Disclaimer: unless otherwise agreed by the Council of UPOV, only documents that have been adopted by the Council of UPOV and that have not been superseded can represent UPOV policies or guidance.

This document has been scanned from a paper copy and may have some discrepancies from the original document.

———

Avertissement: sauf si le Conseil de l'UPOV en décide autrement, seuls les documents adoptés par le Conseil de l'UPOV n'ayant pas été remplacés peuvent représenter les principes ou les orientations de l'UPOV.

Ce document a été numérisé à partir d'une copie papier et peut contenir des différences avec le document original.

———

Allgemeiner Haftungsausschluß: Sofern nicht anders vom Rat der UPOV vereinbart, geben nur Dokumente, die vom Rat der UPOV angenommen und nicht ersetzt wurden, Grundsätze oder eine Anleitung der UPOV wieder.

Dieses Dokument wurde von einer Papierkopie gescannt und könnte Abweichungen vom Originaldokument aufweisen.

———

Descargo de responsabilidad: salvo que el Consejo de la UPOV decida de otro modo, solo se considerarán documentos de políticas u orientaciones de la UPOV los que hayan sido aprobados por el Consejo de la UPOV y no hayan sido reemplazados.

Este documento ha sido escaneado a partir de una copia en papel y puede que existan divergencias en relación con el documento original.

UPOV

TWO/27/15
ORIGINAL : English
DATE : November 30, 1994

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL WORKING PARTY FOR ORNAMENTAL PLANTS AND FOREST TREES

Twenty-seventh Session

Sydney, Australia, September 26 to October 1, 1994

REPORT

adopted by the Technical Working Party for Ornamental Plants and Forest Trees

Opening of the Session

1. The twenty-seventh session of the Technical Working Party for Ornamental Plants and Forest Trees (hereinafter referred to as "the Working Party") was held at Sydney, Australia, from September 26 to October 1, 1994. The list of participants is presented in Annex I to this report.

2. Mr. Mick Lloyd, Registrar of the Plant Variety Rights Office, welcomed the participants to Australia. The session was opened by Mrs. U. Löscher, Germany, Chairman of the Working Party.

Adoption of the Agenda

3. The Working Party unanimously adopted the agenda of its twenty-seventh session which is reproduced in document TWO/27/1, after having agreed to include after item 5(v) an item 5(vi) Kalanchoe, after item 9 an item on resistance as requested by the Technical Committee and to discuss item 13 after item 4. It furthermore deleted items 14a) Iris, c) Limonium, d) Chrysanthemum, i) Geralton Wax Flower and l) Thymus.

Short Reports on Special Developments in Plant Variety Protection in Ornamental Plants and Forest Trees

4. The Working Party received short reports from some of the experts on recent developments in their countries. The expert from Japan reported on 180 plant variety rights for Cymbidium, mainly from 3 companies. The expert from France reported on its first applications for seed propagated ornamental varieties. The expert from South Africa reported that the draft for a new law had not yet passed Parliament. The political opening had resulted in an increase in applications. The list of new ornamental genera and species protected under the plant breeders rights law in South Africa has been extended as can be seen from Annex II to this report. The expert from the United Kingdom reported, that although the new law had not yet passed Parliament, applications from most ornamental species would be considered where a good case could be made for protection. In the ornamental sector the applications have increased by 40%. The first application for a seed propagated variety of sweet pea had been received. Because of the volume of applications the fees could be slightly reduced. The expert from New Zealand reported on an increase of applications and distributed a diagram on the structure of his office as reproduced in Annex III to this report. He reported on an increase of applications, especially for garden perennials. Guidelines had to be prepared for several new genera. In view of climatic differences and differences in reference collections, test reports from other countries could only be used with limitations. The expert from the Netherlands reported on an increase of new species. At present, 1,500 applications were under test, of which 60% were tested in the Netherlands. Electrophoretic characteristics would be used by the controlling service for identification of tulips and for quick assessment of reference varieties. A digital data base was under preparation. The expert from Italy reported on a change in the administration, giving some competence to regional governments. The expert from Australia presented a detailed explanation of the structure of the system in Australia. An updated diagram is reproduced in Annex IV to this report. He reported on the Royal Assent to the new Plant Breeders Rights Act passed on September 5, 1994, which changed the name of the Office to "The Plant Breeders Rights Office." Transgenetic plants would be eligible for protection as would be all fungi and algae. A stronger enforcement procedure was provided. The new Act would be in concordance with the 1991 Act of the UPOV Convention. The expert from Germany reported on an increase of applications of new species, some of them originating from Australia, New Zealand and South Africa. She appreciated close contact with the offices of these countries in order to obtain sufficient information to judge the novelty and distinctness of these varieties.

5. The Working Party was informed on the following action taken by the European Commission to implement the Council Directive 91/682/EEC of December 19, 1991 on the marketing of propagating material (including plants intended to be planted after marketing) of ornamental plants.

(i) The adoption of the European Commission Directives 93/49/EEC, 93/63/EEC and 93/78/EEC dealing respectively with the conditions (including quality plant health) to be met by the material, the supervision and monitoring of suppliers (to be carried out by the responsible official bodies) and the provisions for lists of varieties as kept by the suppliers (see document TWO/26/18 Antibes 1993, paragraphs 31 to 33).

(ii) The postponement of any Community decision whether propagating material produced in third countries should be considered "equivalent" to that produced in the European Union.

(iii) An official interpretation of the scope of the basic Council Directive, in accordance with which "pot or house plants (as commonly understood i.e. sold in pot and intended to remain in the pot) are in principle not covered."

(iv) The organization of comparative trials in the framework of arrangements made within the relevant Standing Committee, carried out in the Netherlands (1993 and 1994), Spain (1993) and Denmark (1994). Similar comparative trials in other areas, (e.g. seeds of agricultural or vegetable species) have the purpose of checking whether the material complies with the Community requirements, including those relating to plant health (quarantine and quality). The trials will be used to harmonize methods for technical examination.

Important Decisions Taken During the Recent Sessions of the Working Party and the Technical Committee

6. Mr. Thiele-Wittig gave a brief report on the main items discussed during the previous session of the Technical Committee, referring for further details to the full report reproduced in document TC/30/6. The main results of the TWC will be reported upon under items 9 and 11.

Cooperation with Breeders in the Testing of Varieties

7. The expert from Australia introduced a report, reproduced in Annex V to this report, on the testing of varieties in Australia. In order that a small office like his could handle the numerous applications, it had to rely on other experts for help. For that purpose, a system of Qualified Person (QP) had been developed. The function of a QP was comparable to that of a patent attorney. He was responsible for the carrying out of the testing. Each QP was accredited for 1 year. QPs' applied for accreditation providing similar information on their qualifications to that in a job application. The PBR office trained the QPs and held yearly workshops in main centers to keep their information and training up to date. At present, 120 QPs have been listed. The applicant could choose a QP in his area with knowledge of the species concerned. The applicant, the QP and the examiner would work together and supervise the test which would be planned on the basis of the information sent in by the applicant at the time of application concerning the origin of the variety (its parents) and whether the variety has been registered or protected elsewhere. It also included a short desciption, including a photo, of the distinguishing characteristics. In total, there would be three publications concerning the variety. The first, at the time of application, would state that there was an application; the second, at the time of conclusion of the test, would give a full description of the variety; the third, at the time of granting protection, would inform the public on the grant of protection. The public was thus well informed and could make objections to the application. In addition to the QP, the examination process also endeavours to identify problems. In the case of applications for the protection of native plants, the Australian Cultivar Registration Authority would be involved to ensure that the candidate variety was new. Central testing existed only for ryegrass varieties in cooperation with the New Zealand authorities. Even here the tests were arranged among the breeders themselves at a research institute. In order that the examiner can visit the tests of candidate varieties with the applicant, the applicant must inform the PBR office a few weeks in advance, when the plants are at stage for observation. On the basis of available facts (on the variety, the applicant, the QP, etc.), the examiner decides whether to visit the trials or to rely completely on the QP. In case of problems or when

requested from overseas, the examiner will always go to see the trials. The applicant must declare where material of the variety will be held and guarantee that material will be available throughout the period of protection. Seed must be placed in a seed bank at the time of the grant of protection. Vegetative material may be in private or government nurseries.

8. The expert from New Zealand reported on the testing system in his country. It was a mixed system comprising certain tests done centrally and others done on the premises of the applicant. For species with few applications, the tests were done on the premises of the applicant. The observations, however, were made by the examiner or, if the testing place was too far away from the Office, by an officially designated person, who would collect the data according to instructions, leaving the decision to the examiner. With the small number of breeders and with mutual understanding, the system worked very well. For ornamental species, often only one application was received. Central testing then entailed unacceptably high costs. At present, applicants were cooperating among themselves and allowing the testing of their varieties on the premises of other applicants. With the increase in the number of applications, however, testing in more and more species would have to be done centrally. In the case of the testing on the premises of the breeder of shrubs and woody plants, the applicant was required to send plant material to be included in a central collection before the final granting of a right.

Working Procedure for Establishing Test Guidelines

9. The Working Party rediscussed its procedure for establishing Test Guidelines and how that procedure could be improved.

10. The expert from Australia particularly regretted that under the present procedure, the absence of the expert of a country leading in the preparation of a given Test Guideline resulted in a delay of one year in the preparation of that document. Smaller offices could not send an expert to all sessions of the different Working Parties every year, therefore other solutions should be sought. The Working Party agreed that more work should be done by correspondence but noted at the same time the practical drawbacks of working by correspondence due to the heavy workload of all experts at their national offices. It therefore proposed that more work should be done in Subgroups and that the Test Guidelines should already have reached a relatively advanced stage before they were presented to the Working Party for discussion. In order to avoid too much additional travel, many of these Subgroups should meet shortly before or after a planned Working Party session. Also parallel meetings of different Subgroups during the session of the Working Party could be envisaged.

11. The Working Party noted, however, that discussions on Test Guidelines and an exchange of views on the documents were necessary in the full session, especially for newer States or States far away from meeting places in order to improve personal communication on UPOV rules and principles and to enable participating experts to inform their colleagues, who have not yet attended or will for financial reasons not attend all sessions of the Working Party, on the basic principles for the drafting of Test Guidelines and on the interpretation of the adopted principles.

12. The Working Party adopted the proposal for parallel meetings during its current session and organized parallel meetings of ad hoc Subgroups on Anthurium, Norway Spruce, Rhododendron, Cymbidium, Serruria, Firelily and Kangaroo Paw.

Request for Photos in the Technical Questionnaire

13. In the context of the discussion of the Test Guidelines for African Violet, the Working Party discussed the usefulness of requesting photos from the breeder at the time of the application for protection. Some States already requested photos, partly to ensure that the candidate variety really existed and partly to obtain additional information helpful for the preparation of the test. The Working Party finally proposed to the Technical Committee that it take a general decision on that matter. The Working Party would prefer a requirement for "a representative photo of the distinguishing characteristics" in the Technical Questionnaire. The Working Party also discussed the standardization of photos prepared by the testing authorities as part of or as an addition to the variety description. The rules laid down by the Australian Office (see Annex X) will form the basis for the drafting of a guideline for the preparation of such photos.

Final Discussions on Draft Test Guidelines

Draft Test Guidelines for African Violet (Revision)

14. The Working Party noted document TG/17/4(proj.) and the fact that no comments had been received in writing on that document. It therefore made only the following main changes in that document:

(i) Conduct of Tests: To have in paragraph 3 the words "propagated material" and "young potted plants" replaced by "propagation" and "plants from propagation."

(ii) Methods and Observations: To have in paragraph 2 the words "at least" deleted.

(iii) Table of Characteristics:

Characteristics

4 To read: "Young leaf: anthocyanin coloration on intervenal lower side"

5 To have the word "intervenal" inserted

9 To read: "Mature leaf/type"

11, 12 To have the corresponding changes as for characteristics 4 and 5

15 To be deleted

(iv) Technical Questionnaire: Paragraph 1 to be presented as for Weigela with the request to indicate the species. The introduction to paragraph 5 to be copied from Weigela.

Draft Test Guidelines for Gentian

15. The Working Party noted the draft Test Guidelines for Gentian as reproduced in document TG/145/1(proj.) and document TWO/27/12 prepared by experts from Japan. It finally made, in addition to the example varieties from document TWO/27/12, the following changes in document TG/145/1(proj.):

(i) Subject of these Guidelines: The Test Guidelines to apply to Gentiana L. To have after "var. buergeri" the author "Miq" added and after "subvar. buergeri" the author "Maxima."

(ii) Material required: Paragraph 1(a) to read: "... sufficient seed to propagate 100 plants."

(iii) Methods and observations: To have after paragraph 4 the paragraph from document TWO/27/12 on the observation on the flower copied.

(iv) Grouping of varieties: To have characteristic 35 added as grouping characteristic.

(v) Table of Characteristics:

Characteristics

4 To be recorded "at mid point"

6 To be recorded "at two thirds from base"

7 To be recorded "at one quarter from base" and to have the states "absent, present"

10, 11, 12, 16, 17, 22, 24, 25 To receive an asterisk

18 To have the order of states as follows: "9, 8, 4, 3, 2, 7, 6, 1, 5"

19 To have the first state read: "folded upwards"

28 To have the first limitation deleted

29 To have also the bracketed limitation from characteristic 28

35 To read: "Corolla: diameter at top"

37, 38 ,50 To receive drawings for explanation

45 To have the states "less than five (1), five (2), more than five (Fukujuhai, 3)"

Ad 32, 33+35, 54+55 To have the drawing replaced by that of document TWO/27/12

(vi) Literature: To receive the list from document TWO/27/12

(vii) Technical Questionnaire: Paragraphs 1 to be changed as for Weigela. Paragraph 4.2 to specify "seed, cuttings, meristem culture." Paragraph 5 to have characteristics 2 and 35 added.

Draft Test Guidelines for Nerine

16. The Working Party noted document TG/146/1(proj.) and the fact that no comments had been received in writing on that document. It therefore made only the following main changes in that document:

(i) Subject of these Guidelines: To have the family name corrected into "Amaryllidaceae" and the name "flexuosa" checked by the experts from South Africa.

(ii) Grouping of Varieties: To have characteristic 37 added.

(iii) Table of Characteristics:

Characteristics

11 To have the states "few, medium, many"

12 To read: "Inflorescence: length of pedicel of outer flower"

14 To receive drawings for explanation

19 To read: "Tepal: position of recurving of longitudinal axis"

24 To have the bracketed content deleted, as well as the example variety

26 To be deleted

29 To have the color compared with the "main color"

36 To have the last state read: "even"

11, 30, 34, 37 The expert from the Netherlands to indicate example varieties

(iv) Technical Questionnaire: Paragraph 1 to be changed as for Weigela as well as the introduction to paragraph 5.

Draft Test Guidelines for Pyracantha

17. The Working Party noted document TG/147/1(proj.) and the fact that no comments had been received in writing on that document. It therefore made only the following main changes in that document:

(i) Subject of these Guidelines: To apply to vegetatively propagated varieties. The species indicated as examples in the Table of Characteristics to refer to commercial clones.

(ii) Table of Characteristics:

Characteristics

3 To have the states "sparse, medium, dense"

5 to 9, 11 To have the words "on mature branch" deleted and replaced by a paragraph reading: "Unless otherwise indicated, all observations on the leaf should be made on the mature branch."

9 To read: "Leaf: shape of blade compared to shape of young branch"

14 To be checked by experts from France whether correlated with characteristic 12

17 To have the states "symmetrically lobed, rounded, asymmetrically lobed and emarginate" and the drawings amended to show only the upper part of the blade

19 To be deleted

31 To read: "Time of full flowering"

(iii) Technical Questionnaire: Paragraph 1 to be amended as for Weigela.

Draft Test Guidelines for Weigela

18. The Working Party noted document TG/148/1(proj.) and the fact that no comments had been received in writing on that document. It therefore made only the following main changes in that document:

(i) Subject of these Guidelines: To include also Weigela marginata

(ii) Material required: To have paragraph 3 deleted

(iii) Methods and Observations: To have the following two paragraphs included: "All observations on the leaf should be made after flowering", and "All observations on the flower should be made at the time of first full flowering."

(iv) Table of Characteristics:

Characteristics

3 To have the order of the last two states reversed

7 To have "crenation" replaced by "incisions"

9, 10 To have the bracketed content deleted

9, 11, 12 To have the examples corrected and checked by French experts

15 To have the first state read: "solitary flower"

16 To have the words "per flower" added; thereafter a new characteristic to be inserted reading: "Flower: secondary flower" with the states "white (1), yellow (2)" to be checked by French experts

17 To read: "Flower: main color on inner side" with the last state to read: "violet-red"

25 To read: "Time of first full flowering"

26 To read: "Duration of first flowering"

(v) Technical Questionnaire: To have the introduction to paragraph 5 corrected and in paragraph 4 the standard sentences on micropropagation.

Draft Test Guidelines for Kalanchoe

19. The Working Party noted document TWO/27/2 and the fact that no comments had been received in writing on that document. It therefore amended in the Technical Questionnaire of that document only paragraph 1 and the introduction of paragraph 5 as for Weigela and paragraph 5.3(ii) to have the first two states read: "light yellow (1), medium yellow (2)." The Working Party asked for the document to be published after its adoption by the Technical Committee

as a Corrigendum to the present adopted Test Guidelines and not as a complete new version of the Test Guidelines as the remaining part of the Test Guidelines had not been checked for possible improvements.

Color Observations

20. The Working Party took note of document TWO/26/17, containing a draft report of the TWO-Subgroup Meeting on Color Measurements held in Antibes, France, on September 30 and October 1, 1993, and of document TWO/27/3 containing a proposal for the grouping of the RHS Colour Chart.

21. Color groups for naming purposes. The Working Party approved the combined grouping for naming purposes as laid down in document TWO/27/3 and asked the Technical Committee to recommend its use inside UPOV for all color namings. It noted the remarks made by Mr. Leslie of The Royal Horticultural Society's Garden at Wisley, United Kingdom, with respect to the use of color charts prepared in the USA. They were described in an article in a publication of the American Rhododendron Society in 1984 edited by D. H. Voss entitled "A Contribution towards standardization of color names in horticulture" by R. D. Huse and K. L. Kelly. The Working Party reconfirmed the decision taken during its last session neither to follow the proposal in the article nor to try to take over part of its wording because its suggested grouping used names from the Universal Color Language which were less neutral. The UPOV proposal used more neutral names and, in addition to the colors or mixed colors, only the intensities: light, medium and dark. The only aim of the grouping was the harmonization of color naming inside UPOV. The grouping was not intended to be used for the purpose of grouping varieties for testing for distinctness. For that purpose, other groups needed to be formed.

22. Measuring of Colors: The Working Party noted that the study on the measuring of color would continue in France. One major problem was the variation caused by differing seasons. The Netherlands would concentrate on the storage of color photos. The United Kingdom and Germany would concentrate their efforts on image analysis leaving little time and efforts for color measurements.

New Methods, Techniques and Equipment in the Examination of Varieties

23. Mr. Thiele-Wittig presented a brief report on the main items discussed during the second session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT), referring to the report reproduced in document BMT/2/9.

24. The expert from New Zealand reported on university studies on the identification of Camelias. An abstract of the study is reproduced as Annex VI to this report. The expert from France reported on a project for the identification of Hortensia varieties in France which would be started this year. He further reported on a study of the microsatellite and RFLP methods, for identification purposes and not for distinctness. The RAPD method had presented problems of reliability in maize and sunflower and the question of uniformity and stability had not yet been studied. He also referred to an article on the identifying of rose cultivars using RAPD Markers which is reproduced in Annex VII to this report. The expert from Italy reported on studies on molecular methods in his Institute. RFLP methods were expensive and therefore lower cost methods such as PCR were under study. The expert from the Netherlands reported that there had been some experience with these

methods, however, all in private companies and thus not available to him. The expert from Germany referred to some publications in Plant Varieties and Seed (Vol 7(1), April 1994). The expert from the United Kingdom referred to an article on the "Rapid Detection of Genetic Variability in Chrysanthemum (Dendranthema grandiflora Tezvlev) using random primers" which is reproduced in Annex VIII to this report. She regretted that there existed much research and many publications, but no follow up to develop reliable, repeatable methods. The expert from Australia reported that studies on agricultural crops would be continued but there were no studies on ornamental species. The expert from Japan reported that in his country only universities were developing methods.

25. The Working Party concluded that in the ornamental field the new methods were not needed as sufficient morphological and physiological characteristics were available. In addition the methods were useful mainly for identification and not for distinctness testing. However, more studies should be made on ornamental species. It was important to have knowledge of the methods in case advice was requested by courts on the use of the methods to judge essential derivation and the genetic distance between two varieties. It was important to bring together, if possible at an international level, breeders who use the methods and institutes, laboratories and scientists, and to find countries which would take the lead in such studies which would have to be made species by species.

26. In November 1994, the international rose breeding institutes will meet in the Netherlands to prepare a guide to the identification of roses. There might be the possibility of encouraging them to look into the study of biochemical and molecular techniques.

Image Analysis

27. The Working Party noted the enquiry made by the TWC on the study and use of image analysis in the different member States. The expert from the Netherlands reported on a study of characteristics such as shape in agricultural crops and vegetables and the possibility of developing quick pictures for storing data. The expert from the United Kingdom reported on studies of Chrysanthemum leaves which were almost completed. The expert from France recalled the studies reported upon during the last session of the Working Party. These were continuing.

28. The studies in the above countries and the study planned in Germany aimed at developing methods to save time and costs in the assessment of existing characteristics. The Working Party agreed that more contacts between offices were necessary in order to avoid developing deviating methods which would be difficult to harmonize at a later stage. It would be useful to collate the aims of the different member States and to discuss these aims and how closer contacts and harmonization could be achieved. The expert from the Netherlands would prepare a questionnaire for distribution to the experts and for answers to be returned before January 31, 1995. A meeting of the Subgroup or a full day of the next session of the Working Party could then be devoted to discussing how further progress in harmonization could be achieved. The following items had already been identified as aims of the study of image analysis:

(i) Faster measuring of characteristics.

(ii) Storage of data collected with image analysis.

(iii) Finding of similar varieties through checking of stored data on image analysis.

(iv) Digitalized storage of photos

List of Species in which Varieties are Tested

29. The Working Party noted document TWO/27/13 comprising a list of species of ornamental plants tested in the UPOV member States. Those countries that had not yet supplied the information on all species were requested to do so before the end of the year. The Office of UPOV would prepare a Circular for that purpose. The Working Party considered the document to be of great use in the contacts between testing authorities. It should be updated at regular intervals, possibly every two years at the end of December.

Distinctness Criteria in Ornamental Species

30. Mr. Brand (France) recalled the problems of uniformity in vegetatively propagated roses where, especially in the case of mutations, certain parts of the plant (one shoot, one flower or one petal) showed instability. This had caused the experts from France some concern as in several cases that instability was observed in material that had already been granted protection in other member States and they had felt under pressure to accept it. In some cases, where results had been bought from another member State, the instability was only observed after the granting of the right.

31. As it had not been possible to study the problems during the last session of the Working Party, on July 12 and 13, 1994, experts from France, the Netherlands and the United Kingdom met in Hanover with German experts as all countries had bilateral agreements and needed to come to a practical solution.

32. The Subgroup discussed at first how to define an off-type. It finally agreed that all plants which showed in part of its organs a mutation were considered to be off-types.

33. Comparing the different testing protocols, it noted that they were rather similar and the differences in the results were mainly caused by different climatic conditions. It agreed to recommend the use of 6 plants for testing. If mutants were found, they would be tested in the following year. Lists of all mutants tested during the last five years as well as lists of candidates with problems requiring a second year of test would be exchanged between the testing authorities as well as lists of the whole reference collection with the main characteristics for each variety. It also agreed on a reduced minimum protocol for submission to breeders and on a multilateral trial in 1995 for 2 years with 3 varieties with mutants. Other countries were welcome to join the trial. The expert from the European Community stated that it was important to elaborate on the relation between the requirements for the granting of rights and varietal purity in the market place in order to avoid a conflict once the protected variety is marketed. It was confirmed that material used in the trial would be taken from the market place.

UPOV Central Computerized Data Base

34. The Working Party noted the history of the discussions concerning a possible UPOV central computerized data base on CD-ROM as set forth in document CAJ/32/2-TC/29/2 and Circulars U 2047 and U 2067 and that the

Council, during its session in October 1993, had approved the preparation of a prototype for a UPOV data base. It also noted the preparation of a UPOV format for the transmission in electronic form to a UPOV central computerized data base on CD-ROM of bibliographic data regarding plant varieties as reproduced in document TWC/12/8. That format, with slight amendments, had been given to a firm (JOUVE) to develop a prototype on the basis of data supplied in that format by the Offices participating in the ad hoc Working Group. The prototype is expected to be ready for checking by October 25, 1994. This would give the experts of the ad hoc Working Group approximately two weeks to check it and submit their findings to the Council of UPOV which would meet on November 9, 1994, and take a decision on the future data base.

35. The Working Party welcomed the progress made and hoped to receive the first results of the testing of the prototype as well as information on the steps to be taken on the basis of those results at its next session. It was important to prepare the data base in such a way that it would also be useful for technical experts. The crop experts should have access to the prototype in order to study it and express their needs.

36. The Working Party noted that certain names of old varieties of ornamental species could be usefully stored in that data base. One should, however, not overload it with too many old names. It was necessary to check at national level which of the older names or the names from breeders' catalogues or other sources should be included. In order to achieve a harmonized approach it was agreed to gain information using the example of Pelargonium. All experts would send all variety denominations of non protected pelargonium varieties they considered to be useful for inclusion in the new data base to the German expert. He would incorporate them into the first transmission of full data to JOUVE to enable all member States to study the usefulness of a collection of these variety denominations.

Uniformity of Vegetatively Propagated Species

37. The Working Party welcomed document TWO/26/19, prepared by the expert from France in the TWC on the request of the TWO, which facilitated the understanding of the above document TWC/11/16. It studied in detail document TWO/27/4, prepared by experts from Germany, and document TWO/27/6, prepared by experts from the Netherlands, providing proposals for population standards and accepted off-types for several ornamental species.

38. Mr. Thiele-Wittig referred to documents TC/30/4 and TWC/11/16 on the COYD and COYU analysis and on the revision of paragraph 28 of the General Introduction to the Test Guidelines. Document TC/30/4 also explained in more detail the connection between the two risks involved, i.e. the alpha risk of wrongly rejecting a homogeneous variety as being heterogeneous and the beta risk of wrongly accepting a heterogeneous variety as homogeneous.

39. The expert from New Zealand explained the philosophy behind the proposal contained in document TW/27/4. For many ornamental species, little knowledge and experience was available. Accordingly it was safer to use an acceptable range for acceptable off-types than to fix a single population standard. In addition, it was not advisable to apply statistics at sample sizes below 10. The Working Party welcomed the proposal but would need to reflect on it at national level and discuss it with their statisticians. It seemed to result in a larger tolerance for crops where insufficient knowledge was available.

40. The Working Party expressed its doubts on the high beta risk indicated for the method in document TWC/11/16. It questioned the validity of the

assumptions for vegetatively propagated species. There was no normal distribution as in seed propagation. Experience of the past showed that only the two ends of the whole scale were applicable. It was thus necessary that the statisticians reconsidered the method for the calculation of the beta risk for vegetatively propagated species.

41. The expert from the Netherlands introduced document TWO/27/6. He recalled the difference between admixtures and genetically related off-types. The majority of the Working Party finally took the position that despite this difference it was not possible to treat them differently, even if admixtures could be easily seen and separated from other off-types. If they were treated differently, the risk that the applicant would misuse that possibility would be too high.

42. Having studied the different proposals for population standards and acceptance probabilities, the Working Party agreed to use in general a population standard of 1% and an acceptance probability of 95% for ornamental varieties. A standard paragraph with these figures would be included in all Test Guidelines. However, in species with a high mutation rate, the population standard should be 2%. The Working Party agreed to set up a list of species with high mutation rates. All experts were asked to send the expert from the Netherlands a list of all species in which more than 10 varieties had been tested or were under test and where the considered population standard of 2% would be justified. A circular from the UPOV Office should state that the information provided for each species should include the number of plants tested as well as further information to be agreed upon in discussions between the Chairmen of the TWC, TWF and TWO on the occasion of the forthcoming session of the Technical Committee.

Uniformity of Species/Varieties which are Propagated both by Seed and Vegetatively

43. The Working Party recalled the requirement for each variety to be judged according to its method of propagation. It had agreed to accept two different requirements on uniformity within one species, provided that it was not possible to propagate a given seed propagated variety vegetatively. The Working Party had foreseen problems if that condition was not included. It had requested further study of the problem.

44. The Working Party noted the report presented by the expert from the Netherlands on Bromelia and Trachelium, reproduced in Annex IX to this report. It finally areed to deal with each variety according to the method of propagation. With regard to document TWO/27/9, the Chairman will contact the author to receive some further explanation. The Working Party noted, however, that for cross pollinated species the COYD and COYU analysis must be applied to measured characteristics. If less than 20 varieties are under test, the long-term LSD method should be applied. This would, however, pose problems for new species where no long-term data were available.

45. The Working Party considered the explanation of the COYD and COYU analyses as not being very user-friendly. The Chairman will contact his national statistician, and will try to obtain an explanation of its application to an ornamental species, e.g. Lobelia.

46. The Working Party noted the Annex to Circular U 2185 containing a report from Denmark on Exacum, the only example where a variety could be propagated vegetatively or by seed. More reflexion on this subject at national level was needed before it could take any position on this matter. All experts were

asked to look for similar cases and to report them to the next session of the Working Party. Mathiola and Cyclamen were in this connection mentioned as other possible species where varieties could be propagated both vegetatively and by seed.

Handling of Visually Assessed Characteristics

47. The Chairman referred to document TWC/11/12 on the handling of visually assessed characteristics. He asked experts to study that document in view of the discussion of this item during the next session.

Testing of Disease Resistances

48. The Working Party noted the request from the Technical Committee to discuss the use of disease resistance characteristics and to study document TWA/23/10 containing a summary of discussions in UPOV on disease resistance in DUS testing. It also noted the following three main questions: (i) whether to use such characteristics only in cases of clear absence or presence; (ii) whether to use only clear resistance or also tolerance; and (iii) whether to include such characteristics in the Test Guidelines but without an asterisk.

49. The Working Party stated that in ornamental species resistance was not used. The Working Party was, however, aware of the fact that the situation was different in other groups of species, and that for example for vegetable species resistance characteristics were in many cases used as grouping characteristics. The decision whether to use resistance characteristics for distinctness would therefore depend very much on the species concerned and on the genetic basis of resistance.

50. Regarding ornamentals, it was too early to take a general decision. As for fruit species, ornamental varieties were mainly vegetatively propagated. Thus the acceptance of resistance characteristics with different degrees of resistance could be imagined. The main criterium was that the method had to be standardized, reliable and repeatable. The experts would check at the national level what had been done so far and whether there was a need for change. It agreed that it was necessary to study the subject in order to be prepared for the case where such a characteristic was claimed by the applicant to be the only distinguishing characteristic. Fortunately, so far, the inclusion of resistance in a given variety had always resulted in several other changes as well.

Test Guidelines for Kangaroo Paw

51. The Working Party noted document TWO/24/3 and document TWO/27/10, containing new proposals prepared by experts from Australia. It finally agreed to the following main changes in document TWO/24/3 as proposed by the ad hoc Subgroup and reported orally during the session:

(i) Subject of these Guidelines: To have the family name "Haemodoraceae" added and the "s" of "Paws" deleted.

(ii) Material required: The material to be submitted to be "10 young plants" and to have the sentence on micropropagation added.

(iii) Conduct of tests: Paragraph 3 to have the words "in the glasshouse" deleted and the number of plants changed to "10."

(iv) Methods and Observations: Paragraph 2 to cover "10 plants or 20 parts of 10 plants at flower anthesis" and to have the following two additional paragraphs included "To determine the number of flowers on the inflorescence, only flowers longer than 5 mm should be included"; and "The width of the perianth tube should be observed at its widest part."

(v) Table of Characteristics:

Characteristics

3 To have a new characteristic included after characteristic 3 reading: "Stem: degree of branching "with the states" weak, medium, strong"

6 To read: "Leaf: attitude" with the states" upright (3), semi-upright (5), spreading (7)"

7 To have the additional state "purplish green (3)"

8 To be deleted

9 To have the first state read: "absent or very slightly pubescent"

11 To have the word "tube" added; thereafter a new characteristic to be included reading: "Flower: length of perianth lobe" with the states "short, medium, long"

13 To have the order of the last two states reversed

15 To read: "Flower: color of inner side of perianth tube" with the states "yellow green (1), medium green (2), dark green (3)"

16, 18 To have the states replaced by the RHS Colour Chart

17, 20, 22 To be deleted

18 To have a new characteristic inserted after characteristic 18 reading: "Flower: color of pubescence on pedical" with the indication of the RHS Colour Chart

For several characteristics the example varieties were amended or new ones indicated. In addition, characteristics 6, 9, 16 require further checking.

(vi) Technical Questionnaire: Paragraph 7.2 to request statement of ambient conditions or greenhouse conditions (outdoors, indoors).

Test Guidelines for Lavender and Lavendine

52. Due to time constraints, document TWO/26/11 was not discussed.

Test Guidelines for Norway Spruce

53. The Working Party noted documents TWO/26/4 and TWO/25/5 prepared by experts from Germany. It finally agreed to the following main changes in document TWO/26/4, proposed by the ad hoc Subgroup and reported orally during the session:

(i) Subject of these Guidelines: The cover page to mention "ornamental varieties of Norway Spruce."

(ii) Material required: To have in paragraph 1 the bracketed part deleted and in paragraph 3 kept.

(iii) Conduct of tests: To have paragraph 3 kept.

(iv) Methods and Observations: Paragraph 2 to be kept. Paragraph 3 to mention "10 parts of 5 plants."

(v) Table of Characteristics:

Characteristics

2, 3, 4, 7, 11 To receive an asterisk

8, 12 To have the asterisk deleted

5 To have the words "annual growth" replaced by "current years shoot"

12 and following To have the figures transferred to the explanations

13 To have the information in brackets completed by "of lateral shoot"

(vi) Literature: To receive several citations

(vii) Technical Questionnaire: To have paragraph 1 corrected. Paragraph 5 to indicate characteristics 1, 2 and 4.

Test Guidelines for Rhododendron (Revision)

54. The Working Party noted document TWO/26/16 and finally made the following main changes in document TWF/26/16, proposed by the ad hoc Subgroup and reported orally during the session:

(i) Subject of these Guidelines: To exclude "Rhododendron simsii Planch. (Pot azalea; varieties covered by document TG/140/3)."

(ii) Material required: To have "normal practice" replaced by "commerce"; the expert from Germany to check a possible influence of the rootstock on the expression of the variety.

(iii) Conduct of tests: To have in paragraph 3 the Figure "20" replaced by "6."

(iv) Methods and observations: Paragraph 2 to apply to "10 typical organs of 6 plants."

(v) Grouping of varieties: The characteristics 1, 2, 26 and 34 to be used for grouping.

(vi) Table of Characteristics:

Characteristics

1 To be exchanged with characteristic 3; characteristic 3 to receive an asterisk

2 To receive an asterisk and to have the order of the states reversed

10 To have the inclusion "shape of" and the second state to read: "straight"

11 To have the addition "of blade"

15 To be placed before characteristic 14, to apply to evergreen varieties with more than 6 flowers per inflorescence only to have the states "flat, slightly domed, strongly domed, conical"

16 To have the order of the last two states reversed

18 To be placed before characteristic 17 and to have the states "absent, present"

19 To receive an asterisk and drawings to be copied from document TG/140/3

20 To be placed after characteristic 24

21 To receive an asterisk and to have the states from "very narrow" to "very broad"

26 To receive an asterisk

32 To have the second state read: "equal"

34 To receive an asterisk and to read: "Time of beginning of flowering"

(vii) Literature: To have several publications cited.

(viii) Technical Questionnaire: Paragraph 1 to be amended as for Weigela. Paragraph 4 to request the mentioning of the rootstock. Paragraph 5 to include characteristics 1, 19, 21, 26 and 34.

Test Guidelines for Firelily (Cyrtanthus)

55. The Working Party noted documents TWO/26/3, TWO/27/5, and TWO/27/14 prepared by experts from South Africa and finally agreed to the following main changes in document TWO/27/14, proposed by the ad hoc Subgroup and reported orally during the session:

(i) Material required: Paragraph 1 to refer to "flowering size."

(ii) Conduct of tests: To have the standard sentence on micropropagation included and to have the sentence on the simultaneous planting deleted.

(iii) Grouping of varieties: Characteristic 36 to be used for grouping.

(iv) Table of Characteristics:

Characteristics

4 To have the second state read: "medium green"

10 To have the first state read: "elliptic"

19 To have the third state read: "narrow campanulate"

20 To be deleted

24, 26, 30 To be checked before the next session

The order of the characteristics will have to be checked whether to indicate first the color of the tube (inner, outer) and then the color of the lobe (inner, outer); the shape of the apex of the perianth lobe to have the Notes "1, 2, 3"; and the position of the widest part of the perianth tube to be deleted.

(v) Technical Questionnaire: To have in paragraph 5 the characteristics 14, 15, 16 deleted. The same characteristics as in paragraph 5 to be used also for grouping.

Test Guidelines for Anthurium (Revision)

56. The Working Party noted document TWO/27/7 prepared by experts from the Netherlands and finally agreed to the following main changes on that document, proposed by the ad hoc Subgroup and reported orally during the session:

(i) Subject of these Guidelines: To have the author of the family deleted.

(ii) Material required: To have "tissue-culture" replaced by "micropropagation."

(iii) Methods and Observations: To have in paragraph 3 "commercial" replaced by "full flowering" and paragraph 4 to read: "Unless otherwise indicated, all observations on the flower should be made shortly before dehiscence of the anthers after the spadix has become sticky."

(iv) Table of Characteristics:

Characteristics

6 To read: "Leaf blade: relative position of lobes"

7 To have the explanations as in document TG/86/2

15 To have the word "coloration" added

16 To have the states 3 and 7 read "slightly below" and "slightly above", respectively

20 To read: "Spathe: relative position of lobes"

21 To read: "Spathe: height of adpressed part of lobes"

22 To read: "Spathe: shape of distil part" and to have the order of the states reversed

23 To read: "Spathe: shape of tip"

28 To read: "Spathe: shape in cross section of middle zone" and to have the second state read: "straight"

29 To have the drawing from document TG/86/2 added

30 The expert from the Netherlands to prepare drawings

33 To be split into two characteristics, to read: "Spathe: curvature of longitudinal axis" with the states from "strongly incurred" to "strongly recurred" and "Spadix: rolling" with the states "absent, present"

35, 36 To have the word "shortly" added before the word "before"

(v) Literature: The expert from the Netherlands to indicate literature

(vi) Technical Questionnaire: To request under paragraph 4 the indication of the propagation method whether "micropropagation," "cuttings" or "divided plants," and to have the introduction to paragraph 5 copied from Weigela.

Test Guidelines for Serruria

57. The Working Party noted document TWO/27/8 prepared by experts from South Africa and agreed to the following main changes in that document, proposed by the ad hoc Subgroup and reported orally during the session:

(i) Methods and Observations: To have paragraph 7 deleted and in paragraph 6 the words "middle part of the current season's growth on" replaced by "upper third of."

(ii) Table of Characteristics:

Characteristics

3 To read: "Plant: diameter" with the states "narrow, medium, broad"

8 To read: "Leaf: attitude" with the states "not always upright (1), always upright (2)"

9 To read: "Leaf: predominant angle in relation to branch (always upright leaves excluded)" with the states "small, medium, large"

11 To have a new characteristic inserted after characteristic 11 reading: "Varieties with bipinnate leaves only: Leaf: degree of pinnation" with the states "weak, medium, strong"

13 To be split into three characteristics as follows: (a) Leaf: color excluding anthocyanin" with the states "grey, grey green, yellow green, medium green, dark green"; (b) Leaf: anthocyanin coloration" with the states "absent, present"; (c) Leaf: intensity of anthocyanin coloration" with the states "weak, medium, strong."

25, 27, 28 To be deleted

26 To read: "Flower head: size" with the states "small, medium, large"

29 To read: "Involucral bract: main color"

29 to 34 To have the order of the characteristics changed as follows: 30, 30, 33, 29, 34"

31 to 33 To bring the words "involucral bract" at the beginning of the characteristic

34 To read: "Floret bract: color of mid rib"

35 To read: "Floret bract: length of fringe on margin"

36 To bring the words "Floret mass" to the beginning of the characteristic

39 To add the words: "excluding apical fringe, if present"

The following additional characteristics to be added at their respective places:

(a) Involucral bract: color of mid rib compared to main color" with the states "the same (1), different (2)"

(b) Involucral bract: color of mid rib if different from main color" with the states "pale pink (1), medium pink (2), dark pink (3), light red (4), medium red (5), dark red (6), purple brown (7)"

(c) "Floret mass: height" with the states "short, medium, long"

(d) "Floret mass: degree of concealment by involucral bracts" with the states "concealed (1), partially exposed (2), fully exposed (3)"

(e) "Floret mass: color of upper part" with the states "whitish (1), pinkish (2), reddish (3), purplish (4)"

(f) "Flower head: number of involucral bracts" with the states "few, medium, many"

(g) "Involucral bract: shape of apex" with the states "acuminate (1), acute (2)"

(h) "Involucral bract: thickness" with the states "thin, medium, thick";

and the following characteristics to be checked for possible inclusion:

(i) "Leaf: shape of apex" with the states "blunt (1), pointed (2)"

(j) "Floret mass: shape of apex" with the states "flat (1), rounded (2), pointed (3)"

(k) "Involucral bract: reflexing of apex" with the states "inconspicuous (1), conspicuous (2)"

(l) "Floret bract: length of fringe of margin" with the states "short, medium, long"

(m) "Involucral bract: shape" with the states "lanceolate (1), ovate (2), oblanceolate (3)"

Test Guidelines for Cymbidium

58. The Working Party noted document TWO/27/11 prepared by experts from Japan. It finally discussed it until characteristic 41 and made the following main changes in that document:

(i) Subject of these Guidelines: To have the words "vegetatively propagated varieties" added.

(ii) Material required: To have in paragraph 1 the bracketed word "meri-clone" added after "clone."

(iii) Conduct of tests: To have in the first sentence of paragraph 3 the words "for the species concerned" added.

(iv) Methods and Observations: To receive two additional sentences reading: "All observations on the leaf should be made on the longest leaf of a flowering shoot bulb"; and "All observations on the length and width should be made on the unextended organ."

(v) Table of Characteristics:

Characteristics

2 To read: "Plant: attitude of bulb" with the states "erect, semi-erect, spreading"

5 To have the third state read: "circular"

11 To have the states "acute (1), obtuse (2), emarginate (3)" and a new characteristic inserted reading: "Leaf, symmetry of apex" with the states "absent, present"

12 To have the first state read: "straight"

13 To be placed after characteristic 3, to receive drawings and to read: "Plant: hight of tip of longest leaf relative to soil level" with the states "far above (1), slightly above (3), same level (5), slightly below (7), far below (9)"

14 To have the Notes "1, 3, 5, 7, 9"

15 To read: "Leaf: green color" with the states "light (3), medium (5), dark (7)"

20 To have the first state read: "terminal flower only"

22 To read: "Varieties with racine only: Flower: length of peduncle"

24 To read: "Flower stalk: rigidity" and to have the Notes "3, 5, 7"

25 To read: "Flower stalk: attitude" with the second state to read: "semi-erect"

29 To read: "Flower: shape of corolla excluding labellum" with the states "all parts incurred (1), some parts incurred, some parts spread (2), all parts spread (3), some parts spread, some parts reflexed (4), all parts reflexed (5), some parts incurred, some parts reflexed (6)"

30 To receive drawings and to read: "Flower: length in full face"

31 To receive drawings and to read: "Flower: width in full face"

32 To have the asterisk deleted

36 To have the states "incurred with reflexed apex (1), strongly incurred (2), slightly incurred (3), straight (4), slightly reflexed (5), strongly reflexed (6), reflexed with incurred apex (7)"

37 To have the states "narrow acute, acute, mucronate, truncate, emarginate"

In the Technical Questionnaire a characteristic to be inserted under paragraph 5 without being included in the Table of Characteristics reading: "Flower: predominant color" with the states "white, yellow, green, pink, red, purple, reddish brown, bicolor."

Status of Test Guidelines

59. The Working Party agreed that the draft Test Guidelines for African Violet (Revision), Gentian, Nerine, Pyracantha and Weigela as well as the Technical Questionnaire and growing conditions for Kalanchoe should be sent to the Technical Committee for final adoption. It agreed that the draft Test Guidelines for Anthurium (Revision), Norway Spruce, Rhododendron (Revision) should be sent to professional organizations for comments and to rediscuss the Working Papers on Test Guidelines for the other species mentioned on the agenda at its next session.

Future Program, Date and Place of Next Session

60. At the invitation of the Netherlands, the Working Party agreed to hold its twenty-eighth session in Wageningen, the Netherlands, from September 25 to 30, 1995. [At the occasion of the session of the Technical Committee the date has been changed to September 4 to 9, 1995]. It was planned that the following items would be discussed during the forthcoming session:

(i) Short reports on special developments in plant variety protection for ornamental plants and forest trees (oral reports).

(ii) Important decisions taken during the recent sessions of the Technical Working Party and the Technical Committee (oral reports).

(iii) Final discussions on Draft Test Guidelines for Anthurium (Revision), Norway Spruce, Rhododendron (Revision).

(iv) Color observations.

(v) Image analysis.

(vi) New methods, techniques and equipment in the examination of varieties (information on DNA methods to be collected by France by the end of November 1994).

(vii) List of species in which varieties are tested (TWO/27/13 + updating).

(viii) Handling of visually assessed characteristics (TWC/11/12).

(ix) Characteristics on disease resistance (TC/30/5).

(x) Central computerized data base (oral report).

(xi) Uniformity of vegetatively propagated species (TWC/11/16, TWO/26/19 + NL to collect lists of species with a population standard of 2% or more)

(xii) Uniformity of species/varieties which are propagated both by seed and vegetatively (all to prepare an example of TWC/11/16; DE to prepare an example of COYD + COYU application of Labelia varieties).

(xiii) Discussion on working papers on Test Guidelines for:

(a) Iris (TWO/26/12 + NL to collect comments by end of May 1995)
(b) Kangaroo Paw (TWO/27/10, UPOV to prepare a new working paper by end of March 1995)
(c) Limonium (TWO/26/14, IL to prepare a new working paper by end of May 1995)
(d) Chrysanthemum (Revision, TG/26/4, GB to prepare a working paper by end of March 1995 (comments to GB by end of April 1995), with a possible Subgroup (DE, FR, NL, GB) meeting in Cambridge in June 1995)
(e) Lavender and Lavendine (TWO/26/11 + FR to prepare a working paper)
(f) Firelily (Cyrtanthus) (TWO/27/5, UPOV to prepare a working paper by end of March 1995)
(g) Geralton Wax Flower (Chamelaucium) (AU to prepare a working paper by end of April 1995)
(h) Serruria (TWO/27/8, UPOV to prepare a working paper by end of March 1995)
(i) Thymus (FR to prepare a working paper by the end of March 1995)
(j) Cymbidium (JP to prepare a working paper by the end of March 1995)
(k) Ficus benjamina (NL to prepare a working paper by the end of December 1994)
(l) Bouvardia (JP+NL to prepare a working paper by the end of February 1995)
(m) Ornamental Apple (Revision) (TG/14/5 + GB to prepare a Working Paper by end of April 1995)

61. It is planned to reserve the first day of the next session for discussions on image analysis. For this purpose, experts on image analysis should also be invited to attend the session on September 25, 1995 [changed to September 4, 1995].

Visits

62. In the evening of September 25, 1994, the Working Party received a short introduction to the Outeniqua Nursery by its proprietor Mr. Roy Rother on the occasion of a dinner sponsored by him. That nursery had already made over 200 applications for plant variety protection and had already received 37 grants for protection.

63. On September 26, 1994, the Working Party visited the Blue Mountains Environs at Leura, Katoomba and Blackheath, accompanied by an extension officer, Mr. Toni Barbolini, of the New South Wales (NSW) National Parks and Wildlife Department, who interpreted the various points of interest and brought the different native species to the attention of the experts during small walks through the endemic flora. At the Jemby-Ringah Lodge, he gave an illustrated talk on the natural history of the Blue Mountains area. In the afternoon of the same day, the Working Party visited the Mt. Tomah Botanic Gardens and received a guided tour of the gardens which are at 1000 m sea level with red volcanic soil and cool and humid climate, making it ideal for the development of collections of cool climate plants.

64. In the evening of September 27, 1994, the Director of Plants Management Australia, Mr. Richard Rose, gave an introduction to the staff of his organization on the occasion of a dinner sponsored by it.

65. In the afternoon of September 28, 1994, the Working Party visited the Swanes Nursery at Dural where it was given an explanation of the different species during a walk through the nursery. All plants offered for sale were produced in the nursery. In the same afternoon the Working Party visited the

Plantmark Wholesale Plant Distribtion Center at Kellyville where it received a guided tour through the expressive display of plants from a large number of nurseries, offered for sale to retailers. The display was a unique possibility to select plants available nowhere else.

66. In the afternoon of September 29, the Working Party visited the New South Wales (NSW) Department Horticultural Research Facility at Navara in the North of Sydney where it was received by its manager, Mr. W.A. Trimmer and received an introduction by the Senior Research Horticulturist, Mr. Ross Worralt, on its breeding activities and a guided visit of the tissue culture laboratory and the glass houses where it was possible to see the research results on Kangaroo Paw, Grevilla, Lechnaultia and Geralton Wax. In the same afternoon the Working Party also visited the Burbank Biotechnology Tissue Culture Laboratory at Tuggerah where it received an introduction to the work done by laboratory manager Mr. Lydi Nashar, the Managing Director Mr. Tatsuo Tanaka and the Marketing Manager, Mr Craig Bryson, and a guided tour through the glasshouses with young plants resulting from tissue culture.

67. This report has been adopted by correspondence.

[Ten annexes follow]

TWO/27/15

ANNEX I

LIST OF PARTICIPANTS AT THE TWENTY-SEVENTH SESSION OF THE TECHNICAL WORKING PARTY FOR ORNAMENTAL PLANTS AND FOREST TREES, SYDNEY, AUSTRALIA, SEPTEMBER 26 TO OCTOBER 1, 1994

I. MEMBER STATES

AUSTRALIA

Mick LLOYD, Plant Variety Rights Office, GPO Box 858, Canberra, Australia 2601, (tel. 06.271.6476, fax 06.272.3650)

Margaret WINSBURY, Plant Variety Rights Office, GPO Box 858, Canberra, Australia 2601 (tel. 06.271.6476, fax 06.272.3650)

Elizabeth PULSFORD, Plant Variety Rights Office, GPO Box 858, Canberra, Australia 2601 (tel. 06.271.6476, fax 06.272.3650)

FRANCE

Richard BRAND, GEVES, B.P. 1, Les Vignères, 84300 Cavaillon (tel. 90.71.26.85, fax 90780161)

GERMANY

Ulrike LOESCHER, Bundessortenamt, Postfach 61 04 40, 30604 Hannover (tel. 0511/95665 or 9566725, fax 0511/563362)

ITALY

Tito SCHIVA, Istituto Sperimentale Floricoltura, Corso Inglesi 508, 18038 San Remo (tel. 0039.184.667.251, fax 0039.184.658.218)

JAPAN

Koji KANAZAWA, Examiner, Seeds and Seedlings Division, Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries, 1-2-1 Kasumigaseki, Chiyoda-ku, Tokyo (tel. 03-3591-0524, fax 03-3502-6572)

NETHERLANDS

Joost BARENDRECHT, CPRO-DLO, Postbus 16, 6700 AA Wageningen (tel. 08370-76893, fax 08370-22994, E mail: C.J.Barendrecht@crpo.agro.nl)

NEW ZEALAND

Chris BARNABY, Plant Variety Rights Office, P.O. Box 24, Lincoln (tel. 64-3-3256355, fax 64-3-3252946

SOUTH AFRICA

Elise BUITENDAG, Plant Quality Control, Citrus and Subtropical Fruit Research Institute, Private Bag X11208, Nelspruit 1200 (tel. 1311 52071, telex 335240 SA, fax 1311 23854)

UNITED KINGDOM

Elizabeth SCOTT, Ornamental Plants Section, NIAB, Huntingdon Road, Cambridge CB3 OLE (tel. 223-342399, fax 223-342229)

II. OBSERVER ORGANIZATION

EUROPEAN ECONOMIC COMMUNITY

Marcantonio VALVASSORI, Pincipal Administrator, European Economic Community, rue de la Loi 200, VI B II.1, Loi 84 1/7, 1049 Brussels (tel. 02-295 6971, fax. 02-296 93 99)

III. OBSERVER

RUSSIAN FEDERATION

Alexey CHERNETSKY, "Agbina", Schelkovskoe shosse 90/A, 105523 Moscow, Russian Federation, (tel. 095-965-56-64, fax 095-468-52-69)

IV. OFFICER

Ulrike LOESCHER, Chairman

V. OFFICE OF UPOV

Max-Heinrich THIELE-WITTIG, Senior Counsellor, 34, chemin des Colombettes, 1211 Geneva 20, Switzerland (tel. 022 7309152, telex 412 912 ompi ch, fax (041-22) 7335428)

[Annex II follows]

ANNEX II

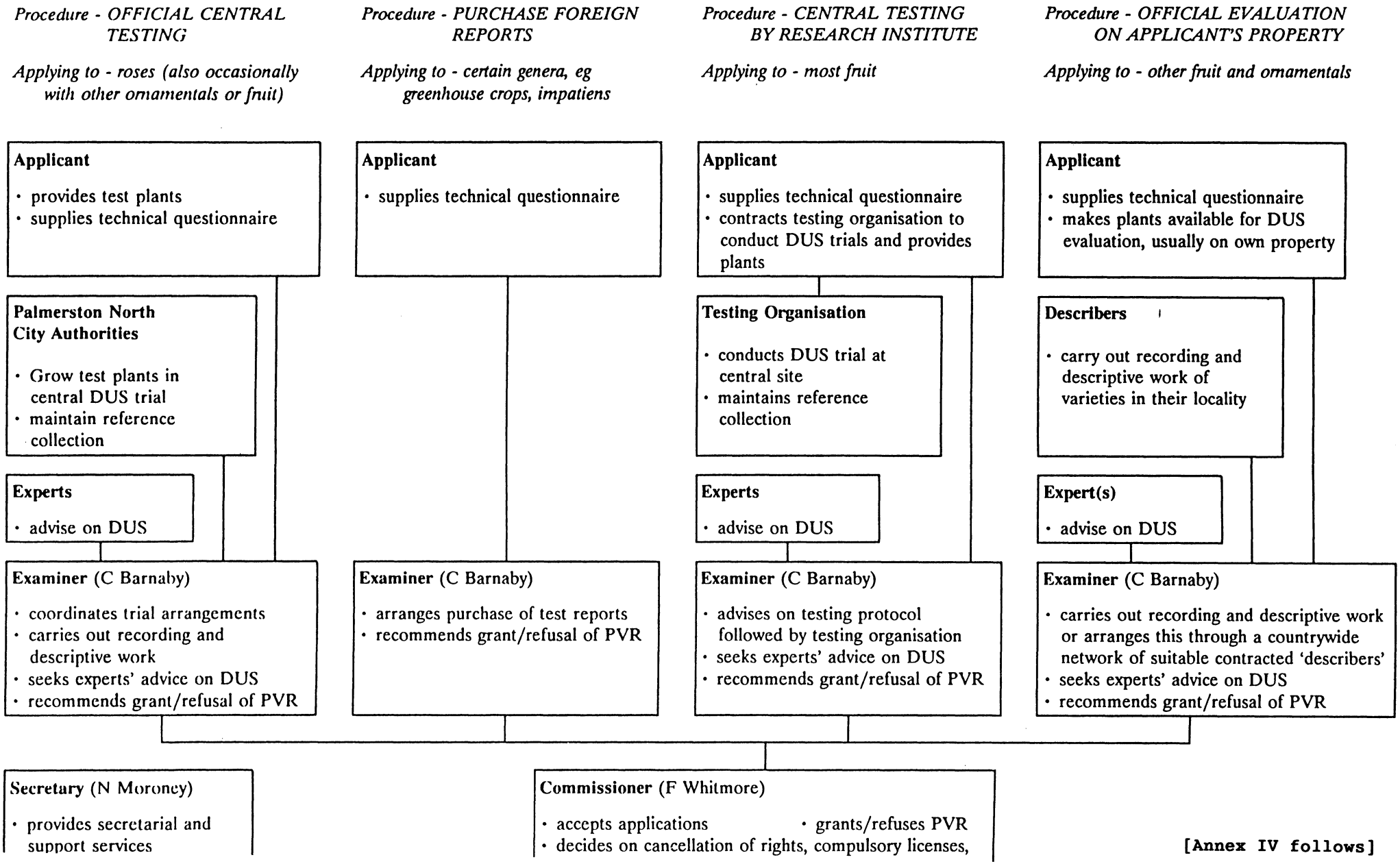
NEW ORNAMENTAL GENERA AND SPECIES DECLARED UNDER THE PLANT BREEDERS' RIGHTS LAW IN SOUTH AFRICA

- Alstroemeria
- Bougainvillea
- Canna
- Dieffenbachia
- Eucalyptus gunnii
- Gardenia
  Hebe
- Hemerocallis
- Hosta
- Impatiens
- Koeleria
- Lathyrus tingitanus
- Petunia
- Plumbago
- Rosmarinus
- Scabiosa
- Scaevola
- Strelitzia
- Zantedeschia

[Annex III follows]

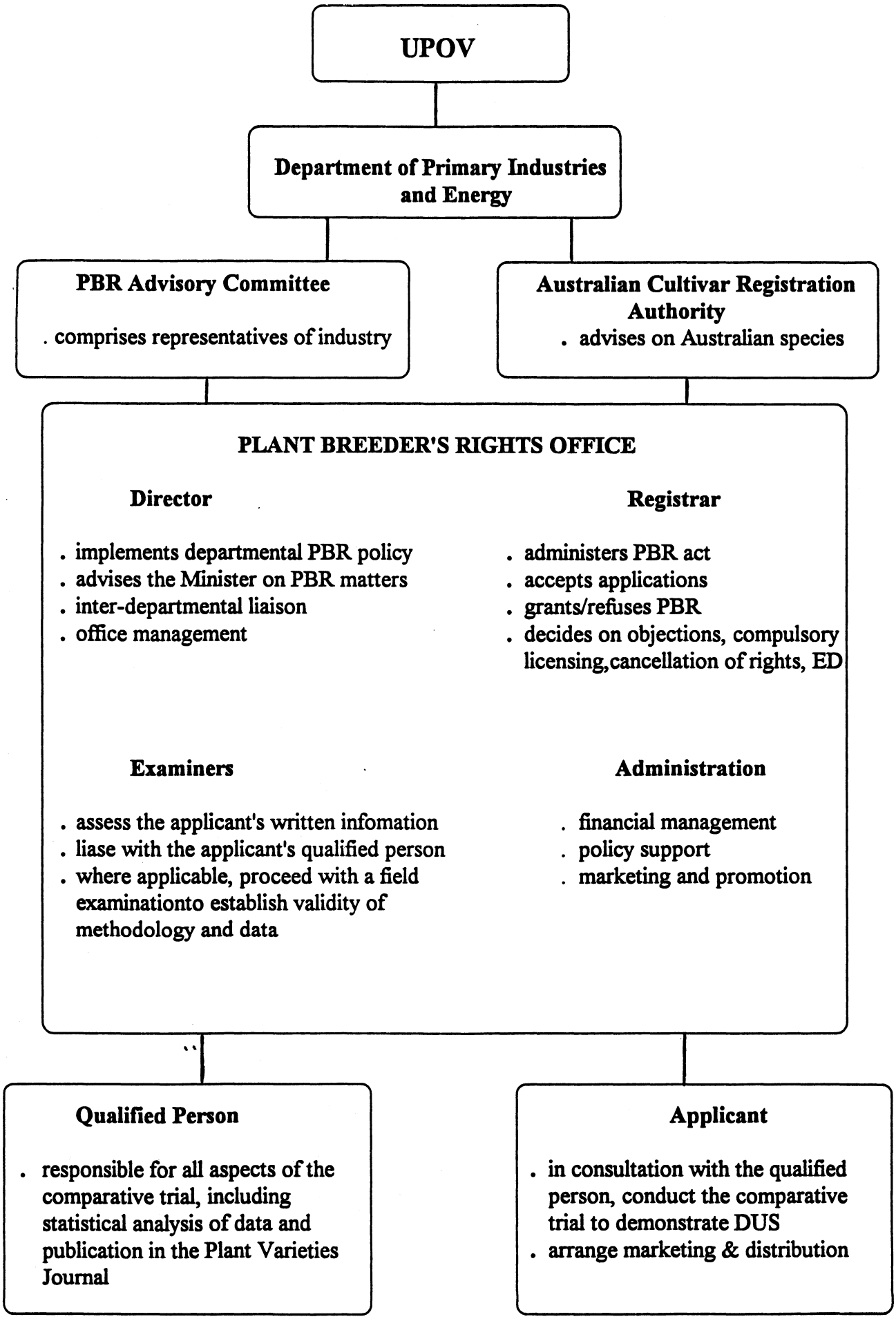
ANNEX III

NEW ZEALAND PLANT VARIETY RIGHTS (PVR) PROCEDURES - FRUIT AND ORNAMENTAL VARIETIES



[Annex IV follows]

ANNEX IV


UPOV
Department of Primary Industries and Energy
PBR Advisory Committee
. comprises representatives of industry
Australian Cultivar Registration Authority
. advises on Australian species
PLANT BREEDER'S RIGHTS OFFICE
Director
. implements departmental PBR policy
. advises the Minister on PBR matters
. inter-departmental liaison
. office management
Registrar
. administers PBR act
. accepts applications
. grants/refuses PBR
. decides on objections, compulsory licensing,cancellation of rights, ED
Examiners
. assess the applicant's written infomation
. liase with the applicant's qualified person
. where applicable, proceed with a field examinationto establish validity of methodology and data
Administration
. financial management
. policy support
. marketing and promotion
Qualified Person
. responsible for all aspects of the comparative trial, including statistical analysis of data and publication in the Plant Varieties Journal
Applicant
. in consultation with the qualified person, conduct the comparative trial to demonstrate DUS
. arrange marketing & distribution

The Australian Plant Breeder's Rights Scheme

The Plant Breeder's Rights scheme in Australia is administered under the *Plant Breeder's Rights Act 1994*. This act conforms with the 1991 revision of the UPOV Convention and replaces the *Plant Variety Rights Act 1987*. Under the new legislation, new varieties of all plant, fungal, algal species and transgenic plants are eligible for protection.

The Plant Breeder's Rights Office (PBRO) is located in the Crops Division of the Commonwealth Department of Primary Industries and Energy. Dr HL (Mick) Lloyd is both Registrar of PBR and Director of the PBRO. A staff of three examiners and two administrative officers support Dr Lloyd in these functions. PBRO receives approximately 300 applications per year.

Australia's PBR scheme uses breeder testing to establish the distinctness, uniformity and stability of new varieties. The breeder or their agent carries out comparative trials, using UPOV technical guidelines, to establish that each new variety satisfies DUS criteria. To ensure technical rigour, the PBRO requires all applicants to engage the services of an accredited qualified person (QP). The QP, in collaboration with the PBR Office, accepts responsibility for all aspects of the comparative trial, including the choice of comparative varieties, experimental design, collection of data, statistical analysis and preparation of a description of the variety. There are over 100 QPs in Australia and New Zealand each of whom is accredited to consult on one or limited range of plant species in which they have expertise.

The QP must apply to the PBRO for accreditation before they can act as PBR consultants and assist applicants. This involves a written application outlining qualifications, experience, and names of referees. The QP must also attend annual training workshops given by the PBRO to retain accreditation. These measures aim to ensure that PBR grants are technically rigorous and legally sustainable in the event of infringement.

A comparative trial in Australia may not always be necessary, providing the variety has been test grown in a UPOV member country using official UPOV guidelines and test procedures, and all the most similar varieties of common knowledge have been included in the trial. If the test indicates the variety is clearly distinct from known Australian varieties, a comparative test may not be warranted. In both of these cases, the PBRO still requires applicants to submit a description for publication in the *Plant Varieties Journal*.

The PBRO publishes a description and photograph of each variety in the *Plant Varieties Journal*. Publication allows a breeder's peers to object to the granting of PBR, informs industry and gives the public an opportunity to comment on individual applications. The PBRO investigates all objections and comments it receives.

The Registrar consults widely on all applications. Specifically, he consults the Australian Cultivar Registration Authority for specialist advice on all applications for new varieties of Australian indigenous species. He also utilises the knowledge and experience of the Plant Breeder's Rights Advisory Committee (PBRAC). The Advisory Committee is comprised of representatives of breeders, producers and consumers, and others with appropriate qualifications and experience.

[Annex V follows]

ANNEX V

Applying to be a PVR Qualified Person

What is a QP?

A qualified person, or QP, acts as a PVR applicant's technical consultant. They accept responsibility for overseeing the comparative trial and for providing evidence that a variety is distinct, uniform and stable. This role may involve the QP consulting on choice of comparative varieties, experimental design, management regime, collection of data, statistical analysis, photography and preparation of the description of the variety .

The QP's technical role helps guarantee that applications for PVR are technically rigorous. It complements the role of PVR Office examiners in ensuring that PVR grants are legally sustainable in the event of infringement and subsequent litigation.

When first applying for PVR, an applicant must nominate a QP. This is done using a **'QP1' form - *'Nomination of a qualified person'*.** This form is intended to provide a guide for the applicant and their QP in determining what functions the QP will play in the preparation of the application. Once the application is complete, the QP must certify the application by completing and signing form **'QP2' - *'Certification by the qualified person'*.** This form outlines exactly which functions the QP undertook or supervised in relation to the application.

There are two categories of QP:

- **Consultant QP** - accredited to act as consultants to PVR applicants. The applicant retains the QP on a mutually agreed basis. A list of consultant QPs appears in each issue of the *Plant Varieties Journal.*

- **Non consultant QP** - accredited to certify applications for which they are the breeder, owner or authorised agent, or, an employee of the breeder, owner or authorised agent.

Note that accreditation is ascribed to the individual - it is not transferable to other people within a company or institution.

As part of the accreditation process, the PVR Office conducts workshops for qualified persons. These workshops cover the principles, practice and developments of the PVR application process. You must attend a workshop in order to validate and maintain your accreditation.

How do I apply?

If you wish to be accredited as a QP, you will need to apply to the PVR Office. Your application should include the following information:

- full name, address and contact numbers;
- current employment details;
- qualifications;
- relevant experience;
- species or group of species for which accreditation is being sought;
- the geographical area in which you are able to act as a QP;
- names, addresses and telephone numbers of three referees; and
- whether or not consultant or non-consultant status is being sought.

Applications should be sent to :

The Registrar
Plant Variety Rights Office
DPIE
GPO Box 858
CANBERRA ACT 2601

s:\QPFORM1.HLL

Form QP1(12/92)

NOMINATION OF A QUALIFIED PERSON

Nomination of a qualified person must accompany part 1 of the application for PVR

- To be completed by the applicant or their agent at the time of the initial application and attached to part 1 of the application for PVR
- If the applicant or agent is not accredited by the PVR Office there are two options available:
  - the applicant or agent can complete this form and simultaneously apply for accreditation or,
  - the applicant can select an accredited consultant qualified person from the list in appendix 3 of Australian *Plant Varieties Journal*
- It is strongly recommended that you contact the selected qualified person and use this form as a guide to come to an understanding with them on what role they will play in the application process

Name of the variety

Name of nominated qualified person (QP)

Please answer all questions by ticking [✓]the correct answer opposite

The nominated QP is accredited by PVRO as a:		
• consultant qualified person	yes [ ]	no [ ]
• non-consultant qualified person	yes [ ]	no [ ]
• As the QP, I will be responsible for, and certify, this application for PVR	yes [ ]	no [ ]
I have contacted the nominated QP and they have agreed to:		
• review the application documents related to the above variety first filed in another UPOV member country and make recommendations to PVRO on their suitability for examination without a test growing in Australia	yes [ ]	no [ ]
• perform those functions ticked [✓] in the box below if PVRO requires a comparative test growing in Australia as part of the application process	yes [ ]	no [ ]

Tick [✓] only those functions that the QP has agreed to perform in relation to this application

[ ] • completion of part 1 of the application form	[ ] • make observations/take measurements to comply with approved DUS test guidelines
[ ] • advice on and availability of the most similar varieties of common knowledge	[ ] • perform the necessary statistical analysis of the measurements to determine DUS
[ ] • planning the test growing(trial)	[ ] • provide observations, data and statistical analysis of the DUS trial for the applicant to complete part 2 of the application form
[ ] • recommending the most appropriate trial site for the varieties in the trial	[ ] • completion of part 2 of the PVRO application
[ ] • choice of the trial site	[ ] • verification of the field trial, observations, data and statistical analysis
[ ] • supervision of the layout and planting of the trial	[ ] • certification of the application
[ ] • planting the trial	[ ] • provision of a standard description of variety in the PVRO-approved format
[ ] • care and maintenance of the trial	[ ] • provision of a slide and eight colour prints of the variety showing distinctness characters
[ ] • instruction to applicant on the timing and nature of observations/measurements needed	

Name of applicant/agent	Signature	Date

QP2/5/94

CERTIFICATION BY A QUALIFIED PERSON

- to be completed and signed by the applicant or the applicant's agent *and* the qualified person
- the qualified person must be officially *accredited* for the species, in writing, by the Plant Variety Rights Office
- this completed form should be attached to, and submitted with, Part 2 of the application form

VARIETY NAME
APPLICATION NUMBER
APPLICANT'S OR AGENT'S NAME
QUALIFIED PERSON'S NAME

Please answer all questions by ticking [ ] the correct answer opposite

I am accredited with the Plant Variety Rights Office as a:		
• consultant qualified person	yes [ ]	no [ ]
• non-consultant qualified person	yes [ ]	no [ ]
As the Qualified Person I have :		
• reviewed the application documents related to the above variety first filed in another UPOV member country and have attached recommendations to PVRO on their suitability for examination without a test growing in Australia	yes [ ]	no [ ]
• performed those functions ticked [ ] in the box below in the comparative test growing in Australia as part of the application process the results of which are reported in part 2 of the application form and attachments	yes [ ]	no [ ]

Tick [ ] only those functions that the QP has performed in relation to this application

[ ]	• completed part 1 of the application form	[ ]	• made observations/taken measurements to comply with approved DUS test guidelines
[ ]	• advised on and availability of the most similar varieties of common knowledge	[ ]	• performed the necessary statistical analysis of the measurements to determine DUS
[ ]	• planned the test growing (trial)	[ ]	• provided observations, data and statistical analysis of the DUS trial for the applicant to complete part 2 of the application form
[ ]	• recommended the most appropriate trial site for the varieties in the trial	[ ]	• completed part 2 of the PVRO application
[ ]	• choice of the trial site	[ ]	• verified the field trial, observations, data and statistical analysis
[ ]	• supervised the layout and planting of the trial	[ ]	• provided a standard description of the variety in the PVRO-approved format
[ ]	• planted the trial	[ ]	• provided a slide and eight colour prints of the variety showing distinctness characters
[ ]	• maintained the trial		
[ ]	• instructed applicant on the timing and nature of observations/measurements needed		

Signature of Qualified Person	Date

If the applicant or agent for the applicant is a different person from the qualified person (QP), please countersign below to confirm that there is a joint understanding on the respective roles of the applicant/agent and QP in this application

Signature of Applicant/Agent	Date

ANNEX VI

IDENTIFICATION OF CAMELLIA SPECIES, CULTIVARS AND THEIR INTERRELATIONSHIPS USING RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS

DNA was extracted from young leaf tissue of more than 50 camellia species and 90 camellia cultivars. Random amplified polymorphic DNA (RAPDS) markers were constructed using polymerase chain reaction. Amplification products were analysed by electrophoresis. The relationships between cultivars of *C. reticulata* and species of genus *Camellia* were investigated using RAPDS. Unique banding patterns of RAPD markers were produced for each camellia species using primer-01 (5'-CGGCCCCGGC-3'), while different varieties or cultivars from the same species usually showed identical bands, especially for *C. reticulata*. At the section level, the morphologically closely related species showed some similar bands. The species in section *Chrysantha*, section *Tea* and section *Archecamellia* showed quite different banding patterns from each other and from the species in section *Camellia* and section *Theopsis*. Identification of the varieties or cultivars from the same species was enhanced using two additional primers, Primer-02 (5'-CGGCCCCTGT-3') and Primer-03 (5'-CGGTCACTGT-3'). RAPD markers can fingerprint camellias rapidly and are a powerful tool for studying systematic relationships between species and for identification of varieties or cultivars in genus *Camellia*.

[Annex VII follows]

HortScience 28(4):333–334. 1993.

Identifying Rose Cultivars Using Random Amplified Polymorphic DNA Markers

A.M. Torres

Departamento de Mejora y Agronomía, Centro de Investigación y Desarrollo Agrario, Apartado 4240, 14080 Córdoba, Spain

T. Millán and J.I. Cubero

Departamento de Genética, Escuela Tecnica Superior de Ingenieros Agrónomos y de Montes, Apartado 3048, 14080 Córdoba, Spain

Additional index words. Rosa hybrids, random amplified polymorphic DNA markers, cultivar identification, patent protection

Abstract. **Five rose (*Rosa* spp.) cultivars were analyzed using random amplified polymorphic DNA (RAPD) markers. Using eight primers, all cultivars were distinguished by comparing differences in DNA banding patterns. The RAPD technique fingerprints rose cultivars rapidly and inexpensively for identification and patent protection purposes.**

Developing highly reliable and discriminatory methods for identifying cultivars has become increasingly important to plant breeders and those in the nursery industry who need sensitive tools to differentiate among and identify cultivars for plant patent protection. In the past, cultivars were identified primarily based on horticultural, morphological, and physiological descriptions. In most cases, the descriptions and measurements varied considerably due to environmental fluctuation and human judgment. The development of new cultivars that lack distinguishing morphological characteristics has furthered the need for more positive identification methods. As an adjunct to morphological and physiological methods, identification tests based on isozyme patterns have been introduced to fingerprint ornamental cultivars of various species (Kuhns and Fretz, 1978; Messeguer and Arús, 1985; Wendel and Parks, 1983). This technique, however, is not powerful enough to distinguish among phenotypically similar cultivars. Tissue must be sampled at comparable physiological states to obtain uniform and repeatable banding patterns. Moreover, some genetic differences among cultivars may not be visualized by isozyme technique, since the number of loci that can be resolved is limited.

Methods developed over the past 20 years can detect differences in DNA sequence between individuals. Compared to isozyme techniques, analyzing DNA has many advantages: it is independent of environmental conditions; DNA sequence is identical, no matter what plant tissue or tissue stage is analyzed; and the number of scorable loci is unlimited. The most widely used technique is restriction fragment length polymorphism (RFLP) analysis. Base substitutions in a restriction endonuclease site or insertions or deletions between sites result in detectable differences in the fragment length of restriction enzyme-digested DNA. These polymorphisms already have helped identify cultivars of some species (Apuya et al., 1988; Gebhardt et al., 1989), including rose (Hubbard et al., 1992). More recently, the random amplified polymorphic DNA (RAPD) technique (Williams et al., 1990) based on the polymerase chain reaction (PCR) has been used to detect polymorphism in some species (Welsh and McClelland, 1990; Welsh et al., 1991; Williams et al., 1990). The technique involves using a single primer ≈10 bases long to generate fingerprints of DNA segments that amplify in DNA preparations from one parent but not the other. Detected polymorphisms are inherited in a Mendelian fashion and can be produced from any species without any other DNA sequence information (Williams et al., 1990). The RAPD technique has similar uses as RFLP analysis—constructing linkage maps, identifying cultivars, and determining parentage are among the most valuable applications (Welsh et al., 1991). Advantages of the RAPD technique over RFLP analysis include the following: 1) the equipment and supplies necessary are inexpensive relative to those needed for RFLP analysis; 2) speed of analysis is <2 days, since Southern blotting and labeled probes are not necessary; 3) minimal quantities of DNA are required; and 4) a high degree of polymorphism is generated when the RAPD technique is used, a result indicating that selecting lines with diverse genetic backgrounds may be less critical than when using RFLP analysis (Weeden, 1991; Williams et al., 1990).

The objective of the present study was to assess the discriminating power of RAPD markers to identify and protect rose cultivar patents. For this purpose, we surveyed variability patterns produced with eight primers and five rose cultivars. Samples of the following cultivars were provided by Universal Plantas S.A. of Sevilla, Spain: 'Cardinal' (A), 'Sonia' (B), 'Carta Blanca' (C), 'Laser' (D), and 'Carta de Oro' (E). DNA of each cultivar was extracted from ≈100 mg of young, unexpanded or partially expanded leaf tissue, because this tissue produced the highest DNA yield. Leaves were stored at –80C if not used immediately. The extraction procedure was as described by Torres et al. (1993), a method based on (but considerably modified from) that of Lassner et al. (1989). Coprecipitated RNA was eliminated by adding 0.7 units of RNase A. The DNA was dissolved in tris-EDTA, and the final concentration was estimated by agarose gel electrophoresis and ethidium staining, using known concentrations of uncut λ DNA as a standard. To check the repeatability of the fingerprint pattern, more than one plant (in most cultivars) was included in the analysis. Thus, a total of 10 plants was analyzed.

Primers and amplification conditions. Eight 10-base-long arbitrary primers (Operon Technologies, Alameda, Calif.) were used for the experiments (Table 1). DNA amplification conditions were standardized for all primers. Amplification reactions were performed in 25 µl of 20 to 40 ng of plant genomic DNA, buffer [50 mM KCl, 10 mM tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, and 0.001% gelatin], and 100 µM each of dNTP, 0.2 µM primer, and 1 unit Taq DNA polymerase (Promega, Madison, Wis.). The reaction was overlaid with mineral oil. Amplification was performed in a Perkin Elmer (Norwalk, Conn.) Cetus DNA Thermal Cycler programmed for 40 cycles with the following temperature profile: 1 min at 94C, 2 min at 35C, and 2 min at 72C, using the fastest available transitions between each temperature. Cycling ended with a final extension at 72C for 8 min. Amplification products were electrophoresed in 1% agarose, 1% Nu-Sieve (FMC BioProducts, Rockland, Maine) agarose, and 1 × tris-borate–EDTA gels and visualized by ethidium staining. Controls lacking template DNA were included on each primer reaction mix used.

Cultivar comparisons. Identifying cultivars by the RAPD technique is possible because each cultivar yields a reproducible DNA band pattern. Since a limitless number of primers can be assayed and several DNA bands can be differentiated for each one, the number of

Table 1. Sequence of the eight oligonucleotide primers used [primer identification (ID) following OPERON's recommendations].

Primer ID	Sequence (5' to 3')
OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA-04	AATCGGGCTG
OPA-05	AGGGGTCTTG
OPA-06	GGTCCCTGAC
OPA-07	GAAACGGGTG
OPA-08	GTGACGTAGG

Received for publication 5 Aug. 1992. Accepted for publication 28 Dec. 1992. This research has been performed within the framework of the Andalusian Biotechnology Institute (Instituto Andaluz de Biotecnología). The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

BIOTECHNOLOGY

possible combinations is infinite. In the present work, differences among cultivars were obvious and expressed consistently with most primers. The most discriminatory primers were OPA-06 (Fig. 1) and OPA-08 (Fig. 2), which gave clear differences in banding patterns among cultivars. Discriminatory bands are indicated by arrows in the corresponding figures. For example, using OPA-06, 'Sonia' was discriminated from all other cultivars by the simultaneous presence of two intense bands of ≈950 and 1100 bp, while 'Carta Blanca' exhibited the 950-bp band and a band of ≈800 bp (Fig. 1). The remaining cultivars were characterized by 1) a combination of four bands ranging between ≈1250 and 950 bp ('Laser'); 2) three bands of ≈800, 1100, and 1150 bp ('Carta de Oro'); and 3) three bands of 950, 1100, and 1250 bp ('Cardinal') (Fig. 1). OPA-08 gave similar results (Fig. 2). With all primers, the DNA banding pattern for each cultivar was consistent. Occasional differences in the intensity of some nondiscriminatory bands between replicates of the same cultivar (e.g., 'Laser' in Figs. 1–3) probably were due to differences in the quality of DNA isolated from the respective samples. The remaining primers gave less conclusive results. OPA-05 (Fig. 3) clearly distinguished 'Sonia' (B), 'Carta Blanca' (C), 'Laser' (D), and 'Carta de Oro' (E), but 'Cardinal' (A) could not be distinguished from 'Laser'. OPA-04 was the only primer that failed to amplify rose genomic DNA and displayed faint, inconsistent bands; hence, OPA-04 was not considered in the analysis. The rest of the primers (Table 1) consistently identified 'Sonia', 'Carta Blanca', and 'Carta de Oro', but 'Cardinal' and 'Laser' were sometimes ambiguously classified (data not shown).

Although additional work is needed to confirm the results obtained in this initial study, our results indicate that the RAPD technique can characterize rose cultivars. This method offers a rapid and relatively inexpensive way to resolve many highly discriminatory bands, thus increasing the probability of correctly identifying cultivars to protect patent rights.

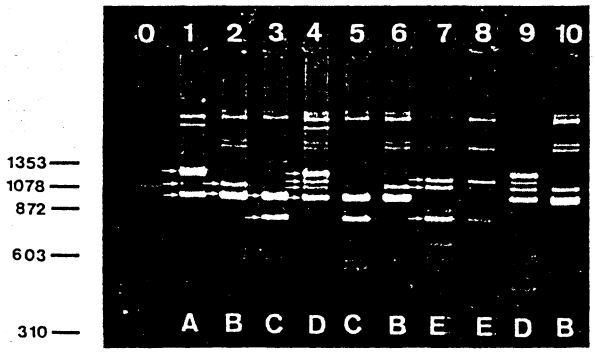


Fig. 1. Amplifying rose cultivar DNA using the OPA-06 primer: (A) 'Cardinal' (lane 1), (B) 'Sonia' (lanes 2, 6, and 10), (C) 'Carta Blanca' (lanes 3 and 5), (D) 'Laser' (lanes 4 and 9), and (E) 'Carta de Oro' (lanes 7 and 8). Lane 0 = control sample lacking template DNA. Discriminatory bands among cultivars are indicated by arrows. Size marker was derived from Øx174/Hind III digest.

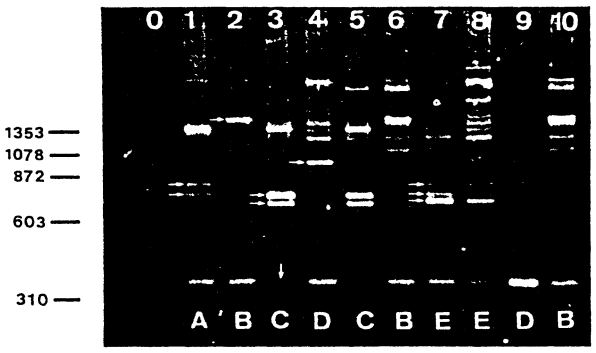


Fig. 2. Amplifying rose cultivar DNA using the OPA-08 primer. Cultivar designations and corresponding lanes are the same as given in Fig. 1. Discriminatory bands among cultivars (or their absence) are indicated by arrows. Size marker was derived from Øx174/Hind III digest.

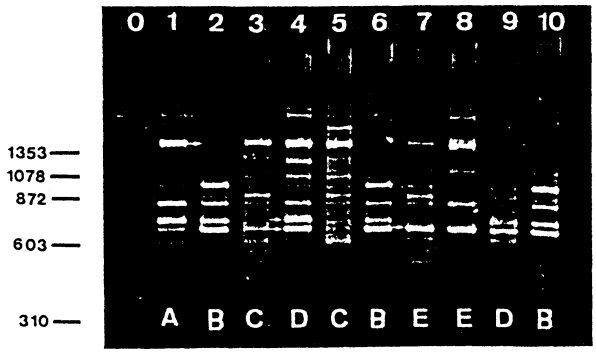


Fig. 3. Amplifying rose cultivar DNA using the OPA-05 primer. Cultivar designations and corresponding lanes are the same as given in Fig. 1. Discriminatory bands among cultivars (or their absence) are indicated by arrows. Size marker was derived from Øx174/Hind III digest.

Literature Cited

Apuya, R., B. Frazier, P. Keim, E.J. Roth, and K. Lark. 1988. Restriction fragment length polymorphisms as genetic markers in soybean, *Glycine max* (L.) Merril. Theoretical Applied Genet. 75:889–901.

Gebhardt, C., C. Glomendahl, U. Schachtschabel, T. Debener, F. Salamini, and E. Ritter. 1989. Identification of 2n breeding lines and 4n varieties of potato (*Solanum tuberosum* ssp. *tuberosum*) with RFLP-fingerprints. Theoretical Applied Genet. 78:16–22.

Hubbard, M., J. Kelly, S. Rajapakse, A. Abbott, and R. Ballard. 1992. Restriction fragment length polymorphisms in rose and their use for cultivar identification. HortScience 27:172–173.

Kuhns, L.J. and A.T. Fretz. 1978. Distinguishing rose cultivars by polyacrylamide gel electrophoresis. II. Isoenzyme variation among cultivars. J. Amer. Soc. Hort. Sci. 103:509–516.

Lassner, M.W., P. Peterson, and J.I. Yoder. 1988. Simultaneous amplification of multiple DNA fragments by polymerase chain reaction in the analysis of transgenic plants and their progeny. Plant Mol. Biol. Rptr. 7:116–128.

Messeguer, R. and P. Arús. 1985. Electrophoretic identification of carnation cultivars. HortScience 20:372–373.

Torres, A.M., N.F. Weeden, and A. Martín. 1993. Linkage between isozymes, RFLP and RAPD markers in *Vicia faba*. Theoretical Applied Genet. 85:937–945.

Weeden N.F. 1991. Chromosomal organization and gene mapping, p. 23–49. In: D. Murray (ed.). Advanced methods in plant breeding and biotechnology. CAB Intl., Wallingford, U.K.

Welsh, J., R.J. Honeycutt, M. McClelland, and B.W.S. Sobral. 1991. Parentage determination in maize hybrids using the arbitrarily primed polymerase chain reaction (AP-PCR). Theoretical Applied Genet. 82:473–476.

Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Res. 18:7213–7218.

Wendel, J.F. and C.R. Parks. 1983. Cultivar identification in *Camellia japonica* L. using allozyme polymorphisms. J. Amer. Soc. Hort. Sci. 108:290–295.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphism amplified by arbitrary primers as useful as genetic markers. Nucleic Acid Res. 18:6531–6535.

[Annex VIII follows]

Heredity 71 (1993) 335–341

Received 5 November 1992

Rapid detection of genetic variability in chrysanthemum (*Dendranthema grandiflora* Tzvelev) using random primers

KIRSTEN WOLFF*† & JENNY PETERS-VAN RIJN†

**University of Leiden, PO Box 9516, 2300 RA Leiden; and † TNO Nutrition and Food Research, PO Box 360, 3700 AJ Zeist, The Netherlands*

Genetic variation in chrysanthemum (*Dendranthema grandiflora*) was studied using a recently developed technique generating Random Amplified Polymorphic DNAs (RAPDs). It appeared that variation between cultivars was high and that the cultivars used could be distinguished from each other by using only two different primers. A family of cultivars, derived from one original cultivar by vegetative propagation, had identical fragment patterns. Because of the high level of polymorphism and clonal stability RAPD fragments are useful for cultivar identification. Genetic variability among related *Dendranthema* species was too high to study genetic distances either among cultivars within chrysanthemum or among species related to chrysanthemum.

Keywords: chrysanthemum, cultivar identification, genetic variability, PCR, RAPDs.

Introduction

Dendranthema grandiflora Tzvelev (*Chrysanthemum morifolium* Ramat.) is a polyploid belonging to a hexaploid species complex comprising six or seven species (Dowrick, 1953). On average 54 chromosomes are present, although counts from 36 to 75 are possible (Langton, 1989). Regular bivalents are formed during meiosis suggesting an allopolyploid origin (Dowrick, 1953; Watanabe, 1977). It is not known whether most characters are inherited in a disomic or a hexasomic way. Bivalent formation suggests a disomic transmission. Presence of carotenoid pigmentation, however, appeared to be transmitted in a hexasomic way (Langton, 1989). Chrysanthemum has a strong self-incompatibility system, thus causing many crosses between related or unrelated individuals to be unsuccessful. Usually only between 5 and 50 per cent of crosses between sibs in an F_1 are compatible (Drewlow *et al.*, 1973; Ronald & Ascher, 1975; Zagorski *et al.*, 1983; Stephens *et al.*, 1984). The genetics of the sporophytic self-incompatibility system is not completely resolved but probably several loci are involved (more than two) and there is dominance of alleles (Zagorski *et al.*, 1983; Stephens *et al.*, 1984). Polyploidy, unknown origin of the species and the self-incompatibility system caused many genetic analyses to be unsuccessful in chrysanthemum and, therefore, little is known about the genetics of the species.

Commercially chrysanthemum is mainly asexually propagated by terminal, vegetative cuttings (Dowrick & El-Bayoumi, 1966; Teynor *et al.*, 1989). Propagation via seeds is usually used only to develop new cultivars since biparental crosses give an extremely variable F_1 differing in many morphological characters (Dowrick & El-Bayoumi, 1966).

In this study we examine genetic variability in the species *Dendranthema grandiflora* and among related species. We expect the variability among cultivars within chrysanthemum to be large considering the outcrossing nature of the mating system (Brown, 1979; Loveless & Hamrick, 1984; Wolff, 1991). We further expect that many loci are in a heterozygous state and these loci may, therefore, show segregation in the offspring of a biparental cross. Our future goal is to use molecular markers for cultivar identification and in mapping studies. The present study is the first one exploring molecular variability in chrysanthemum.

During recent decades several techniques have been introduced that detect molecular variability within and among several species. Three of these widely applied techniques are the use of restriction fragment length polymorphisms (RFLPs) (e.g. Helentjaris *et al.*, 1986),

Correspondence: K. Wolff, TNO, PO Box 360, 3700 AJ Zeist, The Netherlands.

DNA fingerprinting (Jeffreys *et al.*, 1985) and specific amplification of polymorphic DNA fragments with PCR (Weining & Langridge, 1991). Prior knowledge of the DNA composition of the species and/or the presence of useful probes is, however, required. Recently a new and rapid technique to detect polymorphisms was developed using the polymerase chain reaction (PCR) and random primers to amplify DNA (Welsh & McClelland, 1990; Williams *et al.*, 1990). The technique is named RAPD after Random Amplified Polymorphic DNAs (Williams *et al.*, 1990). The DNA fragment patterns generated can discriminate between individuals, cultivars or species (Arnold *et al.*, 1991; Caetano-Anolles *et al.*, 1991; Hu & Quiros, 1991; Quiros *et al.*, 1991; Hadrys *et al.*, 1992). In some cases the transmission of polymorphic fragments and the localization of the amplified DNA fragment on the genome was determined (Williams *et al.*, 1990; Martin *et al.*, 1991). RAPDs have already been used successfully in mapping studies in *Arabidopsis* (Reiter *et al.*, 1992) and in tomato (Aarts *et al.*, 1991; Martin *et al.*, 1991; Klein-Lankhorst *et al.*, 1991). Michelmore *et al.* (1991) showed that the RAPD markers are extremely useful in a bulked segregant analysis to detect disease-resistance genes in lettuce.

The newly developed technique of RAPD seems suitable for studying genetic variability in chrysanthemum and related species as species-specific probes for studying RFLPs are not present at this moment. In a preliminary study we observed that the polymorphic RAPD markers have a genetic basis: all fragments present in the offspring of a biparental cross were clearly transmitted from parents to offspring (Wolff, unpublished results). Only one non-parental band was seen in one of the offspring out of 43 polymorphic fragments scored using nine primers. Variability within *Dendranthema grandiflora* will be studied by looking at genetic variability among cultivars, and at the same time determine whether RAPDs are useful for identifying cultivars. Furthermore, we can test the stability of fragment patterns in mutant members of a clonal family of cultivars that differ in specific morphological and physiological characters. Genetic variability among related chrysanthemum species will be analysed by studying several species from this polyploid species complex.

Materials and methods

Plant material and experimental setup

The plant material was kindly provided by three Dutch chrysanthemum breeding companies as leaf material, freshly cut from adult plants. In chrysanthemum breeding it is often the case that from a successful cultivar other cultivars are obtained by (somatic) mutation with, amongst others, different flower colour. These new cultivars are made by irradiating the successful original cultivar with gamma-rays or by cloning the original cultivar into thousands of clonal derivatives and looking for a spontaneous somatic mutation. In this study we used one specific family, the family derived from CH36. The original cultivar is pink flowered and by now at least 14 different coloured cultivars have been obtained by spontaneous mutation and four derived by irradiation. To study clonal stability 13 members of this family and 27 primers (numbers 1 to 26 and 29) were used.

For cultivar variability 18 different cultivars from three breeders and eight primers (numbers 1, 13, 14, 15, 18, 21, 22 and 23) were used. Designation of the cultivars used and their origin is given with Fig. 1.

Variability among species was studied using six primers (numbers 3, 4, 15, 21, 22 and 23) and 15 individuals, representing 13 different species. Names of the species and their ploidy level, as far as information is available from the literature, are given with Fig. 3.

DNA extraction

Chrysanthemum leaves were ground in liquid nitrogen and freeze dried under vacuum. The dry powder was kept at −20°C. DNA was extracted from the dry powder according to Saghai-Maroof *et al.* (1984) with the modifications of a double chloroform/isoamyl-alcohol (24:1) treatment, the use of cold absolute ethanol to precipitate the DNA, two washes in 76 per cent ethanol/0.2 M NaAc prior to the wash in 76 per cent ethanol/10 mM NH_4OAc and the dissolving of the DNA in 10 mM Tris/1 mM EDTA (*p*H 8.0). Yields were usually 70 μg DNA per 300 mg of powder per extraction.

Primer synthesis

Oligodeoxynucleotide primers were synthesized using a DNA synthesizer 381A from Applied Biosystems. After deprotection of the oligos, two extractions with phenol/chloroform (1:1) and two extractions with chloroform were done. The primers were numbered according to the order of synthesis (Table 1); numbers one to eleven are identical to the primers published by Williams *et al.* (1990).

Amplification reaction conditions

The use of RAPDs was developed by Williams *et al.* (1990). The DNA was amplified under conditions similar to normal PCR, with the exception that only a

Table 1 Nucleotide sequences of the primers used for generating RAPDs

1	5′-TGG TCA CTG A-3′
2	5′-AGG TCA CTG A-3′
3	5′-TCG TCA CTG A-3′
4	5′-TGC TCA CTG A-3′
5	5′-TGG ACA CTG A-3′
6	5′-TGG TGA CTG A-3′
7	5′-TGG TCT CTG A-3′
8	5′-TGG TCA GTG A-3′
9	5′-TGG TCA CAG A-3′
10	5′-TGG TCA CTC A-3′
11	5′-TGG TCA CTG T-3′
12	5′-CGG TCA CTG T-3′
13	5′-CGG CCA CTG T-3′
14	5′-CGG CCC CTG T-3′
15	5′-CGG CCC CGG T-3′
16	5′-TGG TGA GTG T-3′
17	5′-TCA CGA TGC A-3′
18	5′-GCA AGT AGC T-3′
19	5′-GGA ATA AGC G-3′
20	5′-AGG AGA ACG G-3′
21	5′-GCT CGT CGC T-3′
22	5′-GCA TGT CGT G-3′
23	5′-GCC TGT CGA T-3′
24	5′-GCG TGA CTT G-3′
25	5′-TGG TCC TGC G-3′
26	5′-TGC TGG GCG G-3′
29	5′-GGG CGG GGC G-3′

single primer was used and that the nucleotide order of the primer was random, with the only requirement that the G + C content be at least 50 per cent (Williams *et al.*, 1990). In a previous experiment the reaction conditions were optimized (Wolff *et al.*, 1993). Reactions were performed in a volume of 50 μl containing 20 mM Tris-HCl, *p*H 8.3, 50 mM KCl, 3mM $MgCl_2$, 0.001 per cent gelatin, 100 μM each of dATP, dCTP, cGTP and dTTP, 0.2 μM primer, 25 ng of chrysanthemum genomic DNA, and 1 U of *Taq* polymerase (Amplitaq, Perkin Elmer Cetus) using a Perkin Elmer 9600 thermal cycler. After 5 min heating at 94°C 45 cycles were run. Each cycle consisted of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. Ramp time between annealing temperature and 72°C was set at 0.3–0.4°C s^{-1} (a total of 2 min). A ramp time shorter than 1.5 min led to low or no DNA amplification. In a preliminary experiment the influence of DNA and polymerase concentration was tested with three primers and two different DNA templates.

Amplified DNA fragments were separated by electrophoresis in a 1.4 per cent agarose gel with a TBE buffer system (Sambrook *et al.*, 1989). The total reaction volume was loaded on the gel. Gels were stained with ethidium bromide and fragment patterns were photographed for further analysis.

Observations

Different fragments produced with each primer were numbered sequentially and presence or absence of fragments in each individual was scored. Individuals from the same row on one gel were compared with each other. Fragments with a medium or strong signal were taken into account as these fragments are fully reproducible. Fragments with the same mobility on the gel but with different intensities were not distinguished from each other when cultivars or species were compared with each other.

Variability

Variability among cultivars and among species was expressed as the similarity *S*. This is calculated as:

$$S = \frac{2 \times N_{AB}}{N_A + N_B},$$

in which N_{AB} is the number of bands shared by individuals A and B, and N_A and N_B are the number of bands in individuals A and B, respectively. The similarity measure can also be called band sharing. Distance can be calculated as $D = 1 - S$ (Swofford & Olsen, 1990). The chance of finding two individuals with the same fragment pattern can be calculated as the mean similarity ($\overline{S}$) to the power of the mean number of bands ($\overline{N}$) (Nybom & Hall, 1991).

Results

The Dutch cultivars showed strongly differing fragment patterns (Fig. 1). On average, band sharing was 0.66 (range 0.49–0.77). Only a few primers (1–3), depending on the number of polymorphic fragments generated by the primers chosen, were needed to distinguish all cultivars in this study. Each primer gave on average seven bands per individual. This, together with a mean similarity of 0.66, gives a chance of finding two identical patterns in two individuals of 5×10^{-2} for one primer, 3×10^{-3} when using two primers and 1.6×10^{-4} when using three primers. Similarity among cultivars was calculated for within breeding company and a between company comparison (only the two largest groups, from Fides and from CBA were compared). Cultivars obtained from CBA were slightly more similar (*t*-test; $P = 0.028$) to each other ($S = 0.71$) than they were to cultivars from Fides ($S = 0.66$). Cultivars from Fides were as similar to each other

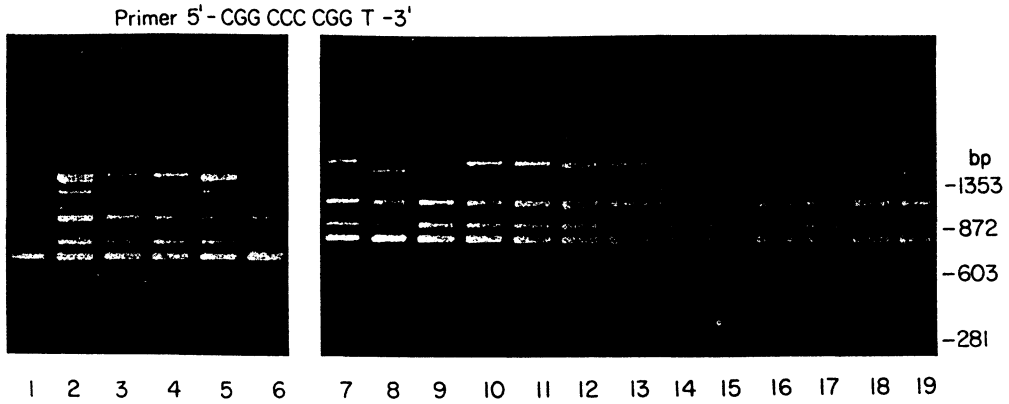


Fig. 1 RAPD fragment pattern of several Dutch chrysanthemum cultivars. From lane 1 to lane 18 cultivars with the following numbers are used: CH1, CH2, CH3, CH4, CH5, CH6, CH7, CH8, CH9, CH10 (all from Fides), CH11, CH12, CH14, CH17 (from CBA), CH53, CH54 (from Fides), CH55, CH57 (from Hoek Breeding).

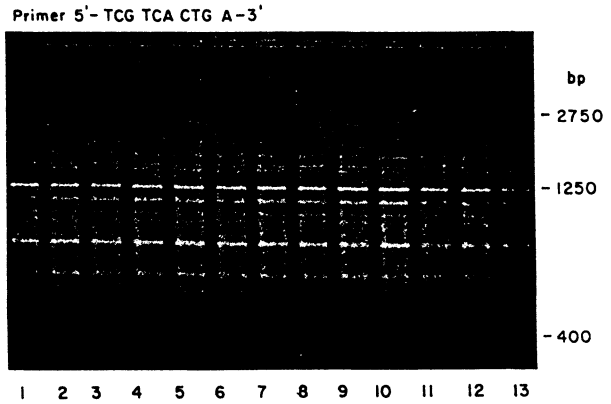


Fig. 2 RAPD fragment of a family of cultivars derived from number CH36.

($S=0.66$) as to cultivars from CBA ($S=0.66$). The cultivars belonging to one family, all derived from CH36, were extremely similar (Fig. 2). Only one primer (number 19) out of the 27 tested gave slightly different patterns for these 13 mutant cultivars. In this case there was marginal variation for the fainter bands only.

The different *Dendranthema* species were studied with six primers. Genetic variability among the species was high (Fig. 3). Some primers yielded extremely different banding patterns in each species; many bands were present in only one of the species. Mean similarity among species was 0.49 (range 0.22–0.77). This is significantly lower than similarity among chrysanthemum cultivars ($P<0.001$, *t*-test). Two of the species were represented by two individuals from a different origin. In both cases the two individuals of one species were as different from each other as from individuals from other species. Therefore, the high variability within chrysanthemums seems to be present within other related species as well. The ploidy level of some of the species is known from the literature (see Fig. 3 and Kawata, 1978). There was no relationship between the ploidy level and the number of RAPD fragments generated (*c.f.* Fig. 3: 4 × species in lanes 1 and 5 and 10 × species in lanes 2 and 3).

Discussion

Genetic variability in chrysanthemum

The chrysanthemum varieties show a high level of genetic variability as was expected from a plant possessing a mating system of strict outcrossing. Apparently the regular introduction of material from Japan and other countries, which is considered a good breeding practice, has resulted in the maintenance of high levels of genetic variability in the gene pool. Levels of variability for RAPD in this species are comparable to some other species studied (Carlson *et al.*, 1991; Hu & Quiros, 1991; Reiter *et al.*, 1992; Van Heusden & Bachmann, 1992; Welsh *et al.*, 1991). Some of the wild and agricultural species studied show far lower levels of variability, probably because of their mating system and the breeding strategy (Arnold *et al.*, 1991; Halward *et al.*, 1992).

Cultivar identification and cultivar relatedness are important issues for horticultural breeders. The application of RAPDs seems very valuable in this regard. If primers are chosen that are known to give highly polymorphic banding patterns in chrysanthemum, only a few primers are needed to distinguish cultivars, as long as these cultivars are not mutants of each other. Therefore, cultivars derived vegetatively from one original cultivar cannot be distinguished from each other using

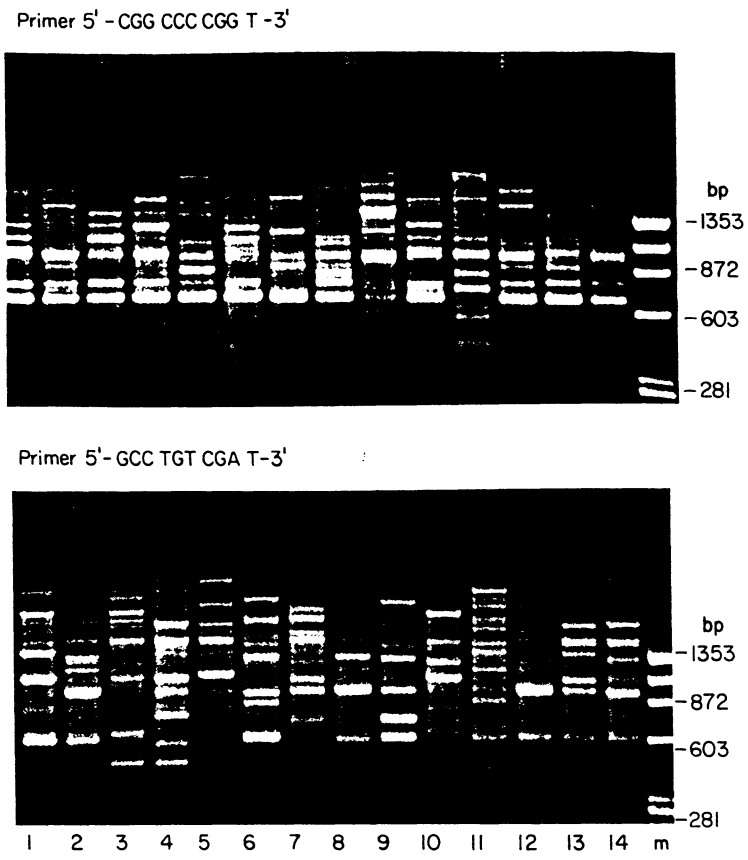


Fig. 3 RAPD fragment pattern of related *Chrysanthemum* and *Dendranthema* species. The species' names and ploidy level, if known, are from lane 1 to 14: 1. *D. yoshinaganthum* Makinoi ex Kitamura (4 ×) (CPRO) 2. *D. arcticum* L. Tzvelev (10 ×) (8 × Dowrick, 1952) (CPRO) 3. *D. pacificum* (Nakai) Kitamura (10 ×) (CPRO) 4. *D. shiwogiku* (Kitamura) Kitamura (8 ×, 10 ×) (CPRO) 5. *D. indicum* L. Des Moul (4 ×, 6 ×) (CPRO) 6. *D. zawadskii* (Herb.) Tzvelev (8 ×, Krogulevich, 1978) (CPRO) 7. *C. nankingense* Handel-Mazetti (Sharman) 8. *C. zawadskii* Herbich ssp. latilobum (Maxim.) Kitagawa 'Pink Procession' (4 ×) (4 ×, 6 × Lee, 1967, 1975) (USDA) 9. *C. yezoense* T. Maek. (10 ×, Dowrick, 1952) 10. *D. pacificum* (Nakai) Kitamura (10 ×) (Sharman) 11. *D. weyrichii* (Maxim.) Tzvelev (8 ×) (6 × Shimotomai, 1932) (CPRO) 12. *D. rubellum* Sealy 'Clare Curtis' (2n = 63 Dowrick, 1952) (Sharman) 13. *C. wakasaense* Shimot. ex Kitamura (4 ×, Dowrick, 1952) (CPRO) 14. *D. grandiflora* Tzvelev. (6 × Dowrick, 1952). Chromosome counts are from Kawata (1978), except if noted otherwise.

this technique. The members of these families originate from each other by mutations. It is not known whether these are point mutations or inversions, deletions or even loss of chromosomes. Observations by Dowrick & El-Bayoumi (1966) that morphological mutations often coincide with a difference in chromosome number, seem not to be confirmed by our observation as no differences in RAPD fragment pattern among somaclonal cultivars are observed in this study. The mutations causing the morphological differences may be localized on a small part of the genome.

Similarity (band sharing) can be used as a measure of relatedness (Nybom & Hall, 1991; Welsh *et al.*, 1991). Hu & Quiros (1991) studied genetic variability in *Brassica oleracea*, and found higher similarities among cultivars from one company than between different company. We found that for one company the cultivars may be slightly more related to each other than to cultivars from another companies. The number of cultivars in this study is, however, too low for definitive conclusions. More cultivars are needed to be conclusive about relatedness within and among these groups.

Genetic diversity among Dendranthema and Chrysanthemum species

Genetic variation among *Dendranthema* and *Chrysanthemum* species, related to the cultivated chrysanthemum, is extremely high, with a very low similarity among species. Besides that, genetic variation within species is also very high. Therefore, RAPDs do not seem useful for determining distances between these species. The usefulness of RAPDs in phylogenetic studies may be different in species that have a low genetic variability within species and where the species are more closely related. In Louisiana irises variation within species was low and natural hybrids among the species were identified because they possessed fragments of both species (Arnold *et al.*, 1991). In *Arachis* species dendrograms were generated using RAPD fragments as characters (Halward *et al.*, 1992). These dendrograms showed relationships among *Arachis* species that were in concordance with species relationships derived from allozymes, RFLPs and morphological characters. Although RAPDs may be useful in studying genetic relationships of closely related species the general use of RAPD fragment patterns for taxo-

nomic purposes is debatable. One should at least check whether fragments are identical by either sequencing or, probably easier, by using the fragments as a probe. In general, other methods, like sequencing or cpDNA RFLP analysis, are preferred in taxonomic studies (see e.g. Dowling *et al.*, 1990). In a forthcoming study we will determine copy number of fragments and test identity to similar fragments from different individuals.

The absence of a correlation between ploidy level and numbers of fragments generated can easily be explained by the fact that PCR reactions are largely based on competition processes among molecules and not solely on the presence or absence of some sequences.

The high levels of variability of RAPD fragments in chrysanthemum renders these RAPD fragments good candidates for cultivar identification and genetic analysis. For specific segregation analyses large numbers of offspring and offspring from several types of crosses are needed; this will be pursued in a subsequent study. The preliminary data on the transmission of polymorphic RAPD fragments seems to point at a diploid-like inheritance; 25 out of 43 polymorphic fragments, present in only one of the parents, segregated in a 1:1 (presence:absence) fashion (Wolff, data unpublished). These fragments are extremely valuable in studies in which markers are used to localize QTL. Furthermore, these polymorphic markers are analogous to single-dose restriction fragments (SDRFs) as described by Wu *et al.* (1992). They describe how SDRFs are extremely useful in mapping studies in polyploids and how they can be used to distinguish allopolyploidy from autopolyploidy. We have to explore the problems that the hexaploidy of the organism and the dominance of the fragments may cause in future research.

Acknowledgements

This is publication number 5 of the PBTS-project 'Rapid biotechnological detection of resistance in chrysanthemum against insect pests'. The project is a joint activity of the University of Leiden (Population Biology), TNO-Zeist (Nutrition and Food Research) and the chrysanthemum breeders CBA, Fides Research and Breeding and Hoek Breeding. It is subsidized by Stipt (Ministry of Economic Affairs). Several *Dendranthema* species were a kind gift of Jan de Jong, CPRO, Wageningen; Joe L. Sharman, Monksilver Nursery, Cottenham, England; and the North Central Regional Plant Introduction Station, USDA-ARS, Ames, Iowa for which we are highly grateful. We thank Robert Campbell, Annelies Hofman, Albert Kamping and Ed van der Meyden and an anonymous reviewer for helpful comments on an earlier version of the manuscript.

References

AARTS, J. M. M. J. G., HONTELEZ, J. G. J., FISCHER, P., VERKERK, V., VAN KAMMEN, A. AND ZABEL, P. 1991. Acid phosphatase-1, a tightly linked molecular marker for root-knot nematode resistance in tomato: from protein to gene, using PCR and degenerate primers containing deoxyinosine. *Plant Mol. Biol.*, **16**, 657–661.

ARNOLD, M. L., BUCKNER, C. M. AND ROBINSON, J. J. 1991. Pollen-mediated introgression and hybrid speciation in Louisiana irises. *Proc. Natl. Acad. Sci., U.S.A.*, **88**, 1398–1402.

BROWN, A. H. D. 1979. Enzyme polymorphism in plant populations. *Theor. Pop. Biol.*, **15**, 1–42.

CAETONA-ANOLLES, G., BASSAM, B. J. AND GRESSHOFF, P. M. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Bio. Technology*, **9**, 553–557.

CARLSON, J. E., TULSIERAN, J. K., GLAUBITZ, J. C., LUK, V. W. K., KAUFFELDT, C. AND RUTLEDGE, R. 1991. Segregation of random amplified DNA markers in F1 progeny of conifers. *Theor. Appl. Genet.*, **83**, 194–200.

DOWLING, T. E., MORITZ, C. AND PALMER, J. D. 1990. Nucleic acids II: restriction site analysis. In: Hillis, D. M. and Moritz, C. (eds) *Molecular Systematics*, Sinauer Ass., pp. 250–317.

DOWRICK, G. J. 1952. The chromosomes of *Chrysanthemum*, I: the species. *Heredity*, **6**, 365–375.

DOWRICK, G. J. 1953. The chromosomes of *Chrysanthemum*, II: garden varieties. *Heredity*, **7**, 59–72.

DOWRICK, G. J. AND EL-BAYOUMI, A. 1966. The origin of new forms of the garden *Chrysanthemum*. *Euphytica*, **15**, 32–38.

DREWLOW, L. W., ASCHER, P. D. AND WIDMER, R. E. 1973. Genetic studies of self incompatibility in the garden chrysanthemum, *Chrysanthemum morifolium* Ramat. *Theor. Appl. Genet.*, **43**, 1–5.

HADRYS, H., BALICK, M. AND SCHIERWATER, B. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molec. Ecol.*, **1**, 55–63.

HALWARD, T., STALKER, T., LARUE, E. AND KOCHERT, G. 1992. Use of single-primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea* L.). *Plant Mol. Biol.*, **18**, 315–325.

HELENTJARIS, T., SLOCUM, M., SCHAEFER, A. AND NIENHUIS, J. 1986. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. *Theor. Appl. Genet.*, **72**, 761–769.

HU, J. AND QUIROS, C. F. 1991. Identification of broccoli and cauliflower cultivars with RAPD Markers. *Plant Cell. Rep.*, **10**, 505–511.

JEFFREYS, A. J., WILSON, V. AND THEIN, S. L. 1985. Hypervariable 'minisatellite' regions in human DNA. *Nature*, **314**, 67–73.

KAWATA, J. 1978. Japanese cultivars and wild species; their useful characteristics for chrysanthemum breeding. In:

Langton, F. A. (ed.) *Eucarpia Meeting on Ornamentals*, Littlehampton, UK, pp. 33–48.

KLEIN-LANKHORST, R. M., VERMUNT, A., WEIDE, R., LIHARSKA, T. AND ZABEL, P. 1991. Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). *Theor. Appl. Genet.*, **83**, 108–114.

KROGULEVICH. 1978. In: Malyshev, L. I. and Peshlcova, G. A. (eds) *Flora of the Prebaikal*, Novosibirsk, pp. 19–48.

LANGTON, F. A. 1989. Inheritance in *Chrysanthemum morifolium* Ramat. *Heredity*, **62**, 419–423.

LEE, Y. N. 1967. Chromosome numbers of flowering plants in Korea. *J. Kor. Cult. Res. Inst.*, **11**, 455–478.

LEE, Y. N. 1975. Taxonomic study on white flowered wild chrysanthemum in *Asia. J. Korean Res. Inst. Better Living*, **14**, 63–79.

LOVELESS, M. D. AND HAMRICK, J. L. 1984. Ecological determinants of genetic structure in plant populations. *Ann. Rev. Evol. Syst.*, **15**, 65–95.

MARTIN, G. B., WILLIAMS, J. G. K. AND TANKSLEY, S. D. 1991. Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. *Proc. Natl. Acad. Sci., U.S.A.*, **88**, 2336–2340.

MICHELMORE, R. W., PARAN, I. AND KESSELI, R. V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci., U.S.A.*, **88**, 9828–9832.

NYBOM, H. AND HALL, H. K. 1991. Minisatellite DNA 'fingerprints' can distinguish *Rubus* cultivars and estimate their degree of relatedness. *Euphytica*, **53**, 107–114.

QUIROS, C. F., HU, J., THIS, P., CHEVRE, A. M. AND DELSENY, M. 1991. Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica*. *Theor. Appl. Genet.*, **82**, 627–632.

REITER, R. S., WILLIAMS, J. G. K., FELDMANN, K. A., RAFALSKI, J. A., TINGEY, S. V. AND SCOLNIK, P. A. 1992. Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. *Proc. Natl. Acad. Sci., U.S.A.*, **89**, 1477–1481.

RONALD, W. G. AND ASCHER, P. D. 1975. Self compatibility in garden *Chrysanthemum*: occurrence, inheritance and breeding potential. *Theor. Appl. Genet.*, **46**, 45–54.

SAGHAI-MAROOF, M. A., SOLIMAN, K. M., JORGENSEN, R. A. AND ALLARD, R. W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci., U.S.A.*, **81**, 8014–8018.

SAMBROOK, J., FRITSCH, E. F. AND MANIATIS, T. 1989. *Molecular Cloning: a Laboratory Manual*, 2nd edition. Cold Spring Harbour Laboratory Press, New York.

SHIMOTOMAI, N. 1932. Eigenartige Vermehrung der chromosomenzahl bei den Artenbastarden von Chrysanthemum. *Bot. Mag. (Tokyo)*, **46**, 789–799.

STEPHENS, L. C., ASCHER, P. D. AND WIDMER, R. E. 1984. Interaction among sporophytic *S* loci in self-incompatible garden chrysanthemums. *Euphytica*, **33**, 623–631.

SWOFFORD, D. L. AND OLSEN, G. J. 1990. Phylogeny reconstruction. In: Hillis, D. M. and Moritz, C. (eds) *Molecular Systematics*, Sinauer Ass. pp. 411–501.

TEYNOR, T. M., ASCHER, P. D., WIDMER, R. E. AND LUBY, J. J. 1989. Inheritance of flower color in *Dendranthema grandiflora* Tzvelev. (*Chrysanthemum morifolium* Ramat.) using cultivars and inbreds II. Vacuole pigmentation. *Euphytica*, **42**, 297–305.

VAN HEUSDEN, A. W. AND BACHMANN, K. 1992. Genotype relationships in *Microseris elegans* (Asteraceae, Lactuceae) revealed by DNA amplification from arbitrary primers (RAPDs). *Pl. Syst. Evol.*, **179**, 221–233.

WATANABE, K. 1977. The control of diploid-like meiosis in polyploid taxa of *Chrysanthemum* (Compositae). *Jap. J. Genetics*, **52**, 125–131.

WEINING, S. AND LANGRIDGE, P. 1991. Identification and mapping of polymorphisms in cereals based on the polymerase chain reaction. *Theor. Appl. Genet.*, **82**, 209–216.

WELSH, J. AND MCCLELLAND, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Acids. Res.*, **18**, 7213–7218.

WELSH, J., HONEYCUTT, R. J., MCCLELLAND, M. AND SOBRAL, B. W. S. 1991. Parentage determination in maize hybrids using the arbitrarily primed polymerase chain reaction (AP-PCR). *Theor. Appl. Genet.*, **82**, 473–476.

WILLIAMS, J. G. K., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A. AND TINGEY, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids. Res.*, **18**, 6531–6535.

WOLFF, K. 1991. Analysis of allozyme variability in three *Plantago* species and a comparison to morphological variability. *Theor. Appl. Genet.*, **81**, 119–126.

WOLFF, K., SCHOEN, E. D. AND PETERS-VAN RIJN, J. 1993. Optimizing the generation of Random Amplified Polymorphic DNAs in chrysanthemum (*Dendranthema grandiflora* Tzvelev.). *Theor. Appl. Genet.*, in press.

WU, K. K., BURNQUIST, W., SORRELLS, M. E., TEW, T. L., MOORE, P. H. AND TANKSLEY, S. D. 1992. The detection and estimation of linkage in polyploids using single-dose restriction fragments. *Theor. Appl. Genet.*, **83**, 294–300.

ZAGORSKI, J. S., ASCHER, P. D. AND WIDMER, R. E. 1983. Multigenic self incompatibility in hexaploid *Chrysanthemum*. *Euphytica*, **32**, 1–7.

[Annex IX follows]

ANNEX IX

CROPS PROPAGATED BOTH VEGETATIVELY AND GENERATIVELY

Trachelium

As Trachelium is a strict self pollinator there are no differences in the rules for the homogeneity with respect to clonal propagated varieties.

Bromeliaceae

Most bromeliads are cross pollinators but self pollination can be found too.
Where possible breeders choose for seed propagation because the costs of that kind of production are lower compared to tissue or cutting production.

In practice the more expensive varieties come from tissue culture and are protected whereas the cheaper ones from seed are seldom protected.

Since the beginning of the protection for Bromeliaceae in NL in 1980 seed propagated varieties have become more homogeneous in general.

In most cases the varieties find their origin in crossings between partially inbred parent lines, one inbred line x one parent plant or sib-like crossings.

In the above mentioned cases a relatively small heterogeneity can be achieved as has been observed recently.

As the methods of breeding are different in Bromeliad species - even within one species - no fixed rules for the establishment of the homogeneity and the description can be given. As a consequence most applications have to be considered seperately as far as the rules are concerned.

Two important points have to be kept in mind:

- Every cloned seedling selected out of and distinct from the protected "parent" population can be granted with rights in principle.
- What is the position of such seedlings in view of the principle of essential derivation?

Wageningen, 15-09-1994

[Annex X follows]

Photography for Plant Breeders Rights

(Rules laid down by the Australian Plant Breeders' Rights Office)

Aims

The photograph is to:

- place the characteristics of the variety on the record;
- show how it is distinct from the most similar variety/ies of common knowledge; and
- assist with identification in the event of infringement of the rights.

Requirements

Part 1:

- A single copy of a colour photograph or photographs showing the distinguishing characteristics of the new variety.

Part 2:

- A single colour transparency, in portrait format, showing the distinguishing characteristics of the new variety
  - the transparency is for publication in *Plant Varieties Journal*

and

- eight (8) colour prints of the same subject
  - these are entered in the Register of Plant Breeders Rights in each capital city and should be between 15cm x 10cm and A4 size.

After exposing the transparency film, change to print film and expose several frames of the same subject. Colour prints must be identical and should be made from a negative. The process of producing prints from transparencies should be avoided as it may compromise colours and the sharpness of the image.

Composition

- Consider arranging the parts of the plants on a flat surface as an alternative to a single view of entire plants
  - this technique is used by experienced breeders to illustrate more than one view of, for example, a flower.

- Use as much of the space in the frame as possible.

- Acceptable variability within the variety can be demonstrated by including more than one specimen in the frame.

- Photographs must be comparative (that is, they must include the most similar variety/ies in addition to the new one)

  - one exception currently allowed applies to rose varieties, which have a large number of characteristics which allows only the new variety to be represented in the photograph.

- Labels in the photograph, indicating the name of each variety, assist by positively identifying the plant material. Labels should:

  - be produced using a personal computer and laser printer or 'Letraset' stencils;

  - give the variety names in upper and lower case letters in single quotation marks, preferably below the specimen; and

  - be in Times Roman type and able to be read when the photograph is reproduced in the Journal.

- Include a cm or mm ruler in the frame as a reference.

Composite Photographs

- To capture more characteristics than would be possible in a single photograph, take photographs at two stages in the life cycle and combine the images in a composite photograph.

Background

- Use an appropriately-coloured plain background. Mid or dark grey or mid-blue often give the most pleasing results.

- A black background may require the use of exposure compensation.

- Patterned background materials are distracting to the viewer and should be avoided.