

TWC/14/19 ORIGINAL: English DATE: September 2, 1996

## INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS GENEVA

## TECHNICAL WORKING PARTY ON AUTOMATION AND COMPUTER PROGRAMS

## Fourteenth Session Hanover, Germany, June 4 to 6, 1996

## 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 fourteenth session in Hanover, Germany, from June 4 to 6, 1996. The list of participants is reproduced in Annex I to this report.

2. Mr. R. Elsner, President of the Federal Office of Plant Varieties (Bundessortenamt), welcomed the participants to his office in Hanover. The session was opened by Mr. S. Grégoire, France, Chairman of the Working Party.

Adoption of the Agenda

3. The Working Party noted that, for several items of document TWC/14/1, no documents were available and therefore deleted those items from the agenda. It adopted a revised agenda as reproduced in Annex II to this report.

Report on Subjects of Special Interest to the Working Party Raised During the Thirty-Second Session of the Technical Committee and on Questions Raised by Other Technical Working Parties

4. Mr. M.-H. Thiele-Wittig gave 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/32/7. Mr. S. Grégoire, France, especially emphasized that he had to correct a misunderstanding that statistics were only applicable to measurements. Statistics might also help experts without measurements.

#### UPOV-ROM Plant Variety Database

5. The Working Party noted the latest stage of preparation of the UPOV Plant Variety Database on CD-ROM (UPOV-ROM) as set forth in Circular U 2347 of December 15, 1995. The Office of UPOV had invited all its member States to submit data for the envisaged disc by the end of January 1996. The disc will cover data from 23 member States. The data from four States, however, will be data already sent in 1995. Only in the case of seven States had it not been possible to obtain data for the first production disc already (Belgium, Chile, Poland, Portugal, South Africa, Switzerland, Ukraine). It is expected that the UPOV-ROM will be issued at the end of June 1996. A request for data for the second production disc had been sent out on April 15 under Circular U 2390 and several countries had already responded to that request.

6. At the request from the Office of UPOV, the Working Party discussed various details of the production disc and agreed that (i) the minimum information should not be changed; (ii) in the beginning, however, it should be applied with great tolerance; (iii) incomplete dates are still valuable and should be maintained, the lacking information replaced by blanks or zeros; (iv) the validation date to be given should be the date the output from the national database was made; (v) names of old varieties for which protection or listing had lapsed should be kept according to the present rules of the State concerned but the crop experts should discuss the question of a possible harmonization of the periods. Some experts thought the names should be kept permanently in the database.

#### Report on New Developments in Member States

7. The Working Party received from some of its experts short reports on recent developments in their countries. Several experts reported on the further inclusion of the DUST package prepared by Mr. C. Weatherup, United Kingdom, in their system. The expert from the United Kingdom explained that the program would be upgraded to run under Windows. The experts from the United Kingdom also reported on a SMART project with advanced training for scientists based on World Wide Web (WWW) technology and another training program to train scientists in variety identification. The expert from France reported on the successful reception of data from VCU trials on diskettes, and on the planned change of the database next year from a centralized system to a multi-location NT/ORACLE client/server system. The expert from Germany reported on the creation of a page on INTERNET in the German Agricultural Network including also a list of Test Guidelines used.

#### TWC/14/19 page 3

It was intended to also make reference to UPOV Test Guidelines. All protected and listed varieties would be placed there as well. He referred to contacts between the European Union (EU) Office and Seed Quest On-line for adapting the Common Catalogue to the Seed Quest on-line computer system. It was planned to form a working group to prepare a concept. The advantage would be that changes would be immediately available everywhere. The expert from Israel reported that he had obtained the Common Vegetable Catalogue on diskette from the NAKG in the Netherlands. The expert from IPGRI (International Plant Genetic Resources Institute) reported on IPGRI's work on crop descriptor lists (currently comprising over 70 titles) and the plan for close cooperation with UPOV in the future. The expert from Poland reported that according to the new Seed Act of November 1995, the number of species for which VCU trials had to be undertaken had been reduced to about 40 but the DUS trials had to be reinforced. Presently, 12 stations were handling them compared to four stations before. Technical courses were given for several eastern European States. The expert from the Netherlands reported on planned courses for Third World countries of DUS testing and statistics. The expert from Israel reported on the entry into force of a new law in accordance with the Act of 1991 of the UPOV Convention. The expert from Japan explained the structure of the National Center for Seed and Seedlings as reproduced in Annex III to this report.

#### Experience in the Testing of Vegetables

8. The Chairman briefly recalled that the Working Party had discussed several methods in the past which might be helpful in judging visually-assessed characteristics but had not made any recommendations on their use. They were just offers for help. If any other Technical Working Party needed help it could approach the TWC. The expert from Germany added that the methods were just explorative methods. So far, winter wheat, pelargonium and broad beans had been chosen as examples.

9. The vegetable expert from Germany reported on his experience with the method when applied to celeriac where Germany was doing the testing for most member States. As a result (i) it appeared that in several characteristics only part of the whole scale was used; (ii) it showed whether the minimum distance was set the right way; (iii) it determined the discriminative power of each characteristic; (iv) it showed in a histogram the distribution of the varieties in the characteristics; (v) it gave a complete biometrical evaluation whereby the COY method might cause less varieties to be declared distinct than the 2 x 1 per cent criterion; (vi) it showed the correlation between characteristics. All results were, however, based on national data only. It was still an open question how to work with data from different countries since the UPOV Test Guidelines should be applicable to all UPOV member States.

10. The expert from the United Kingdom recalled that in the Technical Working Party for Agricultural Crops (TWA), when introducing the COY analysis, the level had had to be chosen in a way to allow a smooth transition. The same had to be done in the vegetable area. The expert from Israel stated that the main aim of the whole procedure was not to reduce the number of characteristics but to understand better the varieties and the characteristics of the species concerned.

11. The Working Party concluded that this confirmed the interest of this kind of work for the crop experts. The main difficulty was that all results were restricted to one country only. Between different countries many other factors would also change. It recommended that, when other Test Guidelines were revised, statisticians should be approached for help in a similar way.

#### Threshold Models for Visually Observed Data

The expert from the Netherlands introduced document TWC/14/12 on threshold models 12. for visually observed characteristics. He pointed out that in DUS tests it was common to differentiate between measured and visually observed characteristics. Visually observed characteristics were often of the so-called ordinal type which could be ranked in categories without the distances having necessarily a binding interpretation. The paper described a class of models especially developed for ordinal data, the threshold models. It allowed the same kind of questions to be answered for ordinal data as standard linear models for measured characteristics. The threshold models were written in so-called generalized linear models and the document described the general features of the generalized linear models and how to formulate threshold models in those terms. The method gave a mean value and a dispersion value per variety. The results of the method could be interpreted in the same way as COY data. The number of internodes in maize and the soil coverage in sugar beet were used as Annex VI to this report contains for completion of document TWC/14/12 a examples. graphical presentation of the threshold model with four categories.

13. The Working Party agreed that the document was just for informing the group and a first introduction. Discussions would have to continue within the Working Party before informing other Working Parties.

#### Testing Uniformity

# View of crop experts on the variation or non-variation of the population standard from year to year

14. The expert from France introduced document TWC/14/11 containing a summary of the answers to the questionnaire addressed to the crop experts in order to know their opinion on the way of fixing the population standard for each crop. She repeated the aim of the questionnaire and the questions as to whether there had been problems encountered and why and whether a variation in the level of uniformity had been found at the same location from one year to another. Fourteen countries had replied to the questionnaire. Those who had encountered problems mentioned that for ornamental crops and some vegetables statistics were not felt useful; practical experience was the basis for the fixing of tolerances. Different experts had different opinions. There was a high variability caused by environmental conditions. It was difficult to change past practice and to think in admissible "P" categories of seed and the way of doing the observations. Those who had no problems nevertheless used different precisions. Some fixed "P" at 1 per cent, others at 3 per cent, others at 2 per cent for predominately self-pollinated varieties and hybrids and 1 per cent for vegetatively propagated or self-pollinated varieties. Some chose "P" from the seed certification norms. Those who

observed yearly variations gave as reasons environmental conditions (the quality of the trials, the expression of characteristics) and the sampling size, the representativity, the quality of the crop expert and the attention given to the plants. Concerning the question whether the population standard should be variable or not, there was a quasi-unanimous answer that there was a variation in the level of uniformity observed for a given sample, and agreement on the origin of that variation, but a fixed population standard was considered preferable to a variable population standard from year to year.

# Some discussion points with respect to fluctuations of the population standard in testing for uniformity in self-fertilized and vegetatively propagated crops.

15. The expert from the Netherlands introduced document TWC/14/13 on some discussion points with respect to fluctuations of the population standard in testing for uniformity in self-fertilized and vegetatively propagated crops. He reported on the difficulty of obtaining sufficient data sets to study the question. The document was based on three three-year cycles of data of winter wheat from Germany. The data were, however, insufficient to give a clear answer to the question of fluctuations of the population standard. There was doubt as to whether there was a need to investigate the influence of the year on the population standard and, even if there was an influence, whether that influence should be accepted. It might be more necessary to study other differences, e.g. between drilled plots and row plots.

16. The expert from France also highlighted the importance of harmonization of the lay-out of trials, the time spent for observation, the use of different categories of off-types (e.g. mutations, physical impurities, accidents in seed production (hybrid in parent lines)) or other special categories to avoid other differences in the population standard. The vegetable expert from Germany also referred to inbred lines in cabbage hybrids. The agricultural expert mentioned the special tolerances fixed in the Test Guidelines for Maize for inbred plants in hybrids. The expert from Israel referred to varieties bred for special purposes, e.g. resistance to diseases, others mentioned differences in the treatment of the plots and in transplanting different impacts on the environment.

17. The Working Party therefore finally agreed that it would assume, although there might be certain differences from year to year, that only one fixed constant population standard be used over the years. However, for different categories of varieties there might be different population standards within one species or genus.

# Tools that may help in finding the right population standard and decision rule for different sample sizes

18. The expert from Spain introduced document TWC/14/9 completed with an addendum as reproduced in Annex V on tools to supply tables for document TWC/11/16 and to find correct population standards for different sample sizes and off-types. The document and the addendum enumerated five different cases which were handled with UNIF, a home-made computer program to build tables of acceptance probabilities for different cases. Case 1 was the same as mentioned in document TWC/11/16 but was open to choose every combination of population standard and acceptance probability, even fractional values. In case 2, tables of acceptance

#### TWC/14/19 page 6

probabilities were calculated with the Poisson distribution which results in rather similar figures to those in document TWC/11/16. In case 3, tables were also based on a binomial distribution but the sample size and the number of off-types were fixed, but not the population standard. It is possible to find a combination of sample size, number of off-types and population standards that give better  $\beta$ -errors than those in document TWC/11/16. In case 4, similar results as in TWC/11/16 were obtained by using a hypergeometric distribution. As the relation between the individuals (seed or plants) of the sample sown and the sample submitted tends to zero, the hypergeometric distribution tends to be binomial. However, in the case that relation was 1/10 or bigger, the number differed from that obtained with the binomial This could be the case for inbred lines, etc., where small samples were distribution. submitted. In connection with case 3, the expert recalled the problems with which different Technical Working Parties had been confronted namely the difficulties in knowing what population standards should be normal in different species. The population standard was a theoretic statistical concept related to the whole crop and not to samples, thus technical experts had no experience with population standards. They might only be able to answer for crops which had some legal restrictions for seed lots or may try to imagine what a consumer would consider unacceptable. The main problem was that technically it was very difficult to fix population standards for a crop. One of the reasons was that even within a crop the theoretic population standard might vary from type to type due to the different selection made in a species for a given type. At present document TWC/11/16 led in addition to too high ßrisks which were not tolerable. For case 5 of the Addendum, the Spanish expert showed the results of UNIF for OC (Operating Characteristic) Curves, which are those functions that relate to P (popular standards), and  $\beta$  (probability of acceptance of a variety), and which are able to find a balanced sample scheme for  $\alpha$  and  $\beta$ -errors.

19. Some experts disagreed with the use of hypergeometric distribution for determining decision rules in uniformity testing. The expert from Israel observed that the source of variation within a variety could be split into the generic variability, which was difficult to change, and the variation caused by the breeder. The testing experts would often not know the population standard but the number of off-types tolerated for different species or different types within a species. It was not the office that fixed the standards but the use of the variety that demanded certain standards.

20. The expert from Spain promised to continue the research for the next session of the Working Party.

#### Acceptance probability curves to define an appropriate sample scheme

21. The expert from France introduced document TWC/14/4 on acceptance probability curves to define an appropriate sample scheme. He explained that the document on the basis of the case of uniformity studies on varieties made for UPOV gave an example to illustrate how the use of acceptance probability curves could help to define a sampling scheme. The points of view of the different persons concerned by the variety were confronted, and a solution which should satisfy everyone was sought. In part 1, a short introduction to UPOV was given; in part 2, two different situations of uniformity studies were explained (biologic heterogeneity between plants in a variety, and plants in a variety which are usually alike); part 3 explained how the studies were done in practice; part 4 reproduced the history of the choice

of the sample size and the decision rule in UPOV, in part 5 on acceptance probability curves, it was explained what the curves showed, how they were computed, what they looked like in an example given and finally a solution was sought by understanding the aims and concerns of different persons (e.g. scientific director, breeder, user, UPOV crop expert); part 6 included a study on whether it was possible to satisfy everyone and in part 7 the question was opened up for other studies. The document concluded that in all cases, unless there had been a law or a regulation already accepted, it was important to obtain the aims and concerns of the different kinds of persons dealing with the problem. Trying to translate this information in acceptance probability curves was a good way to illustrate and permit discussion. The concerns were often more important than the goals when an agreed solution had to be found. Ready access to a computer program to explore the possibilities assessed by the discussion was necessary if people wished to be able to look for solutions or adapt a solution when the conditions differed. The QALSTAT program is appropriate for this.

22. The Working Party appreciated the document with its explanations and agreed to present it to the Technical Committee.

#### COYD-Long-term LSD

23. In the absence of the expert from Denmark, the expert from the United Kingdom introduced document TWC/14/16. After having considered various possible solutions, the Working Party agreed to maintain its basic principle of always using the COYD method if there were more than 20 degrees of freedom, and the long-term LSD if there were less than 20 degrees of freedom available. Thus, in the case under study, where in a third year too few varieties were left, the long-term LSD should be used for these varieties for all years.

#### **QALSTAT Computer Program**

24. The expert from France demonstrated QALSTAT, a program prepared by France and available to national offices through the expert from France. The software allowed different acceptance probability curves for different sampling schemes to be set up, depending on the uniformity or heterogeneity of the species concerned. It could either give the decision rule for a given sample size or search for the sample size for a given decision rule.

#### Sequential Analysis

25. The Working Party welcomed the updated document TC/32/6 on sequential analysis prepared by the Chairman of the Working Party with the help of the experts from Germany, Denmark and the United Kingdom. Furthermore, it noted the recommendations of the Technical Committee that each of the Technical Working Parties should act in conjunction with the TWC and look further into the sequential analysis method, which aimed at reducing the sample size to be used in the testing of uniformity, thereby avoiding the rejection of good varieties or the acceptance of bad varieties, as one of the possible approaches for the future.

#### TWC/14/19 page 8

26. The expert from Germany introduced an article, prepared by D.P. Singh and P.K. Agrawal on sequential sampling plan procedure for testing genetic purity. The method explained in the article had the advantages that a sampling scheme was proposed which could choose "N" for any population, that the type II error was known and that it was easy to apply. The disadvantages, however, were that the type I and type II errors were fixed at 5 per cent, that there was no acceptance of small sample sizes, that there was a need for several samples for a positive decision at an unrealistically high figure and that this sample was likely to lead to rejections and not to acceptances and that for electrophoresis it was necessary to continue in several steps which was not practical.

27. The Working Party therefore saw no utility for the method for DUS purposes. The expert from France considered whether it might be helpful when checking the stability of a hybrid.

#### Image Analysis

28. The expert from the United Kingdom reported that the proposal for the FAIR project had not been accepted by the EU but that a new proposal had been made in the meantime. The Working Party also noted that a subgroup of the TWO on image analysis will meet on September 26 and 27, 1996 [changed to October 1 and 2, 1996] at the Bundessortenamt. It will comprise so far the experts working with image analysis from France, Germany, the Netherlands and the United Kingdom, but other interested experts from other groups are also welcome. The Working Party recommended that a DUS specialist also participate, who should establish a list of general problems which should not be limited to one species only and that the problems be studied on an example.

#### **Detection of Outliers**

29. The expert from the United Kingdom referred to his report during the last session. Studies were under way to improve the document from last year's session. It was hoped that a revised document could be produced for the next session of the Working Party. It was aimed at using a multivariate approach, to detect outliers as soon as possible to enable the expert to return to the field to verify whether it was really an outlier.

#### Improvement of Communication

#### Rewriting of Document TWC/11/16

30. Document TWC/14/3 comprised the rewritten document TWC/11/16. In the absence of Mr. K. Kristensen, Denmark, it was introduced by Mr. M. Talbot, United Kingdom. He explained that the document, after a summary and an introduction, contained a part explaining the different possible errors in the testing for off-types, the testing in more than one year, referred to the sequential test with several examples, followed by a detailed description of the method for <u>one</u> test, a detailed description of the method for <u>more than one</u> test, an introduction to the tables and figures and a definition of the statistical terms and symbols

used, before reproducing the tables and figures for different population standards and acceptance probabilities.

31. The Working Party found that the document needed further study, especially with respect to the incorporation of more than one trial, to some linguistic improvements and to the style. The example of the balls might also be replaced by an example with plants. For cases of more than one trial, the combined test should be used for the time being. But the sequential analysis approach would be studied further in order to find a better solution for cases of more than one trial. Until the rewriting was completed, document TWC/11/16 would remain the applicable document.

32. The Working Party recalled Annex VI of the report on its last session which reproduced all the decisions of the Technical Committee on the use of COYD, COYU and of document TWC/11/16. It considered whether an introductory document should be prepared stating on which occasions or for which species a method should be used. While some experts considered the COYD and COYU analyses to be applicable to cross-fertilized species and document TWC/11/16 to self-fertilized and vegetatively propagated species, other experts could imagine all methods being applicable to both groups of species and especially to those which were not completely cross-fertilized or self-fertilized. The Working Party finally agreed to place on the agenda for its next session an item on a possible document of the above kind, but without requesting anyone to prepare a draft.

#### Rewriting of the COY Analysis

33. Document TWC/14/7 comprised the rewritten document on the COYD method. Mr. M. Talbot, United Kingdom, introduced the document prepared by Mr. S.T.C Weatherup and Mr. S. Watson. After a summary and an introduction, it made reference to the previous UPOV distinctness criterion, explained the combined over years distinctness criterion, made a proviso on the limitations of the method, explained the refinement of the method through the Modified Joint Regression Analysis (MJRA) and explained the implementation of the method, giving also some publications as references. An example of part of the output program TWRP was given in a table, showing variety means and results of analysis of variance of characteristics, a comparison of varieties and the distinctness status of candidate varieties. The statistical details of the COYD analysis and the MJRA refinement method were given in an annex.

34. The Working Party considered the document a good reference paper. It had been considerably improved compared to document TC/30/3 and was shorter and better structured. The Working Party proposed to submit the document to the Technical Committee in order to replace document TC/30/4. Only some small changes at the end of PROVISO should be made and the part on the MJRA should be shortened. The part of document TC/30/4 on the COYU should be reproduced unchanged.

#### Telecommunications, exchangeable software and contacts

35. Discussions were based on document TWC/14/10, introduced by the expert from the United Kingdom, containing a list of electronic mail addresses of experts in the technical bodies of UPOV (TC, TWA TWC, TWF, TWO, TWV and BMT), a table of database

management systems in use in UPOV member States and information on exchangeable software supplied by Denmark, France, Germany, Japan, the Netherlands, Poland, Slovakia and the United Kingdom. The document was noted with appreciation and received some corrections and further electronic mail addresses (see Annex IV to this report). 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.

#### List of statistical documents prepared by the TWC

36. Discussions were based on document TWC/14/5, a list of statistical documents prepared by the TWC and document TWC/14/6 with a topic index to documents produced by the TWC, prepared and introduced by Mr. S. Grégoire, France. The Working Party appreciated the updating of that list and especially the topic index which made it easier to find a particular document on a given subject.

#### <u>Results of running the COYD program distributed on diskette during the TWC session to</u> <u>check whether national implementations are in concordance with the latest version of DUST</u>

37. The expert from France reported that he had used the data on the diskette distributed during the last TWC session in the down-loaded and incorporated program and had come to the same results as reproduced on the diskette. He encouraged other experts again to do a similar check as it was important to ensure that with the same data the same results were obtained in the different member States.

#### Items Resulting From the Last Session of the BMT

#### Review of cluster analysis

38. The expert from Germany introduced document TWC/14/8 comprising a review of methods for cluster analysis of marker data prepared by him and the expert from the Bundessortenamt. He summarized that various molecular techniques were now available for varietal identification, which were more powerful than traditional morphological comparisons and isozyme techniques. Statistical analysis of DNA profile data usually consisted of three steps: (1) scoring the profile; (ii) calculating the genetic distances; (iii) summarizing genetic relationships, e.g. as a dendrogram. Dendrograms were useful for studying the genetic relationships among crops cultivars or inbred lines. The document described the computational steps for generating dendrograms from marker data. The type of distance measure suitable for analyzing a given data set depended on the data. Therefore, the type of data arising from DNA profiles and how to score such profiles were described. A brief account was given of some distance and similarity measures in common usage and a short description of some common clustering algorithms.

39. Under "Type and scale of marker data" the banding data and allelic data were handled; under "Genetic distance measures" binary banding data with measures that ignore negative

matches and measures which treat positive and negative matches alike, and allelic frequency data and band frequency data were treated; under "Clustering methods" the Unweighted Pair-Group Method using Arithmetic average (UPGMA) method, the single linkage (nearest neighbor), the complete linkage (furthest neighbor) were explained as well as other properties of the previous methods abbreviated under the acronym SAHN–Sequential (S), Agglomerative (A), Hierarchic (H) and Non-overlapping (N)–were explained; under "Choice of clustering method" possibilities of choices were mentioned and statements of other authors cited. At the end, references to other articles followed and in an appendix an example for deriving allele frequencies and band frequencies from banding patterns and considering a monomeric single-locus enzyme showing triallelic variations in a cross-pollinating population of a diploid species. He explained the difficulties of working with band frequencies instead of allele frequencies if knowledge of the genetics was missing and also when it was not known whether certain enzymes were monomer or polymer where different numbers of bands would stand for the same locus.

40. When some experts proposed to use these tools for the prescreening of varieties before testing, other reminded them that according to UPOV only tools relating to the phenotype were acceptable for DUS testing and thus also for prescreening.

#### The Use of the Analysis of Molecular Variance (AMOVA) for Distinctness Studies

41. The expert from France introduced document TWC/14/15 prepared by her on the use of the analysis of molecular variance (AMOVA) for distinctness studies. After a short introduction she explained the principles of the method, its application to distinctness studies, special cases, the testing procedure and finally gave some examples of its application. The analysis was established for cases where data were available in samples from different populations or different subdivisions of the same population. It had been developed for haploid data but had been extended for diploid data as well. Pairwise comparisons could be performed to test for significant differences in gene frequencies between two varieties. She concluded that AMOVA was a multilocus alternative to the traditional computation of chisquared distances. A higher weight was given in the method to genotypical combinations. It seemed to be slightly more discriminant but the present testing procedure was not yet The software for AMOVA was available as follows: satisfying. anonymous Ftp, acasun1.unige.ch, directory pub/comp/win/amova.

#### Similarity, Clustering and Dendrograms

42. The expert from the United Kingdom introduced document TWC/14/14 on similarity, clustering and dendrograms. He stated that the document laid down the possible uses but also the possible abuses of dendrograms made by scientists. He reported that there were numerous different methods. Several of them, although with different names or even unnamed, did similar things while others did different things or were applicable only to certain situations or under certain conditions. Thus it had to be carefully studied first whether a given method was applicable to a given situation. Unfortunately in many cases scientists would not do this evaluation before applying a method and would just apply a method and would be satisfied

with it if they liked the results coming from the method. This was a wrong and dangerous approach which unfortunately was very frequent.

43. He then explained some primary questions to be answered before applying a method for grouping: Is there a natural grouping? Are groups/clusters of known shape sought? Are known "controls" available to mark "groups"? Are proposed clustering methods appropriate to the (biological) mechanism that generated the data? Are hierarchical "tree/branch" methods appropriate? Are methods of density search, clumping and partitioning appropriate? How many groups are desired? Are overlapping groups allowed? Have the data been screened for other values? etc.

44. He then continued by explaining the following agglomerative methods and several clustering methods: nearest neighbor (single linkage); furthest neighbor (complete linkage); centroid cluster (UPGMC–unweighted pair group); medium cluster–Gower's method (WPGMC–weighted pair group); group average cluster (UPGMA–unweighted pair group average) and Ward's Method–Orloci (error of sum of squares). Thereafter followed non-hierarchical methods as there were decisive methods; partitioning methods; K-cluster means methods and density search methods. He concluded with dendrograms and alternatives to dendrograms as there were contour intervals, contours with minimum spanning "tree," first and second order distances and the "ball and rod" method.

45. He pointed out the frequent misuse of dendrograms. Dendrograms were just the visualization of data in a non-mathematical way showing a certain relationship. They should be read from top to bottom and not only at the bottom. They could rotate as for example a child's mobile. As for a child's mobile, if a part was taken out the whole mobile would become unstable and dendrograms should not be used with parts left out.

46. The Working Party agreed to the explanations. Several expert stressed again that dendrograms were helpful as a quick screening tool but were not the final result they were often wrongly taken for. They were also not giving a pedigree. In many cases scientists wrongly stopped research when reaching a dendrogram that allowed only a first look at the question. This common approach was a dangerous tendency.

#### Statistical Analysis of Molecular Marker Data

47. The expert from the United Kingdom introduced document TWC/14/18 on statistical analysis of molecular marker data, prepared for non-specialists. Several points in the paper were also covered by the preceding papers, however, the principal coordinate analysis was only reported in that paper. He added that it was necessary to work more with molecular scientists and to improve the link between the methods and statistics.

#### Follow-Up of Documents for the Working Group on Biochemical and Molecular Techniques and DNA Profiling in Particular (BMT) (TWC/14/8, TWC/14/14 and TWC/14/15)

48. The Working Party agreed that documents TWC/14/8, TWC/14/14 and TWC/14/15 be given in the present form to the Chairman of the BMT for comments. In addition they might be slightly amended by adding as needed a list of contents, a summary, the limitations or restrictions of the methods and mentioning which method was applicable for self-fertilized

crops and which to cross-fertilized crops (populations). Also, some parts of the overhead tables or diagrams could be incorporated. The amended versions should be ready by the end of September 1996 for distribution to the BMT. The Working Party stressed that it was important that the documents be placed on the agenda for the next session of the BMT and presented orally during that session, provided that the Chairman of the BMT agreed to it.

#### Application of Gower's Similarity Coefficient to Detect Varieties Which Are Very Similar

49. The expert from Poland introduced document TWC/14/2 on the application of Gower's similarity coefficient to detect varieties which were very similar. He recalled that plant breeders when sending in their applications for the registration of new varieties were advised to state the most similar varieties to their candidates. On the other hand, the registration institutions needed to know those varieties which were closest to the candidate variety. One of the possible tools for selecting those varieties was the coefficient of similarity proposed by Gower and calculated for example by the program GOWRX in the DUST system from Mr. Weatherup. The document explained the use of Gower's coefficient on sowings made in consecutive years of varieties of several different species where the Gower's coefficient of similarity was calculated between all possible pairs of treatment. In the experiment, the mean values for all pairs of treatment were calculated (WP) as well as the mean values for all pairs of associated treatments (WPS) (consecutive sowings in different years of the same variety) and the mean value for all non-associated pairs of treatment (WPN). Because the differences between WPS and WPN values were relatively large, the WPS could be treated as a threshold value. If the coefficient of similarity between pairs of non-associated treatments (varieties) was higher than WPS for this crop, such a pair was suspiciously similar. Since the values seemed to be too high for some crops, after discussion with crop experts, a modified threshold value was also applied. The new threshold value WPSM allowed for coefficients of similarity 25 per cent higher than WPS values. He concluded that the use of the general coefficient of similarity (particularly when the number of observed characters was high) could allow suspiciously similar pairs of varieties to be detected. This would make it possible to place them in neighboring plots in the following year of investigation and to observe the possible differences between such varieties more carefully.

50. The Working Party appreciated the method as another possible tool to help experts find similar varieties.

51. When discussing Gower's similarity coefficient, the Working Party considered expressing more clearly whether a given method discussed was mainly intended to inform the Working Party of an additional useful tool to help in the testing or whether it was eventually intended to become a recommendation for use in all UPOV member States. The experts from France, the Netherlands and the United Kingdom offered to prepare a list of all past documents, in their most recent version, which had led to UPOV recommendations that were still valid.

Developments in the World Wide Web

52. The expert from the United Kingdom explained the SMART project, an *explorapedia* of advanced statistical and mathematical techniques for researchers. It comprised a collaboratory approach to the production and delivery via World Wide Web of advanced statistical and mathematical techniques of interest to researchers. He explained the target audience, how to use SMART, the sequential acceptance sampling, examples of abnormal seedlings, seeds (links with software to several packages), collaborative arrangements (with material freely shared) and application software. More detailed information is reproduced in Annex V to this report.

#### Future Program, Date and Place of Next Session

53. The Working Party held first a short discussion on how to improve progress before agreeing on the program for its next session. Some experts wanted to structure the topics more and improve progress by working in small groups of, for example, three experts, and prepare one common document instead of three separate documents. Others would not agree to that procedure as in their opinion the present procedure provided the whole Working Party with more substantial information. Only as a second step might it then try to prepare one combined document or to use the same example data in all documents.

54. All experts agreed that the Working Party should listen more to the problems and questions of the other Technical Working Parties. To make that more obvious there should be a separate item on the agenda exclusively for questions from the Technical Working Parties and experts should read the reports from other Technical Working Parties and attend their sessions when they took place in their country.

55. In future, documents should be more clearly separated between (i) documents for purposes of learning or information of the Working Party; (ii) documents that might be helpful for crop experts and (iii) documents prepared in view of planned recommendations. To facilitate the separation of documents into these groups they should start with an abstract and a list of contents. As already stated, the experts from France, the Netherlands and the United Kingdom would prepare a list of all documents containing UPOV recommendations that were still valid.

56. At the invitation of the expert from Hungary, the Working Party agreed to hold its fifteenth session at the National Institute for Agricultural Quality Control in Budapest from June 3 to 5, 1997. During the session, the Working Party planned to discuss the following items:

- (a) Report on subjects of special interest to the Working Party raised during the thirtythird session of the Technical Committee
- (b) Questions raised by other Technical Working Parties
- (c) Report on new developments in member States (oral reports)
- (d) Handling of visually-assessed characteristics

#### TWC/14/19 page 15

- Ways to analyze visually-assessed characteristics (NL to prepare a paper)
- Possibilities of using biometry to help in the establishment of guidelines with respect to visually-assessed characteristics
- (e) Testing of uniformity
  - Finding the right population standard and decision rule for different sample sizes (ES to prepare a paper on balanced  $\alpha$  and  $\beta$  risks)
  - Guide to help in finding the right method to be used (draft a paper on the application of COYD, COYU and document TWC/11/16)
- (f) Sequential analysis (TC/32/6)
- (g) Items resulting from the last session of the BMT
  - Use of dendrograms
  - AMOVA
  - Statistical methods to distinguish varieties with data resulting from biochemical or molecular techniques
- (h) Image analysis (report from TWO Subgroup Meeting)
- (i) Improvement of communication
  - Improvement of statistical documents (DK to rewrite document TWC/11/16)
  - Telecommunications, exchangeable software and contacts (GB to receive updated information and prepare updated versions)
  - List of statistical documents prepared by the TWC (FR to prepare an updated list)
  - List of statistical documents containing recommendations or methods of possible interest to the Technical Working Parties (FR, GB, NL to prepare a list)
  - Glossary of definitions
  - Results of the run of the COYD program distributed on diskette during the TWC session to check whether national implementations are in concordance with the latest version of DUST
  - Developments in the World Wide Web.
- (j) Detection of outliers by multivariate analysis to the validation of data (GB to prepare a paper with further results).

#### TWC/14/19 page 16

57. As the chairmanship of Mr. S. Grégoire will terminate at the end of the coming ordinary session of the Council, the Working Party unanimously recommended to the Technical Committee to propose Mr. John Law, United Kingdom, as Chairman of the Working Party for the coming three years.

#### Visits, Demonstrations

58. In the afternoon of June 4, 1996, the Working Party received an introduction to the Computer Systems at the Federal Office of Plant Varieties, the DUS Analysis carried out in that office, the electrophoresis methods applied and the seed processing. In the afternoon of June 5, the Working Party received an introduction to image processing and an overview of the trials of Pelargonia and other ornamental species.

59. This report has been adopted by correspondence.

[Six Annexes follow]

#### TWC/14/19

#### ANNEX I

#### LIST OF PARTICIPANTS

#### I. <u>MEMBER STATES</u>

#### **FINLAND**

Kaarina PAAVILAINEN (Ms.), Inspector, Plant Production Inspection Centre, Seed Testing Department, P.O. Box 111, 32201 Loimaa (tel. +358-21-760 561, fax +358-23-205 6222)

#### **FRANCE**

Françoise BLOUET (Ms.), GEVES, La Minière, 78285 Guyancourt Cedex (tel. +33-1-30 83 35 82, fax +33-1-30 83 36 29)

Sylvain GRÉGOIRE, GEVES, La Minière, 78285 Guyancourt Cedex (tel. +33 -1-30 83 36 00, fax +33-1-30 57 01 47, e-mail: gregoire@jouy.inra.fr.)

Christine DILLMANN (Ms.), GEVES, La Minière, 78285 Guyancourt Cedex (tel. +33 -1-30 83 36 00, fax +33-1-30 57 01 47, e-mail: dillmann@jouy.inra.fr.)

#### **GERMANY**

Rudolf ELSNER, Präsident, Bundessortenamt, Osterfelddamm 80, 30627 Hanover (tel. +49-511-95 66-5, fax +49-511-56 33 62)

Georg FUCHS, Regierungsdirektor, Bundessortenamt, Osterfelddamm 80, 30627 Hanover (tel. +49-511-95 66 639, tx. 9 21 109 bsaha d, fax +49-511-56 33 62)

Friedrich LAIDIG, Bundessortenamt, Osterfelddamm 80, 30627 Hanover (tel. +49-511-95 66 689, fax +49-511-56 33 62)

Christina LIEBIG (Mrs.), Bundessortenamt, Osterfelddamm 80, 30627 Hanover (tel. +49-511-95 66-5, fax +49-511-56 33 62)

Andrea MENNE (Mrs.), Bundessortenamt, Osterfelddamm 80, 30627 Hanover (tel. +49-511-95 66 723, fax +49-511-56 33 62)

Uwe MEYER, Bundessortenamt, Osterfelddamm 80, 30627 Hanover (tel. +49-511-95 66-688, fax +49-511-56 33 62)

#### TWC/14/19 Annex I, page 2

J.P. OHMS, Bundessortenamt, Osterfelddamm 80, 30627 Hanover (tel. +49-511-95 66-5, fax +49-511-56 33 62)

Elmar PFÜLB, Bundessortenamt, Prüfstelle Rethmar, Hauptstraße 1, 31319 Sehnde (tel. +49-5138-2013, fax +49-5138-9112)

Kathrin SIEBERT (Mrs.), Bundessortenamt, Osterfelddamm 80, 30627 Hanover (tel. +49-511-95 66 751, fax +49-511-56 33 62)

Hans-Peter PIEPHO, University of Kassel, Steinstrasse 19, 37213 Witzenhausen (tel. +49-5542-98-1248, fax +49-5542-48-1230, e-mail: piepho@wiz.uni-kassel.de)

#### HUNGARY

Zoltán VERESS, National Institute for Agricultural Qualification, Keleti K. u. 24, 1024 Budapest II (tel. +36-1-212-3127, fax +36-1-212-2673)

#### **ISRAEL**

Baruch BAR-TEL, Plant Breeders' Rights Council, Agricultural Research Organization, POB 6, Bet Dagan 50 250 (tel. +972-3-968 3492, fax +972-3-968 3492)

#### **JAPAN**

Katsumi YAMAGUCHI, National Center for Seeds and Seedlings, Ministry of Agriculture, Forestry and Fisheries, 2-2, Fujimoto, Tsukuba-shi, Ibaraki 305 (tel. +81-298-38-6594, fax +81-298-38-6583, e-mail: katyam@ap-a.cc.affrc.go.jp)

#### **NETHERLANDS**

Fred A. VAN EEUWIJK, CPRO-DLO, Postbus 16, 6700 AA Wageningen (tel. +31-317-477 062, fax +31-317-418 094, e-mail: f.a.vaneeuwijk@cpro.dlo. nl)

#### POLAND

Wieslaw PILARCZYK, COBORU, Research Center for Cultivars Testing, 63-022 Slupia Wielka (tel. +48-667-523 41 ext. 274, fax +48-667 535 58)

### TWC/14/19 Annex I, page 3

#### <u>SPAIN</u>

Mariano DEL FRESNO ALVAREZ-BUYLLA, Instituto Nacional de Semillas y Plantas de Vivero, José Abascal 4, 28003 Madrid (tel. +34-1-347 6927, telex 47698 INSM, fax +34-1-442 8264, e-mail: fresno@ispv.inia.es)

#### UNITED KINGDOM

John LAW, National Institute of Agricultural Botany (NIAB), Huntingdon Road, Cambridge CB3 OLE (tel. +44-1223-276 381, ext. 2254, fax +44-1223-277 602, e-mail: j.law@pvs.maff.gov.uk)

Mike TALBOT, Biomathematics & Statistical Scotland, The University of Edinburgh, James Clerk Maxwell Building, The King's Buildings, Edinburgh EH9 3JZ (tel.: +44-131 650 4890, fax: +44-131-650 4901, e-mail: m.talbot@bioss.sari.ac.uk)

#### II. OBSERVER ORGANIZATION

#### INTERNATIONAL PLANT GENETIC RESOURCES INSTITUTE (IPGRI)

Tom HAZEKAMP, Via delle Sette Chiese 142, 00145 Rome, Italy (tel. +39-6-5189 2236, fax +39-6 575 0309, e-mail: t.hazekamp@cgnet.com)

#### III. TECHNICAL EXPERTS

Jean-Louis LAFFONT, Pioneer France Mais, Chemin de l'Enseigure, 31840 Aussonne, France (tel.: +33-61 06 20 00, fax: +33-61 85 14 22, e-mail: laffont@phibred.com)

Moez R. MEGHJI, CIBA Seeds, Applied Technology Research, 221 Imperial Drive, Bloomington, IL 61701, USA (tel.: +1-309 823 8578, fax: +1-309-823 9468)

#### IV. OFFICER

Sylvain GRÉGOIRE, Chairman

### V. OFFICE OF UPOV

Max-Heinrich THIELE-WITTIG, Senior Counsellor, 34, chemin des Colombettes, 1211 Geneva 20, Switzerland (tel. +41-22-730 9152, telex 412 912 ompi ch, fax +41-22-733 5428)

[Annex II follows]

## TWC/14/19 Annex I, page 4

#### TWC/14/19

#### ANNEX II

#### REVISED DRAFT AGENDA

#### prepared by the Chairman of the TWC and adopted by the TWC

- 1. Opening of the session
- 2. Adoption of the agenda (this document)
- 3. Report on subjects of special interest to the Working Party raised during the thirty-second session of the Technical Committee and on questions raised by other Technical Working Parties (TC/32/7)
- 4. UPOV-ROM Plant Variety Database
- 5. Report on new developments in member States (oral reports)
- 6. Handling of visually-assessed characteristics
  - Experience in the testing of vegetables
  - Threshold models for visually observed data (TWC/14/12)
- 7. Testing of uniformity
  - View of crop experts on the variation or non-variation of the population standard from year to year (TWC/14/11)
  - Some discussion points with respect to fluctuations of the population standard (TWC/14/13)
  - Tools that may help in finding the right population standard and decision rule for different sample sizes (TWC/14/9)
  - Acceptance probability curves (TWC/14/4)
  - COYD-long-term LSD (TWC/14/16)
  - QALSTAT computer program
- 8. Sequential analysis (TC/32/6)
- 9. Image analysis (stage of EU project, TWO/28/13)

### TWC/14/19 Annex II, page 2

- 10. Detection of outliers
- 11. Improvement of communication
  - Rewriting of document TWC/11/16 (TWC/14/3)
  - Rewriting of the COY analysis (TWC/14/7)
  - Telecommunications, exchangeable software and contacts (TWC/14/10)
  - List of statistical documents prepared by the TWC (TWC/14/5, TWC/14/6)
  - Results of the run of the COYD program distributed on diskette during the TWC session to check whether national implementations are in concordance with the latest version of DUST
- 12. Items resulting from the last session of the BMT
  - Review of the cluster analysis (TWC/14/8)
  - AMOVA (TWC/14/15)
  - Statistical methods to distinguish varieties with data resulting from biochemical or molecular techniques (TWC/14/14)
  - Statistical analysis of molecular marker data (TWC/14/18)
  - Follow-up of documents for the BMT
- 13. Application of Gower's Similarity Coefficient to detect varieties which are very similar
- 14. Developments in World Wide Web
- 15. Future program, date and place of next session
- 16. Closing of the session.

[Annex III follows]

TWC/14/19 Annex II, page 3

#### TWC/14/19

## ANNEX III

TWC/14/19 Annex III, page 2

TWC/14/19 Annex III, page 3

[Annex IV follows]

#### TWC/14/19

### ANNEX IV

## E-mail Addresses of Participants in UPOV Technical Working Party and Technical Committee (TC) Sessions

## (June 14, 1996)

Country	Working Party/ TC	Department/Unit	Name	E-mail address
AU	BMT		C. Buller	buller@rsbs-central. anu.edu.au
AU	BMT		M. Morell	morell@rsbs-central. anu.edu.au
AU	BMT		R. Peakall	rod.peakall@anu.edu.au
СА	TWA, TWC, TWF, TWO, TWV, BMT	Agriculture Canada	L. Mougeot	lmougeot@em.agr.ca
СА	TWA, TWC, TWF, TWO, TWV, BMT	Agriculture Canada	B. Cole	bcole@em.agr.ca
DE	TWC	University of Kassel	HP. Piepho	piepho@wiz.uni-kassel.de
DK	TWC	ABI/DIPS	K. Kristensen	kk@dina.sp.dk
ES	ТС	JNSPV	J.M. Elena	jmer@ispv.inia.es
ES	TWA	JNSPV	L. Salaices	salaice@ispv.inia.es
ES	TWC	JNSPV	M. del Fresno	fresno@ispv.inia.es
FI	TC, TWA, TWC, BMT	PPIC/STD	K. Paavilainen	kaarina.paavilainen@mmm.agrifin. mailnet.fi
FR	TWA	GEVES	Joëlle Lallemand	joëlle@calvanet.calvacom.fr
FR	TWC, TWO	GEVES	M.H. Gandelin	gandelin@antibes.inra.fr
FR	TWC	GEVES	S. Grégoire	gregoire@jouy.inra.fr
FR	TWC	INRA	Christine Dillmann	dillmann@jouy.inra.fr
GB	BMT	SASA	K.O'Donnell	odonnell@sasa.gov.uk
GB	BMT	NIAB	R. Cooke	r.j.cooke@pvs.maff.gov.uk
GB	BMT	NIAB	J. Reeves	j.c.reeves@pvs.maff.gov.uk
GB	ТС	PVRO	A. Bould	a.bould@pvs.maff.gov.uk
GB	BMT, TWA	DANI	M. Camlin	camlinm@.dani.gov.uk

## TWC/14/19 Annex IV, page 2

Country	Working Party/ TC	Department/Unit	Name	E-mail address
GB	TWC	DANI	C. Weatherup	acjb0341@agvl.qub.ac.uk
GB	TWC	NIAB	G. Pullen	f.g.pullen@pvs.maff.gov.uk
GB	TWC	SOAEFD/BioSS	M. Talbot	m.talbot@bioss.sari.ac.uk
GB	TWF	WYE	P. Dodd	p.dodd@wye.lon.ac.uk
GB	TWV	SASA	N. Green	green@sasa.gov.uk
GB	TWC	NIAB	J. Law	j.law@pvs.maff.gov.uk
GB	TWO	NIAB	E. Scott	e.scott@pvs.maff.gov.uk
GB	TWC	PVRO	D. Staward	d.stavard@maff.pvs.gov.uk
GB	TC	MAFF/PVRO	All staff	(name)@maff.pvs.gov.uk
GB	TWC	DANI	S. Watson	swatson@alpha.dani.gov.uk
JP	TC	SSD/MAFF	T. Maekawa	maekawa@sc.maff.go.jp
JP	TWC	NCSS/MAFF	K. Yamaguchi	katyam@ap-a.cc.affrc.go.jp
NL	TC	CPRO-DLO	H.C.H. Ghijsen	h.c.h.ghijsen@cpro.dlo.nl
NL	TWO, TWF	CPRO-DLO	C.J. Barendrecht	c.j.barendrecht@cpro. dlo.nl
NL	TWO, TWV	CPRO-DLO	N.P.A. Van Marrewijk	n.p.a.van.marrewijk@cpro.dlo.nl
NL	TWC	CPRO-DLO	F. van Eeuwijk	f.a.vaneeuwijk@cpro. dlo.nl
NL	TWC	CPRO-DLO	G. van der Heijden	g.w.a.m.van.der.heijden@cpro.dlo. nl
NZ	TWA	PVRO	P. Rhodes	pvro@lincoln.cri.nz
NZ	TWF	PVRO	C. Barnaby	pvro.@lincoln.cri.nz

[Annex V follows]

TWC/14/19

ANNEX V

TWC/14/19 Annex V, page 2

TWC/14/19 Annex V, page 3

[Annex VI follows]

TWC/14/19

ANNEX VI

[End of document]