on Biochemical and Molecular Techniques and DNA Profiling in Particular (BMT) and asked to present it after slight modifications as a BMT document to the BMT with copy to the Working Party. The title should refer to prescreening instead of DUS testing.

Threshold Methods

35. Application of COYD and COYU Analysis: The Working Party noted that the Technical Committee, while agreeing that several experts had still to gain experience with the application of COYD and COYU analysis for further species, had insisted that the document as reproduced in TC/33/7 had been adopted for use for cross-fertilized species and that no alternative strategy should remain and efforts should rather be made to apply the document. Where there were too few varieties, the document would offer an alternative with the criterion of the long-term LSD. The Committee especially asked the Technical Working Party for Vegetables (TWV) to rediscuss the subject and study the application of the analysis to further vegetable species. The Committee also asked the TWC to consider means of explaining the method better or making it more user-friendly. Moreover, if TWC experts were sent to sessions of other Technical Working Parties, the method would finally gain better acceptance by the various Technical Working Parties.

36. The Chairman recalled his summary made on the session of the Technical Committee that had requested to know (a) whether there were valid reasons for not applying COYD (too few varieties, incomplete tables, large interactions, problems with setting an appropriate probability level), (b) what should be used instead of COYD and whether the two-out-of-three rule was still applicable in any situation, (c) whether there were similar valid reasons for not

using COYU (e.g. too few degrees of freedom, incomplete tables of data, difficulties in setting probability levels, etc.), (d) what was recommended in these cases, whether it was suggested to look at non-forage species, e.g. onions or leeks, as an additional crop to assess the potential application of COYD/COYU, (f) whether COYD was applicable to data from cross pollinating as well as self-pollinating species for both measured data and other recordings, (g) whether COYD could be used in countries where contemporary candidates were under test.

Distinctness and Genotype x Environment Interaction

37. The Working Party noted document TWC/16/3 on distinctness and genotype x environment interaction, prepared by experts from Germany. The document refers to document TWC/14/7 which introduces the combined over years distinctness (COYD) criterion. According to this criterion, two varieties are distinct, when their difference exceeds a least significant difference (LSD), which uses the variety x year interaction mean square as an error term. This criterion had been adopted to replace the earlier UPOV distinctness criterion (the "2 x 1% criterion"), which requires the varieties to 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 2 x 1% criterion uses the plot error mean square as an error term. The document argued that the COYD criterion treats genotype x year and genotype x location interaction differently. Specifically, genotype x year interactions are considered as random, while genotype x location interaction is implicitly taken as fixed. That does not seem to be consistent. By contrast, the 2 x 1% criterion regards both interaction components as fixed. It is argued that regarding interaction effects as fixed is appropriate for assessing distinctness. Therefore, the document suggests that TWC critically evaluate the COYD criterion relative to the 2 x 1% criterion.

38. The document then studies whether the genotype x environment interaction determines distinctness and the different handling of genotype x location and genotype x year interaction by COYD. The document concludes in arguing that both genotype (G) and genotype x environment (GE) effects are a result of the operation of the genotype. Any difference in the expression of the genotype among varieties, i.e. any differences in the phenotype among varieties, reflects differences in the genotype and is thus indicative of distinctness. Thus, distinctness should be defined in terms of both G and GE effects. In this connection, it cites Falconer (1981), who was of the opinion that observations on the same trait in different environments might be regarded as two different traits, because of the expected differences in physiological processes and the sets of genes leading to the expression of the genotype.

39. Defining distinctness in terms that involve interaction effects implies that a statistical procedure should be based on a model with fixed GE effects. It would seem desirable that such definition should treat genotype x location (GL), genotype x year (GY) and genotype x location x year (GLY) interaction alike, i.e. as fixed effects. The 2 x 1% criterion is consistent with this requirement, while the COYD criterion is not. Therefore, the TWC should critically re-evaluate the COYD criterion relative to the 2 x 1% criterion.

40. The Working Party agreed with the finding but not with the conclusions. According to UPOV a variety needs to be distinct in at least one location. Therefore most member States use only one testing location and grant protection if the variety is distinct in that place. That means, however, that it is possible that the variety is not distinct in another place, which,

however, is irrelevant for the granting of rights. It is on the other hand necessary that distinctness is consistent and repeatable in the following year. Therefore not just any difference is acceptable, but only those that are expected to be found in the next year. The Offices were able to choose locations but cannot choose years. The Working Party was therefore satisfied with the present procedure of COY.

41. The Working Party noted document TWC/16/4, containing some remarks on the combined over years distinctness criterion, prepared by experts from Germany. The document states that the combined over years distinctness (COYD) criterion is based on a mixed model, which implies that the variance-covariance structure has the so-called Compound Symmetry (CS) form. The LSD computed for COYD produces a valid test only when the CS assumption is met. To measure the departure from the CS assumption the document suggested and computed for *Lolium perenne* a data set of the *Bundessortenamt*. The results indicate appreciable departure from CS. They show that under departure from CS, an increased sampling variation is to be expected among variety x year mean squares for different groups of years. This may partly explain observations reported in past TWC documents. The document concluded that the validity of the LSD procedure suggested for COYD may be hampered by departure from the CS assumption. The document encouraged the TWC to search for powerful and simple alternatives to COYD for data that violate the CS assumption. The document concludes that (a) if the CS assumption is tenable, it would seem best to use as much data as possible, i.e. long-term data, to estimate variance components, provided the CS assumption can be extended to long-term data. This ensures maximum attainable precision for the variance estimates. Analysis may be done by the method of fitting constants (FITCON). However, since it cannot be ruled out that long-term time trends of variability (which are difficult to detect) and the effect of a changing composition of the set of varieties under test invalidate the CS assumption for long-term data, it seems safer to use generally only three-year data. Inspection of several traits in a *Lolium* data set indicates that three-year data tend to have a somewhat smaller residual mean square ($MS_{int}$) and to meet the CS assumption required for FITCON analysis better. When balanced two-year or three-year subsets can be formed with more than 20 residual degrees of freedom, such subsets may be used for computing LSDs. If balanced subsets have less than 20 residual degrees of freedom, an unbalanced three-year data set can be analyzed using FITCON. It should be borne in mind that all of these procedures are based on the assumption of CS; (b) if there is a marked departure from CS, only the paired t-test can be recommended as a valid procedure. A problem with this approach is the extremely small number of degrees of freedom. Three years of paired data appears to be the minimum requirement for this test. To compensate for the loss of power, reducing the significance level from 1% to 5% might be considered. No satisfactory alternative to the paired t-test can be suggested at this stage, and it is unclear whether a simple and powerful alternative under departure from CS is forthcoming.

42. The Working Party made it clear that 20 degrees of freedom were not considered to be a fixed margin between the COYD method and the long-term LSD. In the beginning it was stated “about 20,” but the word “about” had been lost. Twenty degrees of freedom was a very conservative approach to be on the safe side. All depended on the quality of the data with long-term results. Three years of incomplete data might be used instead of long-term results but more studies were necessary before firm recommendations could be made. The expert from Germany offered to prepare another paper for the next session.

43. The Working Party agreed to make it clearer to the experts applying COYD that it was important to obtain consistent results. The experts should stick to one method and not change it if in one year the total number was higher or lower than the level of about 20 degrees of freedom. The number of varieties grown should also not be artificially increased to reach the level of 20 degrees of freedom. A few degrees less would not affect the precision of COYD, especially as it was only intended to support the opinion of the expert. In some publications a level of 12 degrees of freedom was considered the critical level for the application of certain methods. It was important to bring this information to the attention of the crop experts to change their obviously wrong impression of 20 degrees of freedom as a fixed borderline. The revised document TG/1/2 should also be clear in that respect.

COYD for Measured Characteristics in Self-fertilized Varieties.

44. The Working Party noted the request from the Crop Working Parties to find a simple method for the treatment of measured characteristics in self-fertilized species or varieties. It noted document TWA/27/9 Rev. on COYD for measured characteristics in self-fertilized varieties, prepared by experts from Germany for the next session of the Technical Working Party for Agricultural Crops and highlighting some problems of establishing uniformity. In certain types of varieties it was easier to observe the off-types visually, in some others it was easier to apply COYD.

45. The Working Party agreed that all depended on the uniformity of the genotype. In the case of little variation a visual assessment of off-types might be easier, in the case of more variation it might be better to apply the COYD analysis.

46. The COYD analysis gave a statistically firm approach for the handling of measured data irrespective of whether they result from cross-fertilized species or from self-fertilized species. The Working Party therefore recommended the use of COYD where an expert looked for a method to handle measured data of self-fertilized species. However, it did not want to impose its use, if the crop expert preferred not to do so.

47. The Working Party agreed that nothing prevented the use of COYD for any measurements as long as the normal conditions for the application of the analysis of variance were fulfilled, e.g. these were measurements from more than one year, a certain number of plants, there existed some difficulties to observe distinction, there was a philosophy to wish to be able to distinguish the variety consistently in the following years, there was a normal distribution of the figures, there was a constant variance, the observations were independent, the trial lay-out was randomized, there existed at least one replication.

Handling of Visually-assessed Characteristics

48. The expert from Hungary reported on a study on visually-observed characteristics as reproduced in Annex III to this report. The study reported on the calculation of adjusted over-years values of data from winter barley. Because of the more continental climate, the varieties showed a higher influence of the year with larger extremes which did not allow the use of the observed data directly for variety descriptions. Some experts in the Working Party raised some possible problems which might occur with the linear adjustment, e.g. if the adjustment

lead to negative notes or if there was a larger variability in the center of the scale than at its ends.

Testing of Uniformity

49. The Working Party considered that document TC/34/5 on uniformity for self-fertilized varieties adopted by the Technical Committee did not at present call for further discussions, despite some criticism by one expert of the difficulties encountered by crop experts in finding the right population standard and decision rule for different sample sizes.

Items Resulting from the Fourth Session of the Working Group on Biochemical and Molecular Techniques and DNA Profiling in Particular (BMT)

50. During its previous session, the Working Party had already noted a brief report on the main items discussed during the last session of the BMT and that the full report was reproduced in document BMT/4/19. It had also noted Circular U 2532 of April 28, 1997, listing the proposed plans as a result of the above session. The Working Party had agreed that experts from Belgium, France, Germany, Israel, the Netherlands and the United Kingdom would consider supplying molecular example data sets to the Chairman for study by a special interest group in order to be in a better position to continue discussions during the next session and to come to some substantive conclusions to the specific points in that Circular.

51. The Chairman recalled that in Circular U 2685 he had reported that binary data sets from Belgium, the United Kingdom and the United States, on beet, flax, azalea, ryegrass (AFLP as well as allele frequency data on dip/tet ryegrass) and maize (many DNA methods, pedigree and morphological data) had been circulated both by e-mail and on a floppy disc by post, and that the BMT had asked the TWC to consider for the next BMT session the following: (a) tools to estimate confidence interval around “distance estimators,” (b) alternatives to dendograms, (c) refinements to AMOVA to formally quantify, within and between variety variation, (d) to consider methods for combining DNA data to enable comparisons to be made for the “classifications” from molecular and existing morphological data. In addition, to assess the discrimination “power” from the different approaches, (e) to establish a library of “working data sets” to aid method exploration and development working on common data from as wide a user base as practicable.

52. The Working Party agreed on the transmission of the two documents mentioned in the paragraphs 29 to 34 above to the BMT.

Incomplete Plot Design, Reduction of Reference Collection

53. The Working Party noted that the Technical Committee had rediscussed the question of prescreening and noted the differing views of the different Working Parties. In order to make progress in the discussions, the Technical Committee agreed that some concrete cases would have to be selected and the whole problem further investigated on the basis of them. It proposed to ask all Technical Working Parties to rediscuss the question of prescreening and to cite examples that would support their position. For the Technical Working Party for

Agricultural Crops (TWA) the species *Poa* and potato were mentioned as possible examples, and for the Technical Working Party for Ornamental Plants and Forest Trees (TWO) roses. For roses there was already a good deal of additional information that would be helpful. In addition, it would underline the importance of ornamental varieties and the international trade of them. For the Technical Working Party for Fruit Crops (TWF) the species peach was mentioned.

54. The Working Party noted document TWC/16/12 on the efficiency of different designs in spring rape prepared by experts from Denmark. The document recalls that in the DUS testing spring rape is one of the major crops in Denmark, which means that there are many reference varieties grown each year. At the same time some difficulties have been encountered in the establishment of distinctness of new candidates. An investigation was therefore started in order to examine whether the designs could be improved in order to lower the critical differences necessary to distinguish new candidates from established varieties. The investigation consisted of two parts: in the first part some existing trials laid out as complete block designs with many entries (varieties) were analyzed in order to access the possibly effect if the trials had actually been laid out using an incomplete block design. This was done by imposing incomplete blocks on the actual designs. In the second part a single resolvable incomplete block design was analyzed using the incomplete block structure and compared with the result when the trial were analyzed as a randomized complete block design.

55. The results of the investigation showed that for each of the 76 possible combinations of trials and characteristics the design with the smallest LSD value were found. This showed that designs with blocks restricted to plots in just one row were smallest in 49 out of 76 cases. In a table it was shown that a block restricted to be in just one row was preferable for all examined block sizes except complete blocks. Here complete blocks of 120 plots divided over two rows were preferable to blocks of 120 continuous plots in 52 out of 60 (8 + 52) examined cases. The average relative LSD values also showed that dividing the blocks over two rows resulted in average relative LSD values greater than 100 for all block sizes. When the block were formed by plots in just one row the results indicated that a block size of 10-12 seemed to be preferable The average relative LSD values were smallest for block size 12. The block sizes 10, 12 and 20 were the block sizes which most frequently were the best block size. Very small block sizes (less than 5) were very rare among the best block sizes. Complete blocks were also very rarely the best design.

56. The Working Party noted a report of the expert from Poland on some optimum problems in planning DUS trials. Because of the rapid increase in the number of varieties in the testing it was questionable whether the basic assumptions of the analysis of variance were still fulfilled. It was studied how to optimize the costs of trials and how to reduce the costs. Compared were the number of years, the number of replicates and the number of characteristics and their optimum relationship. The study concluded that the number of replicates was too small, the number of measurements too high and the number of years to small. If possible, more than two years should be used.

57. The Working Party noted document TWC/16/2 on the possibility of application of incomplete blocks in DUS trials, prepared by experts from Poland. The document recalls that the necessity of proving distinctness from all known varieties causes the necessity of comparing a growing number of varieties within the same trial. For example in DUS trials on maize conducted in 1996 at the experimental station Slupia Wielka in Poland, 212 varieties

were compared. As a rule, the randomized complete block design was used in such experiments. In view of such a high number of varieties one could doubt whether the basic assumptions of analysis of variance were fulfilled. In particular, the assumption concerning uniformity of plots within complete blocks (replicates) could not be fulfilled. The document therefore studied the possibility of application of incomplete blocks in DUS trials. Basing on the results of three trials on maize conducted in 1995-1996, the post-blocking technique was used in order to find the "optimum" size of blocks (the best number of plots within blocks). The analysis of the results lead to the following conclusions (a) application of incomplete blocks instead of complete ones can give high increase of effectiveness (reduction of variance of comparisons); (b) the effectiveness of incomplete blocks depends on the characteristics involved; (c) in some experiments the reduction of variance of comparisons may be as high as 50%; (d) application of resolvable incomplete blocks ($\alpha$-designs) gives the possibility to choose between complete and incomplete block versions of analysis; (e) application of incomplete blocks besides of reduction of variance of comparisons leads also to some complications in interpreting results caused by more complicated structure of covariance matrix of treatment means that the analysis of more extensive trial data is necessary to draw more general conclusions. In particular the results of additional crops and characteristics should be included in further investigations.

58. The Working Party agreed that incomplete block design could allow some gains in the testing costs by reducing the number of plants observed without losing precision (e.g. 40 instead of 60 plants). Care should, however, be taken with varieties with large border/neighbor effects like rape seed as the method could only compensate different soil conditions but not side effects. The incomplete block design was a good tool to show the precision with increased number of plants.

59. The Working Party noted, however, that it might not be possible to realize a gain as the varieties had also to be tested for uniformity and for those tests more plants were needed. Only if a large number of varieties (over 200) was tested, was it possible to work with fewer plants. Therefore the testing should be continued with complete plots and incomplete plots should be used only if problems arose.

60. For the next session the expert from Denmark offered to prepare a paper on the follow-up on COYD with incomplete plots.

Improvement of Communication

Telecommunications, Exchangeable Software and Contacts

61. The expert from the United Kingdom introduced document TWC/16/7 on database management systems in use in UPOV member States and document TWC/16/9 on exchangeable software. The Working Party regretted that only a small number of member States had supplied information. More countries were invited to supply information and to check the information they had given in the past. Changes and new information should also be sent by e-mail to Mr. Ian Nevison (United Kingdom) (e-mail: ian@bioss.sari.ac.uk.). The information would also be available on Internet (http:// www.bioss.sari.ac.uk/links/upov/).

62. The expert from the United Kingdom introduced document TWC/16/8 on electronic mail addresses of UPOV technical experts. The document was noted with appreciation. He invited more countries to supply information and to check the information they had given in the past and report all changes by e-mail to Mr. Ian Nelson.

List of Statistical Documents Prepared by the TWC

63. The contents of documents TWC/15/2 and TWC/15/3 prepared for the last session by experts from France containing a list of documents produced by the Technical Working Party on Automation and Computer Programs and a top index to those documents is now available on Internet and will be updated by experts from the United Kingdom (see paragraph 61 above). The Working Party appreciated the updating of those lists and especially the topic index which made it easier to find a particular document on a given subject. It proposed, however, to continue for some years with the updating of printed documents. The Office of UPOV would download the information about four to five weeks before the next session and distribute it to the TWC experts.

List of Statistical Documents Containing Recommendations or Methods of Possible Interest to the Technical Working Parties

64. The Working Party agreed to prepare a list of statistical documents still of broader interest and/or containing recommendations from the TWC to the other Technical Working Parties which would still be valid or prepare a summary of information which might be contained in different documents. It would also be tried to indicated in the reports of the sessions which documents prepared for a given session had a longer lasting interest.

Development of Computer Programs for DUS Testing (responses on DUST9, DUSTW and other possible programs)

65. The Working Party noted that the Technical Committee had welcomed the new version of the DUSTX package and the prototype produced for Windows. The Technical Committee recommended broader use of that freely available software which would ensure more harmonized evaluation of data. It was recalled that the prototype version of DUSTX or DUSTW (for Windows) was available from Ms. Sally Watson, Biometrics Division, DANI, Newforge Lane, Belfast, BT9 5PX, United Kingdom, and that in order to broadcast the free availability of the DUST Program, document TWC/15/17 reproduced the content of the manual for the DUSTW prototype with a simplified introduction.

66. The Working Party received some updated information on corrections to DUST 9 and DUSTW as reproduced in Annex II to this report and also a demonstration of the working of DUSTW during the coffee breaks. It was expected that DUSTW would be completed by the end of this year.

Developments in the World Wide Web

67. The Working Party noted document TWC/16/6 on the UPOV TWC WWW information pages, prepared by experts from the United Kingdom. As discussed at the UPOV TWC meeting in Budapest in June 1997 some WWW pages had been created which brought together information about the work of the TWC. The main aim of the pages was to provide a readily-accessible reference source for information about the work of the TWC in particular, but of other groups also, if they wished to participate. Information that was held in the pages included: (a) e-mail addresses of participants at all UPOV Technical Working Parties; (b) a list of UPOV TWC participants and their addresses; (c) basic information about TWC meetings; (d) an indexed list of all past TWC working papers; (e) descriptions of the procedures COYD/COYU; (f) details of UPOV database systems; (g) details of statistical software available from UPOV TWC participants. The pages were accessible at: http://www.bioss.sari.ac.uk/links/upov/. In the meantime the Office of UPOV had opened an official Website which could be accessed at: http://www.upov.int. UPOV would also provide for links from its Website to those pages. The expert from the United States reported that information on plant patents and especially on images of the US plant patents of the last 20 years were available at http://www.patents.ibm.com/ibm.html which was a private Website. He wondered whether UPOV could make available UPOV documents on the Internet, if needed with password protection. The Office of UPOV reported that plans were under way to produce UPOV documents on the Internet, either under a password or free, but it was mainly a question of workload when that would happen.

68. The Working Party noted document TWC/16/5 on the e-mail bulletin board for varieties and seeds technical matters, prepared by experts from the United Kingdom. The document explains that an electronic bulletin board for the discussion of technical matters concerned with plant varieties and seeds would serve as a form of on-line special interest discussion group. Newcomers could join the group by registering as members. Discussion would take place by e-mail. If a member is seeking advice on a problem he/she can send a message to the bulletin board address and the message is automatically distributed to all current members. Replies can be sent directly to the individual initiating the inquiry or to the bulletin board. Typically bulletin boards are used for posing and answering questions or for dissemination of information, e.g. notices of meetings. There are several mailbase service agencies which provide the infrastructure that supports bulletin boards but they all operate in a similar way. Each bulletin board must have a list owner who is responsible for its operation. However, once set up, a bulletin board operates automatically with little input required from the list owner.

69. The annex to document TWC/16/5 contained a “template” which describes the aims of the proposed list and its expected membership. The aims outlined are not exclusively UPOV-related as (a) the interests of most plant variety specialists extend beyond UPOV-related issues and (b) experience suggests that bulletin boards with overly narrow (or too broad a) focus are not used.

70. The Working Party welcomed the proposal to set up a bulletin board. It stressed that the good practice guidelines for contributors as laid down in document TWC/16/5 should be strictly followed. It was expected that the bulletin board would start in about one month. It would be announced to those experts who are on the e-mail list in document TWC/16/8.

Experts who wish to participate should register their participation. The registration is free of charge.

Standardization of Attachments of E-mail Intended as Basis for TWC Documents

71. The Working Party noted the difficulties encountered by the Office of UPOV in the opening of attachments to e-mails, in giving them some sense or a reasonable shape to be used as a basis for a UPOV document. Despite a number of conversion programs available and powerful hardware, the opening or saving of electronic documents caused numerous computer breakdowns. Some documents could not be even saved at all despite the help of computer experts. In other documents drawings were overlapping, or depending on the conversion program used, either only the drawing was there but not the text or the text was there and only a square, indicating that there might be something else. In one case all the automatic numbering of headings and paragraphs of a document were completely different to the original version sent. Having noted the problems encountered, the Working Party agreed to a few recommendations for the submission of e-mail attachments. As far as possible the following should be observed:

The software or the program used should always be specified when sending an electronic document:

1. A hard copy should always be sent to enable a comparison with the sometimes incomplete electronic version. If time was short, a hard copy should be sent by telefax.

2. The size of each single attachment should not be too large.

3. Images should not be saved as pictures but as drawings in order to save capacity.

4. Certain still to be recommended software programs should be used if available (e.g. the text of documents should be converted to Word 2.0 or rich text format (rtf)).

Future Program, Date and Place of the Next Session

72. At the invitation of the expert from Finland, the Working Party agreed to hold its seventeenth session in Helsinki from June 29 (9 a.m.) to July 2, 1999 (5 p.m.), with the possibility of meeting during this period for one or two days in Loimaa (Turlu-Tampere) about 200 km from Helsinki. During that session, the TWC plans to discuss or rediscuss the following items:

(a) Report on subjects of special interest to the Working Party raised during the thirty-fifth session of the Technical Committee

(b) Questions raised by other Technical Working Parties

(c) Report on new developments in member States (oral reports)

(d) UPOV-ROM Plant Variety Database (oral report)

(e) Revision of the General Introduction to Test Guidelines

(f) A range of analysis for electrophoresis data (the Chairman to prepare a paper before February 1, 1999)

(g) Image analysis (report on results of the ring test on roses (Netherlands), image analysis used for electronic measurements (a paper to be prepared before April 1, 1999, by experts from the Netherlands and Germany))

(h) Efficient methods of dealing with reference variety selection (the experts from the Netherlands to prepare before April 1, 1999, a paper in cooperation with the experts from the United Kingdom)

(i) Threshold methods (the experts from the Netherlands to prepare a paper before April 1, 1999)

(j) Critical assessment of assumptions underlying COYD (the experts from Germany to prepare a paper before April 1, 1999)

(k) Handling of visually-assessed characteristics (summary paper to be prepared by experts from Germany before February 1, 1999)

(l) Items resulting from the fifth session of the Working Group on Biochemical and Molecular Techniques and DNA Profiling in Particular (BMT) (report by the Chairman)

(m) Examination of the number of distinctness of incomplete blocks in DUS trials (the expert from Denmark to prepare a paper before April 1, 1999)

(n) Experiences of using incomplete blocks for the testing of French beans (the expert from Poland to prepare a paper before April 1, 1999)

(o) Questionnaire on VCU tests and data storage (the expert from Poland to prepare a questionnaire to be circulated by UPOV for answers to be sent before the end of the year to Poland for a document to be prepared by May 1, 1999).

(p) Telecommunications, exchangeable software and contacts (the United Kingdom to receive updated information and prepare updated versions of documents)

(q) Developments in the World Wide Web (the United Kingdom to report on the experience of the E-mail discussion group)

(r) Experience with the DUSTW computer program for DUS testing (a paper to be prepared by experts from the United Kingdom before May 1, 1999)

(s) List of statistical documents prepared by the TWC (the United Kingdom to prepare updated lists on the Internet to be downloaded four to six weeks before the session for printing and distribution on paper)

(t) List of statistical documents containing recommendations or methods of possible interest to the Technical Working Parties

Visits, Demonstrations

73. In the afternoon of June 17, 1998, the Working Party visited the Department of Crop Husbandry and Ecophysiology (DFE) where it received a short introduction to the work of the DFE by its head, Mr. Heursel, and more detailed information on the testing for DUS and VCU by Dr. J. Van Waes. This was followed by a field visit of the testing of chicory and flax.

74. In the afternoon of June 18, 1998, the Working Party visited the glasshouses of the Department of Plant Genetics and Breeding (DvP) where it received an introduction to the crossing and breeding of roses and azaleas and to the program with respect to biotechnology.

*75. This report has been adopted by correspondence.*

[Three annexes follow]

ANNEX I

# LIST OF PARTICIPANTS

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