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TWF/22/4

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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

**TECHNICAL WORKING PARTY
FOR
FRUIT CROPS****Twenty - second Session
Bordeaux, France, June 11 to 14, 1991**

REPORT

adopted by the Technical Working Party for Fruit CropsOpening of the Session

1. The twenty-second session of the Technical Working Party for Fruit Crops (hereinafter referred to as "the Working Party") was held in Bordeaux, France, from June 11 to 14, 1991. The list of participants is given in Annex I to this report.

2. Mr. J.M. Bove, President of the Cellular and Molecular Biology Station, and Mrs. F. Dosba, Director of the INRA Fruit Research Station at the Domaine de la Grande Ferrade at Villenave d'Ornon near Bordeaux, welcomed the participants to their Research Station. The session was opened by Dr. B. Spellerberg (Germany), Chairman of the Working Party.

Adoption of the Agenda

3. The Working Party adopted the agenda of its twenty-second session which is reproduced in document TWV/22/1, after having agreed to insert a new item reading: "Report on the Results of the Diplomatic Conference for the Revision of the UPOV Convention."

Short Report on New Developments in the Member States and Plant Variety Protection in Fruit Species

4. The Working Party received short reports from some of the experts on recent developments in their countries. The expert from the Netherlands reported on the reorganization of variety testing which had led to the Centre for Plant Breeding and Reproduction Research (C.P.R.O. = Centrum voor Plantenveredelings- en Reproductie-Onderzoek). The experts from Germany reported on the changes in their country as a result of unification, leading to the creation of eight new testing stations in the former German Democratic Republic. The expert from Czechoslovakia reported on the entry into force on January 1, 1990, of the Law on Plant Variety Protection and on the beginning of testing as from the autumn of the current year. So far 32 applications for varieties of fruit species had been received. Applications were to be addressed to Ing. Ivan Branzovsky, Ministry of Economy, Nábrezi Kpt. Jarose, Prague 7, Czech and Slovak Federal Republic. The testing would be done by the Central Institute for Control and Testing in Agriculture, Branch of Variety Testing, U Topiren 4, 17600 Prague 7, telephone 0042-2-875-014, fax 0042-2-875-014 (Head of PBR Division: Mr. Jiri Soucek; fruit crops (PBR): Mr. Ludek Krehlik). The expert from the United Kingdom reported that the national fruit collection at Brogdale Farm in Faversham would now be maintained by a charitable trust fund. The trials for PVR would be financed by the Ministry of Agriculture. The experts from South Africa reported that the office for PVR had been renamed Directorate of Plant and Quality Control.

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

5. Dr. M.-H. Thiele-Wittig gave a brief report on the main items discussed during the last session of the Technical Committee, referring for further details to the full report reproduced in document TC/26/5.

6. Amended Standard Technical Questionnaire and Variety Description Form. The Working Party noted document TC/26/6, which reproduced the amended UPOV standard Technical Questionnaire and also the UPOV Variety Description Form.

7. Harmonization of States of Expression. The Working Party also noted the request of the Technical Committee that the examples and rules in document TC/26/4 Rev., on the Harmonization of States of Expression and Notes of Characteristics, be taken into account when Test Guidelines were drawn up or revised.

8. Plant Material From Tissue Culture. The Working Party noted paragraph 34 of document TC/26/5, and the request from the Technical Committee to report back to it on any problems involved with the different methods of propagation and their possible effect on testing. It considered that when this propagation was properly done, the mutation rate was not higher than with other methods of propagation, and so no increase in sample size was necessary. In addition, any effect that propagation by tissue culture might have on fruits with a much longer testing period would be lost after a few years, and therefore would not interfere with the test results.

9. Quantity of Plant Material to be Supplied by the Applicant. The Working Party noted paragraph 43 of document TC/26/5, on the differences in the indication in the Test Guidelines of the quantity of plant material to be supplied by the applicant. It saw no problem in those different approaches, and no conflict in the fact that the first sample sent in by the applicant was the sample representing the variety. For most fruit species plants would in any event be requested only once.

10. Combined Over-Years Distinctness (COYD) Analysis. The Working Party stated that for most fruit species COYD analysis did not apply, because the measured characteristics were so few. For pineapple and banana, however, studies were under way that would take more time. The problems associated with clones and mutants in certain species might in the future lead the Working Party to reconsider its position vis-à-vis certain species. The measuring of certain characteristics could lead to smaller minimum differences.

11. Electronic Exchange of Data. On the basis of an oral report on the previous session of the Technical Working Party on Automation and Computer Programs given by Dr. M.-H. Thiele-Wittig and completed by Mr. Grégoire (France), the Working Party discussed the possibilities of the international exchange in electronic form of data published in official gazettes, and repeated its wish for such an exchange, which would be an improvement on the present exchange on paper of the lists of varieties under test. The Working Party would prefer to have the data collected and incorporated in a single data base, which would be done on an international basis by UPOV, as that would be cheaper than if each member State were individually to collect and transfer into its own data base all the information published in the various gazettes. The internationalization of plant variety protection would require member States to keep abreast of the international situation. It would be necessary to have easy access to and combine all published information with respect to a given variety or species. That could be most easily ensured if all information were collected centrally. It could then be made available periodically via electronic mail or optical disc to all UPOV member States or via direct access to the data base. This kind of electronic exchange would enable the offices of member States to have a faster and less labor-intensive access to data already published in the official gazettes. At present, national offices already received requests for information on particular varieties or species that were difficult, if not impossible, to satisfy.

12. Cooperation with Breeders. Having noted the results of the discussion on cooperation with breeders in the testing of varieties held within the Technical Committee and other Technical Working Parties, the Working Party discussed the possibilities for the species in its field of competence. It finally concluded that it was important for offices not to align themselves with specific breeders in order to remain independent. The possibilities of cooperation depended on the species. For many species it was dangerous to leave testing to the breeders, and only official growing tests would be acceptable. For certain other species, the breeder or applicant could be contacted for details or additional knowledge on the species concerned or for the indication of comparable varieties. In its field of competence, the Working Party did not expect many applications for varieties of new species as a result of the extension of protection to the whole plant kingdom. Growing tests done by breeders would not necessarily be cheaper for breeders.

Report on the Results of the Diplomatic Conference for the Revision of the UPOV Convention

13. Dr. M.-H. Thiele-Wittig informed the Working Party on the main results of the Diplomatic Conference for the Revision of the UPOV Convention which had taken place from March 4 to 19, 1991, and which on March 19, 1991, unanimously adopted a new text for the UPOV Convention. He highlighted the definition of variety, the increased scope of protection, its application after a certain period to all plant genera and species, the introduction of the so-called "farmer's privilege," the possibility for organizations that had their own plant breeders' rights systems to become members, and the introduction of the system of dependency for essentially derived varieties. He closed with the remark that, during the Diplomatic Conference, a Resolution had been adopted requesting the Secretary-General of UPOV to lay down guidelines on "essentially derived varieties."

14. The Working Party noted that in its field of competence the new criteria of "essentially derived variety" would have an important impact on the creation of new varieties. All depended on the way in which varieties were normally bred. The species that would be most affected were those with which mutation breeding was common, such as apples, where most new varieties were mutants of existing varieties and so in future might have to be considered essentially derived. That might carry the risk of any mutants found in future being just ignored and no longer leading to new varieties, as the finder would not be able to produce an independent variety, so that society would be deprived of such improvements.

15. Another open point would be how to prove that a new mutant was derived from a protected variety which itself was a mutant from an unprotected variety, but not from that which the applicant was claiming.

16. The Working Party finally agreed to collect information on apple varieties that at present were included in national lists, either protected or as candidates under test, and ascertain whether those varieties would have to be considered essentially derived if the new criteria had already been in force. If so, the variety from which they had to be considered essentially derived should be indicated. All information should be sent to the Chairman by the end of July 1991 for the preparation of a document for the next session of the Working Party.

Final Discussion of Draft Test Guidelines

Draft Test Guidelines for Blueberry

17. The Working Party noted that no comments in writing had been received regarding the Draft Test Guidelines for Blueberry as reproduced in document TG/137/1(proj.). All it did therefore, was include in the Table of Characteristics, after characteristic 8, a new characteristic with asterisk (*) reading: "Fruit: blue color of skin (after removal of bloom)" with the states "light, medium, dark," the example varieties to be indicated by the experts from Germany.

Test Guidelines for Jostaberry

18. The Working Party noted that no comments had been received regarding the Draft Test Guidelines for Jostaberry as reproduced in document TG/138/1(proj.), and that therefore there were no changes to be made in that document.

Test Guidelines for Lingonberry

19. The Working Party noted that no comments had been received regarding the Draft Test Guidelines for Lingonberry as reproduced in document TG/139/1(proj.) and that therefore there were no changes to be made in that document.

New Methods, Techniques and Equipment in the Examination of Varieties

20. The Working Party noted document TC/26/5, paragraphs 45 and 46, and document TWF/XXI/7, paragraphs 17 to 22, and had a further exchange of views on the possibilities for new technology in the fruit species area.

21. The Working Party noted a report from Mr. R. Monet (France) on polymorphism of morphological characteristics and isoenzymes in peach. He presented the main morphological characteristics deriving from natural mutations that had been preserved in peach populations. If a mutation produced two distinct phenotypes, n mutations allowed $P = 2^n$ phenotypes to be distinguished in the population. Isozymes arose also from natural mutations, in which case the mutation affected the physical properties (e.g. electric charge) of the enzyme, the catalytic property remaining unaffected. If an enzyme solution migrated within an electric field, a separation would occur owing to differences in electric charges. In this way it was possible to visualize different isozymes of a same enzyme. The isozyme pattern was a genotypic characteristic and could be used to differentiate cultivars.

22. Mr. Barendrecht (The Netherlands) reported on the results of a subgroup meeting on color measurements, held in The Netherlands in the presence of experts from France, the United Kingdom, Germany and The Netherlands. The experts had concluded that color measurements might be a reliable way of assessing colors. Some equipment needed further checking, however. The assessment was based on the three-coordinates system. A link to the visible system of color charts would still have to be established. It was not intended that the minimum distance in colors should be reduced, but only that the assessment should be made more objective. The Working Party concluded that the measuring of colors in its field of competence was of less importance than in the field of ornamental species. For fruit species image analysis might be more important, especially for example to separate apple mutants.

23. The Working Party further noted short reports on the study of electrophoresis, image analysis, RFLPs and color measurements in some of the member States. It agreed to improve that information, in that all member States would send a summary to the Chairman with information on their studies on the above or any other methods by the end of October 1991 for the preparation of a document for the next session.

Statistical Methods, Similar Varieties

24. The Working Party recalled its discussions on the meaning of similar variety as reproduced in document TWF/XXI/7, paragraphs 23 to 26, and noted the clarification given by the Technical Committee in paragraph 18 of document TC/26/5, according to which the indication of similar varieties in the variety description was meant primarily to be helpful in the testing of varieties, and that a similar variety therefore had to be selected from within the same group on the basis of grouping characteristics. It also noted the example given by the Technical Committee, which was that a similar variety for a white mutant of a red variety would not be the otherwise genetically closest red variety, but another white variety.

25. The Working Party noted that further progress had been made in South Africa in the determination of sample sizes required to detect differences between radial vectors of leaf profiles and fruit profiles of mango varieties. A report distributed during the session is reproduced in Annex II to this report.

26. The Working Party considered that, at present, statistical methods did not play an important role in the fruit species area, as most observations were made visually and in many cases the number of plants observed was too small. With the application of new methods, the evaluation of results by statistical means would become necessary. The Working Party would therefore continue its discussions on that subject during its next session. The question was raised whether or not, in the testing of clones, each plant could be considered a replicate.

Discussion of Working Papers on Test Guidelines

Test Guidelines for Citrus (Revision)

27. The Working Party noted document TWF/22/2, which contained six tables for different groups of species of Citrus, as well as Poncirus. It had a lengthy discussion on the inclusion of further species and agreed to include Citrus aurantium, Citrus bergamia, Citrus medica and Citrus madurensis. The document should not however be separated into six separate tables. As far as possible, all characteristics should be used in the same way and included in one single table. Where it was impossible to cover all groups with one single characteristic, that characteristic should be split as necessary for the different groups. The Working Party therefore went characteristic by characteristic through the Table of Characteristics, and decided to split characteristics 1, 3, 34, 35, 43, 55, 71, 73, 74, 76, 77, 101 and 116. With respect to characteristics 15, 41, 44, 47, 52, 78, 87 and 100, the need for splitting would have to be checked further. In addition, the Working Party made the following changes to the Table of Characteristics:

Characteristics

- 5,6,7,8,9 to refer to the blade of the leaf
- 10 to have the word "buckling" replaced by "blistering"
- 14 to have the states "absent (1), weakly undulated (2), strongly undulated (3)"
- 16 to have the additional state "acuminate (2)"
- 29 to ascertain whether a clear absence exists
- 32 to ascertain whether it was correlated with characteristic 20
- 39,40,41 to have the words "basal end" replaced by "stalk end"
- 39 to have state 4 deleted
- 44 to have the words "in relation to diameter of fruit" deleted
- 53,60,80 to have "in relation to diameter of fruit" added
- 65,97 to be checked

- 81 to be deleted
- 100 to be separated into two characteristics reading:
 "(a) Fruit: length of juice vesicles" with the states "short, medium long" and
 (b) Fruit: thickness of juice vesicles" with the states "thin, medium, thick";
 to be checked to ascertain whether both characteristics should be split into different groups
- 104 to have "in relation to fruit length" added
- 105 to read: "Fruit: ratio length/diameter of navel (as for 104)"
- 108 to have the method indicated
- 110 to receive an asterisk, to have the word "presence" replaced by "number" and the states to read from "absent or very few" to "very many"
- 115 to receive the bracketed addition "when fresh"
- 116,118,119 to be observed as for 115
- 122 after this characteristic a new characteristic to be inserted reading:
 "Plant: self-incompatibility"

The expert from South Africa would check the groups and their numbers for the characteristics to be split, and try to indicate example varieties by the end of March 1992.

Test Guidelines for Prunus Rootstocks

28. The Working Party noted document TWF/XXI/5 and made the following main changes in that document:

(i) Subject of these Test Guidelines. The Test Guidelines to apply to all varieties used as rootstocks of Prunus (spec.) and their hybrids.

(ii) Methods and Observations. The possibility of the inclusion of a paragraph on off-types for seed-propagated varieties to be checked. All observations on the plant and the leaf to be made during early summer on fully developed leaves. The varieties to be grown as normal fruit varieties and not in stool beds.

(iii) Table of Characteristics:

Characteristics

- 3 the second state to read: "spreading"
- 6 the word "hairiness" to be replaced by "pubescence"
- 7 to be deleted
- 9,10,11 the word "wood" to be replaced by "vegetative"
- 10 to have the states "adpressed, slightly held out, clearly held out"

12,13,14 to be observed on the current year's shoot, characteristics 12 and 13 in autumn, characteristic 14 in early summer

15 to read: "Leaf: attitude" with the states "upwards, horizontal, downwards"

19 to receive drawings for explanation and to have the second state reading "acute"

21 to read: "Leaf blade: intensity of green color of upper side" with the states "light (3), medium (5), dark (7)"; before this characteristic a new characteristic to be inserted reading: "Leaf blade: color of upper side" with the states "green (1), reddish (2), reddish brown (3)"

Lack of time prevented discussion of the document beyond characteristic 21. All experts were therefore asked to send their comments to the Office of UPOV by the end of the year for distribution to the Working Party.

29. In connection with the discussions on the Test Guidelines for Prunus Rootstocks, the Working Party addressed the problem of the submission of plants of seedlings of a seed-propagated rootstock. The seed of rootstock varieties would need to be sown as fresh seed, immediately after harvesting. It was not possible, therefore, to ask for seed to be sent in for testing. When selecting seedlings and marketing his variety, the applicant would obviously choose rather homogeneous plants and so the sample sent in for testing would be a representative sample of the variety marketed, but not of the variety as a whole. However, the Working Party saw no alternative to accepting the submission of plant seedlings.

Test Guidelines for Apple

30. The Working Party noted circular U 1716, and had a general discussion on a possible revision of the Test Guidelines for Apple (TG/14/5). It finally agreed to the following:

(i) it would prepare separate Test Guidelines for fruit varieties, rootstocks and ornamental varieties;

(ii) it would limit the rootstocks for the testing of fruit varieties to one single rootstock important for the market;

(iii) it would require submission of propagating material as bud sticks for further grafting and the production of plants of the varieties to be tested and of the varieties for comparison, at the place of testing;

(iv) it would apply strict rules on the sanitary status of plant material.

The expert from Germany would prepare two drafts for revised Test Guidelines for Apple on the basis of the above principles for the next session.

Test Guidelines for Japanese Pear

31. The Working Party noted document TWF/22/3, which contained a working paper on draft Test Guidelines for Japanese Pear, and made the following main changes in that document:

(i) Material Required: To be checked to ascertain which rootstocks are to be used.

(ii) Conduct of Tests: To have the word "artificially" deleted in paragraph 3.

(iii) Methods and Observations: To have the word "blueberry" in paragraph 1 replaced by "Japanese Pear"; to have the words "at least" in paragraph 2 deleted; to have the words "on unpruned bushes" in paragraph 3 replaced by "before pruning"; to have paragraph 4 checked; to have the words "of the first bud burst" in paragraph 6 deleted.

(iv) Grouping of Varieties: To have characteristic 48 as only grouping characteristic.

(v) As lack of time prevented discussion of the individual characteristics, all experts were asked to send their comments on the document to Mr. Yamaguchi (JP) by the end of October 1991 for the preparation of a new working paper by the end of March 1992. When checking the document, special attention should be given to resistance characteristics and the possibility of reducing the total number of characteristics.

Status of Test Guidelines

32. The Working Party agreed that the draft Test Guidelines for Blueberry, Jostaberry and Lingonberry should be sent to the Technical Committee for final adoption.

33. Discussions on working papers on Test Guidelines for Citrus (Revision), Prunus Rootstocks, Apple (Revision) and Japanese Pear would have to be continued during the next session.

Future Program, Date and Place of Next Session

34. At the invitation of the expert from South Africa, the Working Party agreed to hold its twenty-third session in Nelspruit, South Africa, from August 24 to September 2, 1992. The meeting would start on August 24 at 9 a.m. and close on August 27, to be followed by visits to research, breeding and testing installations for fruit and ornamental varieties. The visits would be organized both for the Working Party and for the Technical Working Party for Ornamental Plants and Forest Trees, which would be holding its twenty-fifth session in South Africa from August 27 to September 7, 1992. During the session, the Working Party plans to discuss the following items:

(a) Short reports on new developments in member States in plant variety protection for fruit species;

(b) Important decisions taken during the previous sessions of the Working Party, the Technical Committee and the Technical Working Party on Automation and Computer Programs;

(c) Color observations (report from the Subgroup);

(d) New methods, techniques and equipment in the examination of varieties (DE to collect information by the end of October 1991);

- (e) Statistical methods;
- (f) Sanitary status of plant material (ZA to collect comments by the end of October 1991);
- (g) Mutations and minimum distances (DE + GB to prepare a working paper by the end of the year);
- (h) Essentially derived varieties (DE to collect information by the end of October 1991);
- (i) Electronic exchange of data (DE to collect information on protected, listed and candidate varieties by the end of the year);
- (j) Discussions on working papers on Test Guidelines for:
- (i) Citrus (Revision) (ZA to prepare new working paper by the end of March 92)
 - (ii) Prunus Rootstocks (UPOV to collect comments by the end of the year)
 - (iii) Apple (DE to prepare two working papers by the end of March 1992)
 - (iv) Japanese Pear (JP to collect comments by the end of October 1991 and to prepare a new working paper by the end of March 1992)
 - (v) Pear (TG/16/4 and a working paper to be prepared by FR by the end of March 1992)
 - (vi) Cherry (TG/35/3 and a working paper to be prepared by FR by the end of March 1992).

Visits

35. On the morning of June 12, the Working Party visited the trial field for chestnut and the tissue culture laboratory of the Fruit Research Station at La Grande Ferrade. In the afternoon, it visited the experimental fields and the certification of fruit varieties on the premises of the Centre Technique Interprofessionnel des Fruits et Légumes (CTIFL) at Lanxade, Bergerac. In the afternoon of June 13, it visited INRA's Domaine Expérimental d'Arboriculture Fruitière des Jarres, near Langon, and its Domaine Vitivinicole de Couhins.

36. This report has been adopted by correspondence.

[Two annexes follow]

ANNEX I

LIST OF PARTICIPANTS AT THE TWENTY-SECOND SESSION
OF THE TECHNICAL WORKING PARTY FOR FRUIT CROPS
BORDEAUX, FRANCE, JUNE 11 TO 14, 1991I. MEMBER STATESFRANCE

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[Annex II follows]

DETERMINATION OF SAMPLE SIZES REQUIRED TO DETECT DIFFERENCES BETWEEN RADIAL VECTORS OF LEAF- AND FRUIT PROFILES OF MANGO (*Mangifera indica* L.) VARIETIES

by

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Abstract.

Determining the sample size, to prove that a true difference exists at a specified significance level, is often a problem. The objective of this project was to develop a simple program to calculate the minimum sample size. A formula from the literature was adapted and integrated with the program to calculate sample size by means of iteration. To prove statistical differences between varieties, sample sizes for measurement of mango leaf- and fruit radial vectors were calculated by running the program on a microcomputer. Calculated sample sizes varied depending on the number of varieties compared and the magnitude specified for the smallest true difference of the mean radial vector to be detected. Data reported in this paper are theoretical computations for specified mango varieties and the results will be verified by comparing reference varieties used in the reported computations, with different mango varieties.

Additional Index words: Analysis of variance, iteration, radial vector, replication, smallest true difference, UPOV

Abbreviations :

ANOVA	= Analysis of Variance	CV	= Coefficient of variation
H_0	= Null hypothesis	IBM	= International Business Machines
k	= Number of treatments (varieties)	Mb	= Megabyte
MSDOS	= Microsoft Disk Operating System	n	= Sample size (replications)
n_c	= Calculated sample size	n_e	= Estimated sample size
RAM	= Random access memory	v	= Degrees of freedom
\bar{Y}	= mean of sample population		
$P < 0.01$	= Probability that the observed differences will occur with 99% certainty in a population		
UPOV	= International Union for Protection of new Varieties of Plants		

INTRODUCTION

Determining the optimal number of replications in experiments is essential because too few replicates result in inaccuracy, while an experiment with too many replications is not cost effective. Using too few replications in an experiment where the treatments are such that the nul hypothesis (H_0) should have been rejected, results in the occurrence of a TYPE II ERROR. However, the probability of accepting a TYPE II ERROR can be used to determine the number of replications required to reject H_0 . An increase in the number of replications result in a decrease in the probability for occurrence of TYPE II ERROR. However, various other factors should also be considered in resolving the number of replications. Federer (1955) refers to the following factors:

1. Required accuracy
2. Variation in the experimental material
3. Availability of sophisticated equipment and skilled labour
4. Size of the trial
5. Experimental design

The general question with statistical analysis of experimental data is "What is the sample size to show that a true difference exists at a significance level α , with a probability P that the significance will be found?" Sokal and Rohlf (1981) described a simple equation to estimate the sample size n required for a test. The significance level at which two means will be considered significantly different, will be α and the probability that a significant difference will be found if it does exist, and if it is as small as δ , is P . This probability is the power $1-\beta$ of the significance test. (β = probability for TYPE II ERROR = 80%)

A method which involves measurement of radial vectors of fruit and leaves relative to an orientation vector, was developed by Buitendag (1990) for the purpose of describing of fruit and leaf profiles of mango varieties (*Mangifera indica* L.). Radial vectors are measured at specified angles. Data from the study of Buitendag (1990) was used to calculate the optimal number of replications (sample size) required for each of the specified angles at which the radial vectors of mango fruit and leaf profiles are measured.

MATERIAL AND METHODS

An iterative computer program was developed by using the programming (macro) commands of a commercial spreadsheet program, SuperCalc 4. The program was developed on an IBM-compatible microcomputer with the following configuration: 80286 Processing unit, 8087 mathematical co-processor, 3 Mb RAM, 40 Mb data storage disk and MS-DOS disk operating system.

The iterative formula used in the program was adapted from Sokal and Rohlf (1981). This iterative procedure is helpful when a direct solution for a term is difficult or impossible to calculate. The basic formula is:

where
$$n \geq 2 \left(\frac{\sigma}{\delta} \right)^2 \left\{ t_{\alpha[v]} + t_{2(1-P)[v]} \right\}^2$$

n = number of replications

σ = true standard deviation

δ = the smallest true difference of the population mean

v = degrees of freedom of the sample standard deviation
with k groups and n replications per group

α = significance level

P = desired probability that a difference will be found to be
significant, if it is as small as δ

$t_{\alpha[v]}$ and

$t_{2(1-P)[v]}$ = values from a two tailed t-table with v degrees
of freedom and corresponding to probabilities of α and $2(1-P)$
respectively.

In order to calculate the optimal sample size required to prove statistical differences between mango varieties, a preliminary ANOVA was executed. This is essential in order to specify the true standard deviation σ . For this purpose the value of the coefficient of variation (CV) from a preliminary analysis of variance is used to calculate variance s ($s = CV \times \bar{Y}/100$), with \bar{Y} being an estimate of population mean. An estimate of the smallest difference desired to be detected is essential in order to calculate the smallest true difference (δ), eg. = 5% of the mean, then $\delta = 5\bar{Y}/100$. It is obvious that for a small true difference, a large sample size will be required and vice versa. (Sokal and Rohlf (1981), Steel and Torrie (1982)).

The equation is not too sensitive to changes in α and P , but is very sensitive to changes in the ratio σ/δ , which means that a large sample size is required to detect small differences. It is also possible to reduce the required sample sizes by refinement of experimental techniques resulting in the reduction of σ . The formula requires input values for CV and the magnitude of the smallest true difference (%) of the \bar{Y} which is desired to be detected. Furthermore it is not essential to have actual values for σ and δ , but rather the ratio σ/δ (Sokal and Rohlf (1981)).

The first calculated value of n (n_c) is calculated by the formula by estimating the value of n (n_e) which is also needed in order to calculate v , the degrees of freedom. Values for s and \bar{Y} are extracted from the preliminary ANOVA of radial vectors of mango leaf and fruit profiles measured at various angles. Buitendag

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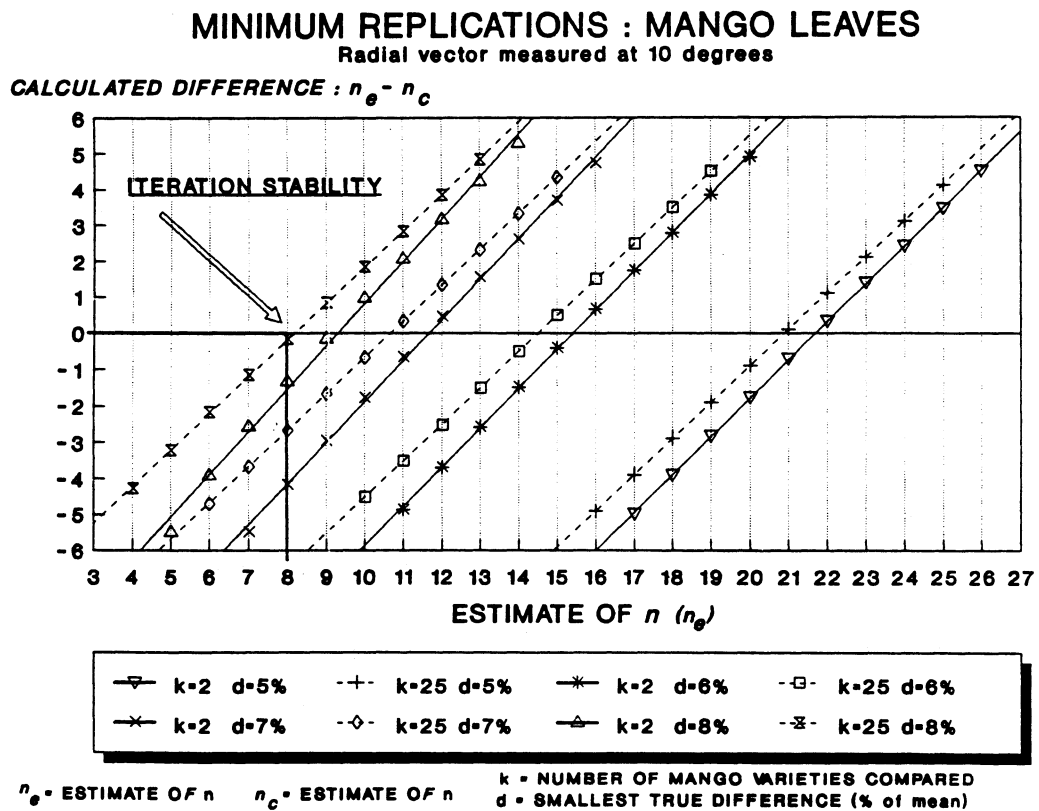


FIG.1 Calculated minimum replications for detection of statistical difference ($P < 0.05$) at four levels of the smallest true difference in the mean length of radial vectors of mango leaves measured at an angle of 10° .

(1990) measured radial vectors of leaf profiles at 2,5°, 10°, 90°, 165° and 175° relative to the orientation vector and fruit profiles were measured at 10°, 225° and 315°.

Once the program is activated and the angle of radial vector measurement is specified, values for s and \bar{Y} are extracted from a pre-programmed data table incorporated in the skeleton spreadsheet. The first step of iterative calculation involves an initial estimate of the number of replicates. Values for the number of varieties to be compared (k) and the required smallest true difference of \bar{Y} to be detected are respectively set at 2 and 1% before the onset of final iteration. From these input values v and \bar{Y} are calculated with automatic and simultaneous recording of values for $t_{[v]}$ and $t_{2(1-P)[v]}$ from a pre-programmed t-table (values programmed from Fisher and Yates, 1957 and Van Ark, 1981). The first iterative value for (n_c) is then calculated. Calculations are simultaneously performed for $P < 0.01$ and $P < 0.05$. However, from the initial estimate of n (n_e), the first iterative calculation of n (n_c) is used as a base value for estimated values of n (n_e) in the iterative computations. The value of $n_c - 7$ is used as the first value for n_e in the series of 15 iterative computations. Calculations are executed for an iterative range of $n_c - 7, n_c - 6 \dots n_c + 7$ which equals $n_e, n_e + 1 \dots n_e + 14$, which proved to be a suitable range for iteration to a stable sample size (n). Stability of n is reached when $n_e - n_c$ equals zero. The corresponding value of $n_c + 1$ when $n_e - n_c \geq 0$ is then accepted as a conservative roundup for sample size n .

RESULTS

Mango leaf profiles

The values of n were determined by means of iterative computation of n . Iteration stability was reached when the calculated difference of $n_e - n_c$ approached zero. The point of iteration stability and the minimum replications n required to detect statistical difference ($P < 0.05$) at four selected levels of the smallest true difference of the mean radial vector are illustrated in FIG. 1. Figure 1 illustrates the increase in n with a reduction in the number of mango varieties compared, and also with a reduction in the specified magnitude of the smallest true difference of the radial vectors to be detected.

MINIMUM REPLICATIONS FOR MANGO LEAVES
Radial vectors measured at 2.5 degrees

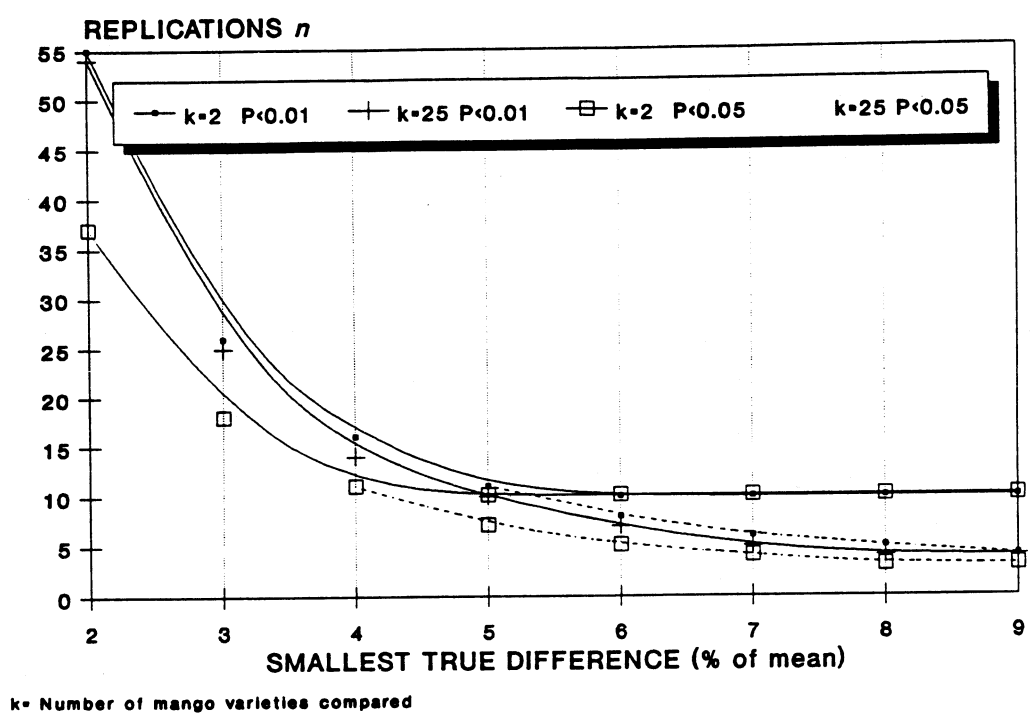


FIG. 2 Minimum sample size required to prove statistical difference between mango varieties for radial leaf vectors measured at 2.5°. ($P<0.01$ and $P<0.05$).

MINIMUM REPLICATIONS FOR MANGO LEAVES
Radial vectors at various angles: $P<0.05$

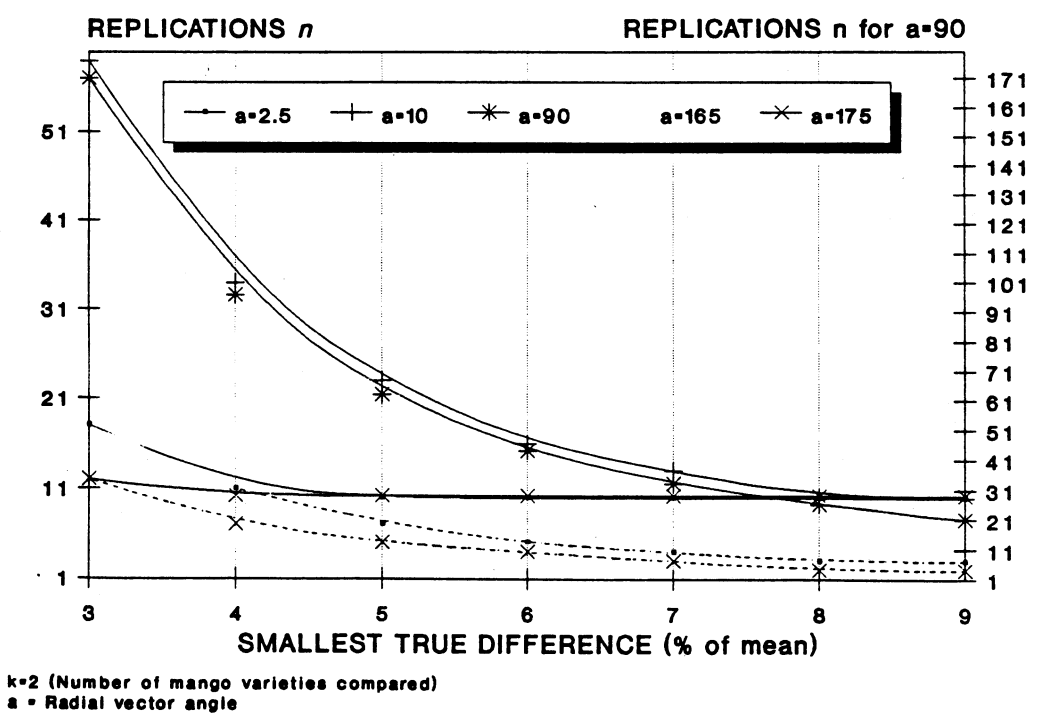


FIG. 3 Minimum sample size required to prove statistical differences between mango varieties for radial leaf vectors measured at various angles. ($P<0.05$).

The minimum replications required for statistical proof of differences between radial vectors of mango leaves were calculated for the number of varieties to be compared (k), which ranged from 2 to 25 varieties. The magnitude of the smallest true difference of the radial vectors varied between 1% and 12% with 1% increments of the mean radial vector length. The calculated sample sizes (n) for mango leaves are tabulated in Tables 1 to 5 ($P < 0.01$ and $P < 0.05$).

Data presented in Tables 1 to 5 prove that the calculated number of replications decrease with an increase in k and with an increase in the magnitude of the specified smallest true difference of the mean radial vector. However, it was necessary to adjust the calculated number of replications to comply with the requirement of a minimum of 18 degrees of freedom. Figures 2 and 3 respectively illustrate the adjusted sample sizes for radial leaf vectors measured at 2.5° if $v < 18$ ($P < 0.01$; $P < 0.05$) and for radial leaf vectors measured at various angles ($P < 0.05$).

Mango fruit profiles

The values for the minimum sample size required to prove statistical differences between mango varieties for radial vectors of fruit profiles were calculated by the same method used for calculation of sample sizes for leaf radial vectors. The results for mango fruit profiles showed the same trends as for the leaf profiles.

Data for the minimum replications required for mango fruit profiles are tabulated in Tables 6 to 8. Figure 4 illustrates the minimum replications required to prove statistical differences ($P < 0.05$) between mango cultivars with radial vectors measured at 10° . Figure 5 illustrates the minimum sample size for radial vectors of mango fruit measured at various angles ($P < 0.05$).

DISCUSSION

The formula used to calculate sample sizes for descriptive characteristics of mango leaf- and fruit profiles is quite simple. However, accurate estimates of n can only be obtained by means of iteration for which the use of a microcomputer

MINIMUM REPLICATIONS : MANGO FRUIT
Radial vector measured at 10 degrees

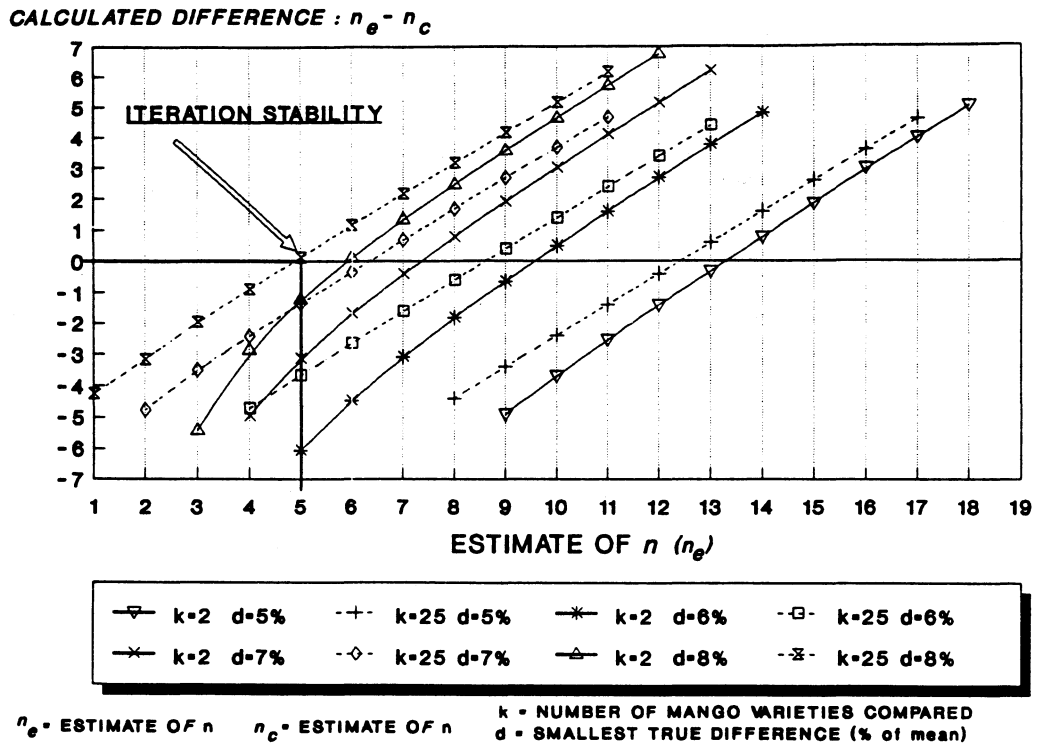


FIG. 4 Calculated sample size for detection of statistical differences ($P < 0.05$) between mango varieties for radial vectors of fruit profiles measured at 10° .

MINIMUM REPLICATIONS FOR MANGO FRUIT
Radial vectors at various angles: $P < 0.05$

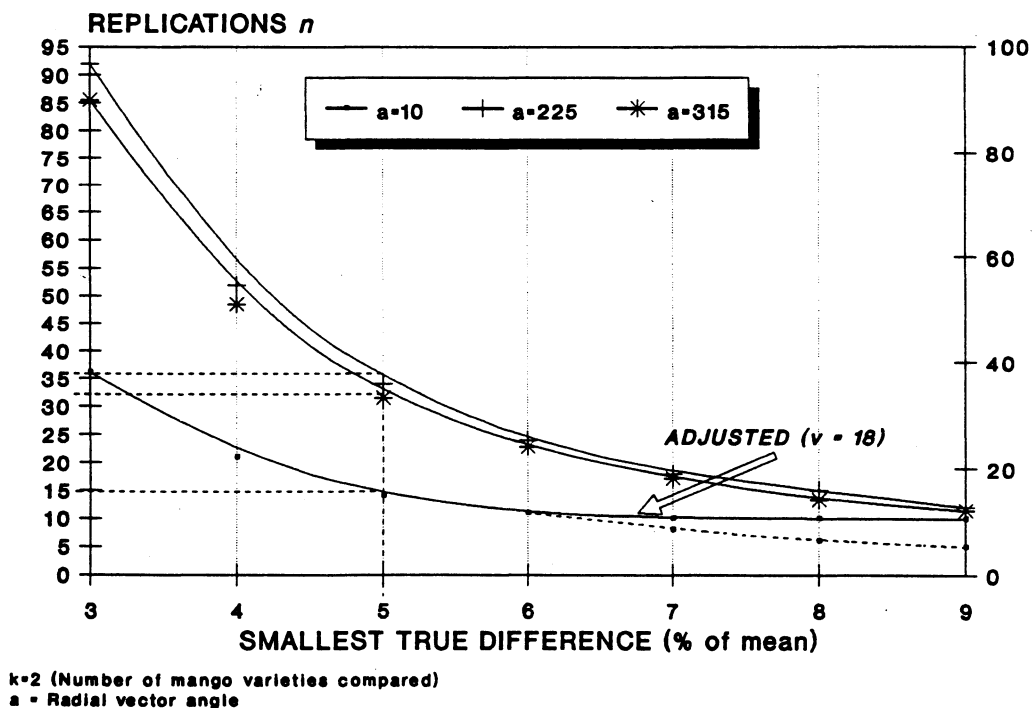


FIG. 5. Calculated sample size for detection of statistical differences ($P < 0.05$) between mango varieties for radial vectors of fruit profiles measured at various angles.

is recommended. In this investigation a total of 15 iterative calculations were performed, but during execution of the program it seemed that with 7 iterations stability of n was reached and the problem was solved.

The magnitude of sample sizes of radial vectors of mango leaf- and fruit profiles measured at the specified angles, was mainly influenced by the degree of variation observed between replications. High calculated coefficients of variation result in large calculated sample sizes and *vice versa*. This phenomenon is stated in statistical textbooks (Clarke 1969; Fisher 1934; Little and Hills 1978; Montgomery 1984; Snedecor and Cochran 1980, Sokal and Rohlf 1981; Steel and Torrie 1982; Treloar 1942; Van Ark 1981).

ANOVA of radial vectors of mango leaf profiles measured at angles of 2.5° and 175° proved that little variation was observed and resulted in coefficients of variation of 2.91% and 2.47% respectively. The smallest CV for fruit profiles was calculated for radial vectors measured at 10° (CV=4.39%). Calculated sample sizes for $k < 4$, were less than 6 and because a minimum of 18 degrees of freedom is required for a reliable statistical analysis, sample sizes were adjusted to comply with $v \geq 18$.

The effect of an increase in k on the sample size proved to be negligible. In general, sample sizes for all descriptive characteristics (angles of measurement) of mango leaf and fruit profiles showed no inchange or a maximum increment of 1 for $k \geq 6$. The reason for this is probably that with increasing values for k , calculated values for v are usually greater than 120. Values for $t_{[v]}$ and $t_{2(1-P)[v]}$ are almost constant with a calculated value for degrees of freedom larger than 120.

Changes in the magnitude of the smallest true difference which is desired to be detected resulted in prominent differences in sample sizes. Detection of very small true differences (eg. 1% of \bar{Y}) requires very large sample sizes.

In conclusion, the objective of this investigation was to calculate sample sizes for descriptive characteristics leaf and fruit profiles. The magnitude of sample size for detection of statistical differences between mango varieties depends on the selected significance level (P) and on the desired smallest true difference

Table 1 Calculated sample sizes (n) required for statistical analysis of normalised values of radial vectors of mango (*Mangifera indica* L.) leaves measured at an angle of 2.5° relative to the orientation vector.

k	Smallest true difference (% of \bar{Y})					P<0.01						
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%
2	216	55	26	16	11	10 (8)	10 (6)	10 (5)	10 (4)	10 (4)	10 (3)	10 (3)
3	216	55	26	15	11	8	7 (6)	7 (5)	7 (4)	7 (3)	7 (3)	7 (3)
4	215	55	25	15	10	7	6	6 (5)	6 (4)	6 (3)	6 (3)	6 (3)
5	215	55	25	15	10	7	6	5	5 (4)	5 (3)	5 (3)	5 (3)
6	215	55	25	15	10	7	6	4	4	4 (3)	4 (3)	4 (3)
7	214	55	25	15	10	7	6	4	4	4 (3)	4 (3)	4 (3)
8	212	55	25	15	10	7	6	4	4	3	3	3
9	212	55	25	15	10	7	5	4	4	3	3	3
10	212	55	25	15	10	7	5	4	4	3	3	3
11	212	55	25	14	10	7	5	4	4	3	3	3
12	212	55	25	14	10	7	5	4	4	3	3	3
13	212	55	25	14	10	7	5	4	4	3	3	3
14	212	55	25	14	10	7	5	4	4	3	3	3
15	212	55	25	14	10	7	5	4	4	3	3	3
16	212	55	25	14	10	7	5	4	4	3	3	2
17	212	55	25	14	10	7	5	4	4	3	3	2
18	212	55	25	14	10	7	5	4	4	3	3	2
19	212	55	25	14	10	7	5	4	4	3	3	2
20	212	55	25	14	10	7	5	4	4	3	3	2
21	212	55	25	14	10	7	5	4	4	3	3	2
22	212	55	25	14	10	7	5	4	4	3	3	2
23	212	54	25	14	10	7	5	4	4	3	3	2
24	212	54	25	14	10	7	5	4	4	3	3	2
25	212	54	25	14	10	7	5	4	4	3	3	2

k	Smallest true difference (% of \bar{Y})					P<0.05						
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%
2	145	37	18	11	10 (7)	10 (5)	10 (4)	10 (3)	10 (3)	10 (3)	10 (2)	10 (2)
3	145	37	17	11	7	7 (5)	7 (4)	7 (3)	7 (3)	7 (3)	7 (2)	7 (2)
4	144	37	17	10	7	6 (5)	6 (4)	6 (3)	6 (3)	6 (2)	6 (2)	6 (2)
5	144	37	17	10	7	5	5 (4)	5 (3)	5 (3)	5 (2)	5 (2)	5 (2)
6	144	37	17	10	7	5	4	4 (3)	4 (3)	4 (2)	4 (2)	4 (2)
7	144	37	17	10	7	5	4	4 (3)	4 (3)	4 (2)	4 (2)	4 (2)
8	144	37	17	10	7	5	4	3	3	3 (2)	3 (2)	3 (2)
9	144	37	17	10	7	5	4	3	3	3 (2)	3 (2)	3 (2)
10	144	37	17	10	7	5	4	3	3	3 (2)	3 (2)	3 (2)
11	143	37	17	10	7	5	4	3	3	3 (2)	3 (2)	3 (2)
12	143	37	17	10	7	5	4	3	3	3 (2)	3 (2)	3 (2)
13	143	37	17	10	7	5	4	3	3	2	2	2
14	143	37	17	10	7	5	4	3	3	2	2	2
15	143	37	17	10	7	5	4	3	3	2	2	2
16	143	37	17	10	7	5	4	3	3	2	2	2
17	143	37	17	10	7	5	4	3	3	2	2	2
18	143	37	17	10	7	5	4	3	3	2	2	2
19	143	37	17	10	7	5	4	3	3	2	2	2
20	143	37	17	10	7	5	4	3	3	2	2	2
21	143	37	17	10	7	5	4	3	3	2	2	2
22	143	37	17	10	7	5	4	3	3	2	2	2
23	143	37	17	10	7	5	4	3	3	2	2	2
24	143	37	17	10	7	5	4	3	3	2	2	2
25	143	37	17	10	7	5	4	3	3	2	2	2

() : Values in parenthesis are the rejected calculated sample size (n_c) if v (degrees of freedom) < 18.
k = Number of treatments (mango varieties) to be compared

of population means to be detected. As plant material is usually limited for the purpose of UPOV descriptions, it would be impractical and expensive to choose high significance levels (99%) and small true differences (1%). One option is to specify $\delta = 6\%$ and ($P < 0.05$) and use the largest sample size of the descriptive characteristics concerned. (eg. for $\delta = 6\%$ and $k=5$, $n=65$ ($P < 0.01$) and for $P < 0.05$, $n=44$. However, it would be more practical to preselect the sample size n , eg. $n=25$ ($k=5$) and then specify the smallest true difference of \bar{Y} (δ), which can be detected. In this regard a sample size of 25 for measuring radial vectors of mango leaf profiles, will detect significant differences between mango varieties for differential magnitudes of the smallest true difference of \bar{Y} .

Example: Mango leaf profiles (n=25, k=5):

Angle of measurement	Smallest detectable true difference of \bar{Y}	
	$P < 0.01$	$P < 0.05$
3°	3%	3%
10°	6%	5%
90°	10%	8%
165°	6%	5%
175°	3%	2%

Results of this investigation can be used as a guideline for choosing sample sizes for detection of differences between mango varieties. The data reported in this paper will be verified by comparing reference varieties with different (new hybrids) mango varieties.

Table 2 Calculated sample sizes (n) required for statistical analysis of normalised values of radial vectors of mango (*Mangifera indica* L.) leaves measured at an angle of 10° relative to the orientation vector.

k	Smallest true difference (% of \bar{Y})					P<0.01							
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%	
2	769	197	88	51	33	24	18	15	12	10	10 (9)	10 (7)	
3	769	196	88	50	33	23	18	14	12	10	8	7	
4	769	196	88	50	33	23	18	14	11	9	8	7	
5	769	196	88	50	32	23	17	14	11	9	8	7	
6	769	196	88	50	32	23	17	14	11	9	8	7	
7	769	195	88	50	32	23	17	13	11	9	8	7	
8	769	193	88	50	32	23	17	13	11	9	8	7	
9	769	193	88	50	32	23	17	13	11	9	8	7	
10	769	193	88	50	32	23	17	13	11	9	8	7	
11	769	193	88	50	32	23	17	13	11	9	8	7	
12	769	193	88	50	32	23	17	13	11	9	8	6	
13	769	193	88	50	32	23	17	13	11	9	8	6	
14	769	193	87	50	32	23	17	13	11	9	8	6	
15	769	193	87	50	32	23	17	13	11	9	7	6	
16	769	193	87	50	32	23	17	13	11	9	7	6	
17	769	193	87	50	32	23	17	13	11	9	7	6	
18	769	193	86	50	32	23	17	13	11	9	7	6	
19	769	193	86	50	32	23	17	13	11	9	7	6	
20	769	193	86	50	32	23	17	13	11	9	7	6	
21	769	193	86	50	32	23	17	13	11	9	7	6	
22	769	193	86	50	32	23	17	13	11	9	7	6	
23	769	193	86	50	32	23	17	13	11	9	7	6	
24	769	193	86	50	32	23	17	13	11	9	7	6	
25	769	193	86	50	32	23	17	13	11	9	7	6	

k	Smallest true difference (% of \bar{Y})					P<0.05							
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%	
2	522	132	59	34	23	16	13	10	10 (8)	10 (7)	10 (6)	10 (5)	
3	517	132	59	34	22	16	12	10	8	7	7 (6)	7 (5)	
4	517	132	59	34	22	16	12	10	8	6	6	6 (5)	
5	517	131	59	34	22	16	12	9	8	6	5	5	
6	517	131	59	34	22	16	12	9	8	6	5	5	
7	517	131	59	34	22	16	12	9	8	6	5	5	
8	517	131	59	34	22	16	12	9	8	6	5	5	
9	517	131	59	34	22	16	12	9	8	6	5	5	
10	517	131	59	34	22	16	12	9	8	6	5	5	
11	517	131	59	34	22	16	12	9	8	6	5	5	
12	517	130	59	34	22	16	12	9	7	6	5	5	
13	517	130	59	34	22	16	12	9	7	6	5	5	
14	517	130	59	34	22	16	12	9	7	6	5	5	
15	517	130	59	34	22	16	12	9	7	6	5	5	
16	517	130	59	34	22	16	12	9	7	6	5	5	
17	517	130	59	34	22	16	12	9	7	6	5	5	
18	517	130	59	34	22	16	12	9	7	6	5	5	
19	517	130	59	34	22	16	12	9	7	6	5	5	
20	517	130	59	34	22	16	12	9	7	6	5	5	
21	517	130	59	34	22	16	12	9	7	6	5	5	
22	517	130	59	34	22	16	12	9	7	6	5	5	
23	517	130	59	34	22	16	12	9	7	6	5	5	
24	517	130	59	34	22	16	12	9	7	6	5	5	
25	517	130	59	34	22	16	12	9	7	6	5	5	

() : Values in parenthesis are the rejected calculated sample size (n_c) if v (degrees of freedom) < 18.
k = Number of treatments (mango varieties) to be compared

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Table 4 Calculated sample sizes (n) required for statistical analysis of normalised values of radial vectors of mango (*Mangifera indica* L.) leaves measured at an angle of 165° relative to the orientation vector.

k	Smallest true difference (% of \bar{Y})					P<0.01							
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%	
2	695	176	79	45	30	22	17	13	11	10 (9)	10 (8)	10 (7)	
3	687	176	79	45	29	21	16	13	11	9	7	7 (6)	
4	687	175	79	45	29	21	16	13	10	8	7	6	
5	687	175	79	45	29	21	16	12	10	8	7	6	
6	687	175	79	45	29	21	16	12	10	8	7	6	
7	687	175	79	45	29	21	15	12	10	8	7	6	
8	687	174	79	45	29	20	15	12	10	8	7	6	
9	687	172	78	45	29	20	15	12	10	8	7	6	
10	687	172	78	45	29	20	15	12	10	8	7	6	
11	687	172	78	45	29	20	15	12	10	8	7	6	
12	687	172	78	45	29	20	15	12	10	8	7	6	
13	687	172	78	45	29	20	15	12	10	8	7	6	
14	687	172	78	45	29	20	15	12	10	8	7	6	
15	687	172	78	45	29	20	15	12	10	8	7	6	
16	687	172	78	45	29	20	15	12	10	8	7	6	
17	687	172	78	45	29	20	15	12	10	8	7	6	
18	687	172	78	45	29	20	15	12	10	8	7	6	
19	687	172	78	45	29	20	15	12	10	8	7	6	
20	687	172	77	45	29	20	15	12	10	8	7	6	
21	687	172	77	45	29	20	15	12	10	8	7	6	
22	687	172	77	44	29	20	15	12	10	8	7	6	
23	687	172	77	44	29	20	15	12	10	8	7	6	
24	687	172	77	44	29	20	15	12	10	8	7	6	
25	687	172	77	44	29	20	15	12	10	8	7	6	

k	Smallest true difference (% of \bar{Y})					P<0.05							
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%	
2	466	118	53	31	20	15	12	10 (9)	10 (7)	10 (6)	10 (5)	10 (5)	
3	465	118	53	30	20	14	11	9	7	7 (6)	7 (5)	7 (4)	
4	462	118	53	30	20	14	11	9	7	6	6 (5)	6 (4)	
5	462	117	53	30	20	14	11	9	7	6	5	5 (4)	
6	462	117	53	30	20	14	11	8	7	6	5	4	
7	462	117	53	30	20	14	11	8	7	6	5	4	
8	462	117	53	30	20	14	11	8	7	6	5	4	
9	462	117	53	30	20	14	11	8	7	6	5	4	
10	462	117	53	30	20	14	11	8	7	6	5	4	
11	462	117	53	30	20	14	11	8	7	6	5	4	
12	462	117	53	30	20	14	11	8	7	6	5	4	
13	462	117	53	30	20	14	11	8	7	6	5	4	
14	462	116	53	30	20	14	11	8	7	6	5	4	
15	462	116	53	30	20	14	11	8	7	6	5	4	
16	462	116	53	30	20	14	11	8	7	6	5	4	
17	462	116	53	30	20	14	11	8	7	6	5	4	
18	462	116	53	30	20	14	11	8	7	6	5	4	
19	462	116	53	30	20	14	11	8	7	6	5	4	
20	462	116	53	30	20	14	11	8	7	6	5	4	
21	462	116	53	30	20	14	11	8	7	6	5	4	
22	462	116	53	30	20	14	11	8	7	6	5	4	
23	462	116	53	30	20	14	11	8	7	6	5	4	
24	462	116	53	30	20	14	11	8	7	6	5	4	
25	462	116	53	30	20	14	11	8	7	6	5	4	

() : Values in parenthesis are the rejected calculated sample size (n_c) if v (degrees of freedom) < 18.
k = Number of treatments (mango varieties) to be compared

Table 3 Calculated sample sizes (*n*) required for statistical analysis of normalised values of radial vectors of mango (*Mangifera indica* L.) leaves measured at an angle of 90° relative to the orientation vector.

k	Smallest true difference (% of \bar{Y})					P<0.01						
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%
2	2257	572	256	145	93	65	49	38	30	25	21	18
3	2257	565	256	145	93	65	48	37	30	25	21	18
4	2257	565	255	144	93	65	48	37	30	24	20	17
5	2257	565	255	144	93	65	48	37	29	24	20	17
6	2257	565	252	144	93	65	48	37	29	24	20	17
7	2257	565	252	144	93	65	48	37	29	24	20	17
8	2257	565	252	144	93	65	48	37	29	24	20	17
9	2257	565	252	144	93	65	48	37	29	24	20	17
10	2257	565	252	144	93	65	48	37	29	24	20	17
11	2257	565	252	142	93	65	48	37	29	24	20	17
12	2257	565	252	142	92	65	48	37	29	24	20	17
13	2257	565	252	142	92	65	48	37	29	24	20	17
14	2257	565	252	142	92	65	48	37	29	24	20	17
15	2257	565	252	142	92	65	48	37	29	24	20	17
16	2257	565	252	142	92	65	48	37	29	24	20	17
17	2257	565	252	142	91	65	48	37	29	24	20	17
18	2257	565	252	142	91	64	48	37	29	24	20	17
19	2257	565	252	142	91	64	48	37	29	24	20	17
20	2257	565	252	142	91	64	48	37	29	24	20	17
21	2257	565	252	142	91	64	48	37	29	24	20	17
22	2257	565	252	142	91	64	48	37	29	24	20	17
23	2257	565	252	142	91	64	48	37	29	24	20	17
24	2257	565	252	142	91	64	48	37	29	24	20	17
25	2257	565	252	142	91	64	48	37	29	24	20	17

k	Smallest true difference (% of \bar{Y})					P<0.05						
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%
2	1517	384	171	97	63	44	33	26	21	17	15	13
3	1517	383	171	97	62	44	33	25	20	17	14	12
4	1517	380	171	97	62	44	32	25	20	17	14	12
5	1517	380	171	97	62	44	32	25	20	17	14	12
6	1517	380	171	97	62	44	32	25	20	16	14	12
7	1517	380	171	97	62	44	32	25	20	16	14	12
8	1517	380	171	97	62	44	32	25	20	16	14	12
9	1517	380	169	97	62	44	32	25	20	16	14	12
10	1517	380	169	97	62	44	32	25	20	16	14	12
11	1517	380	169	97	62	44	32	25	20	16	14	12
12	1517	380	169	97	62	44	32	25	20	16	14	12
13	1517	380	169	96	62	44	32	25	20	16	14	12
14	1517	380	169	96	62	44	32	25	20	16	14	12
15	1517	380	169	96	62	44	32	25	20	16	14	12
16	1517	380	169	96	62	44	32	25	20	16	14	12
17	1517	380	169	96	62	44	32	25	20	16	14	12
18	1517	380	169	96	62	44	32	25	20	16	14	12
19	1517	380	169	96	62	44	32	25	20	16	14	12
20	1517	380	169	96	62	44	32	25	20	16	14	12
21	1517	380	169	96	62	44	32	25	20	16	14	12
22	1517	380	169	96	62	43	32	25	20	16	14	12
23	1517	380	169	96	62	43	32	25	20	16	14	12
24	1517	380	169	96	62	43	32	25	20	16	14	12
25	1517	380	169	96	62	43	32	25	20	16	14	12

k = Number of treatments (mango varieties) to be compared

Table 5 Calculated sample sizes (n) required for statistical analysis of normalised values of radial vectors of mango (*Mangifera indica* L.) leaves measured at an angle of 175° relative to the orientation vector.

k	Smallest true difference (% of \bar{Y})				P<0.01							
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%
2	141	37	18	11	10 (8)	10 (6)	10 (4)	10 (3)	10 (3)	10 (3)	10 (2)	10 (2)
3	141	36	17	11	7	7 (5)	7 (4)	7 (3)	7 (3)	7 (3)	7 (3)	7 (3)
4	141	36	17	10	7	6 (5)	6 (4)	6 (3)	6 (3)	6 (2)	6 (2)	6 (2)
5	141	36	17	10	7	5	5 (4)	5 (3)	5 (3)	5 (2)	5 (2)	5 (2)
6	140	36	17	10	7	5	4	4 (3)	4 (3)	4 (2)	4 (2)	4 (2)
7	140	36	17	10	7	5	4	4 (3)	4 (3)	4 (2)	4 (2)	4 (2)
8	140	36	17	10	7	5	4	3	3	3 (2)	3 (2)	3 (2)
9	140	36	17	10	7	5	4	3	3	3 (2)	3 (2)	3 (2)
10	140	36	17	10	7	5	4	3	3	3 (2)	3 (2)	3 (2)
11	138	36	17	10	7	5	4	3	3	3 (2)	3 (2)	3 (2)
12	138	36	17	10	7	5	4	3	3	3 (2)	3 (2)	3 (2)
13	138	36	17	10	7	5	4	3	3	2	2	2
14	138	36	17	10	7	5	4	3	3	2	2	2
15	138	36	17	10	7	5	4	3	3	2	2	2
16	138	36	17	10	7	5	4	3	3	2	2	2
17	138	36	17	10	7	5	4	3	3	2	2	2
18	138	36	17	10	7	5	4	3	3	2	2	2
19	138	36	17	10	7	5	4	3	3	2	2	2
20	138	36	17	10	7	5	4	3	3	2	2	2
21	138	36	17	10	7	5	4	3	3	2	2	2
22	138	36	17	10	7	5	4	3	3	2	2	2
23	138	36	17	10	7	5	4	3	3	2	2	2
24	138	36	17	10	7	5	4	3	3	2	2	2
25	138	36	17	10	7	5	4	3	3	2	2	2

k	Smallest true difference (% of \bar{Y})				P<0.05							
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%
2	94	25	12	10 (7)	10 (5)	10 (4)	10 (3)	10 (3)	10 (2)	10 (2)	10 (2)	10 (2)
3	94	25	12	7	7 (5)	7 (4)	7 (3)	7 (3)	7 (2)	7 (2)	7 (2)	7 (2)
4	94	25	12	7	6 (5)	6 (4)	6 (3)	6 (3)	6 (2)	6 (2)	6 (2)	6 (2)
5	94	24	12	7	5	5 (4)	5 (3)	5 (3)	5 (2)	5 (2)	5 (2)	5 (2)
6	94	24	12	7	5	4	4 (3)	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)
7	94	24	12	7	5	4	4 (3)	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)
8	94	24	12	7	5	4	3	3 (2)	3 (2)	3 (2)	3 (2)	3 (2)
9	94	24	11	7	5	4	3	3 (2)	3 (2)	3 (2)	3 (2)	3 (2)
10	94	24	11	7	5	4	3	3 (2)	3 (2)	3 (2)	3 (2)	3 (2)
11	94	24	11	7	5	4	3	3 (2)	3 (2)	3 (2)	3 (2)	3 (2)
12	94	24	11	7	5	4	3	3 (2)	3 (2)	3 (2)	3 (2)	3 (2)
13	94	24	11	7	5	4	3	2	2	2	2	2
14	94	24	11	7	5	4	3	2	2	2	2	2
15	94	24	11	7	5	4	3	2	2	2	2	2
16	94	24	11	7	5	4	3	2	2	2	2	2
17	93	24	11	7	5	4	3	2	2	2	2	2
18	93	24	11	7	5	4	3	2	2	2	2	2
19	93	24	11	7	5	4	3	2	2	2	2	2
20	93	24	11	7	5	4	3	2	2	2	2	2
21	93	24	11	7	5	4	3	2	2	2	2	2
22	93	24	11	7	5	4	3	2	2	2	2	2
23	93	24	11	7	5	4	3	2	2	2	2	2
24	93	24	11	7	5	4	3	2	2	2	2	2
25	93	24	11	7	5	4	3	2	2	2	2	2

() : Values in parenthesis are the rejected calculated sample size (n_c) if ν (degrees of freedom) < 18.
k = Number of treatments (mango varieties) to be compared

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Table 6 Calculated sample sizes (n) required for statistical analysis of normalised values of radial vectors of mango (*Mangifera indica* L.) fruit measured at an angle of 10° relative to the orientation vector.

k	Smallest true difference (% of \bar{Y})					P<0.01						
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%
2	464	117	53	31	21	15	12	10	10 (8)	10 (6)	10 (6)	10 (5)
3	462	117	53	31	20	15	11	9	7	7 (6)	7 (5)	7 (5)
4	457	117	53	30	20	15	11	9	7	6	6 (5)	6 (4)
5	457	117	53	30	20	14	11	9	7	6	5	5 (4)
6	457	117	53	30	20	14	11	9	7	6	5	4
7	457	117	53	30	20	14	11	8	7	6	5	4
8	457	117	53	30	20	14	11	8	7	6	5	4
9	457	117	53	30	20	14	11	8	7	6	5	4
10	457	117	53	30	20	14	11	8	7	6	5	4
11	457	116	53	30	20	14	11	8	7	6	5	4
12	457	116	53	30	20	14	11	8	7	6	5	4
13	457	116	53	30	20	14	11	8	7	6	5	4
14	457	115	53	30	20	14	11	8	7	6	5	4
15	457	115	52	30	20	14	11	8	7	6	5	4
16	457	115	52	30	20	14	11	8	7	6	5	4
17	457	115	52	30	20	14	10	8	7	6	5	4
18	457	115	52	30	20	14	10	8	7	6	5	4
19	457	115	52	30	20	14	10	8	7	6	5	4
20	457	115	52	30	20	14	10	8	7	6	5	4
21	457	115	52	30	20	14	10	8	7	6	5	4
22	457	115	52	30	20	14	10	8	7	6	5	4
23	457	115	52	30	20	14	10	8	7	6	5	4
24	457	115	52	30	20	14	10	8	7	6	5	4
25	457	115	52	30	20	14	10	8	7	6	5	4

k	Smallest true difference (% of \bar{Y})					P<0.05						
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%
2	311	79	36	21	14	11	10 (8)	10 (6)	10 (5)	10 (4)	10 (4)	10 (3)
3	310	79	36	21	14	10	8	7 (6)	7 (5)	7 (4)	7 (4)	7 (3)
4	310	79	36	21	14	10	8	6	6 (5)	6 (4)	6 (4)	6 (3)
5	307	79	35	21	14	10	8	6	5	5 (4)	5 (4)	5 (3)
6	307	78	35	20	14	10	7	6	5	4	4	4 (3)
7	307	78	35	20	14	10	7	6	5	4	4	4 (3)
8	307	78	35	20	14	10	7	6	5	4	4	3
9	307	78	35	20	13	10	7	6	5	4	4	3
10	307	78	35	20	13	10	7	6	5	4	4	3
11	307	78	35	20	13	10	7	6	5	4	4	3
12	307	78	35	20	13	10	7	6	5	4	4	3
13	307	78	35	20	13	10	7	6	5	4	4	3
14	307	78	35	20	13	10	7	6	5	4	4	3
15	307	78	35	20	13	10	7	6	5	4	4	3
16	307	78	35	20	13	10	7	6	5	4	4	3
17	307	78	35	20	13	10	7	6	5	4	4	3
18	307	78	35	20	13	10	7	6	5	4	4	3
19	307	78	35	20	13	10	7	6	5	4	4	3
20	307	78	35	20	13	10	7	6	5	4	4	3
21	307	78	35	20	13	10	7	6	5	4	4	3
22	307	78	35	20	13	10	7	6	5	4	4	3
23	307	78	35	20	13	10	7	6	5	4	4	3
24	307	78	35	20	13	10	7	6	5	4	4	3
25	307	78	35	20	13	10	7	6	5	4	4	3

() : Values in parenthesis are the rejected calculated sample size (n_c) if v (degrees of freedom) < 18.
k = Number of treatments (mango varieties) to be compared

Table 7 Calculated sample sizes (*n*) required for statistical analysis of normalised values of radial vectors of mango (*Mangifera indica* L.) fruit measured at an angle of 225° relative to the orientation vector.

k	Smallest true difference (% of \bar{Y})					P<0.01						
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%
2	1201	306	137	78	51	36	27	21	17	15	13	11
3	1201	306	137	78	50	35	27	21	17	14	12	10
4	1201	305	137	77	50	35	26	21	17	14	12	10
5	1201	301	137	77	50	35	26	20	16	14	12	10
6	1201	301	136	77	50	35	26	20	16	14	11	10
7	1201	301	136	77	50	35	26	20	16	13	11	10
8	1201	301	136	77	50	35	26	20	16	13	11	10
9	1201	301	136	77	50	35	26	20	16	13	11	10
10	1201	301	136	77	50	35	26	20	16	13	11	10
11	1201	301	136	77	50	35	26	20	16	13	11	10
12	1201	301	134	77	50	35	26	20	16	13	11	10
13	1201	301	134	77	50	35	26	20	16	13	11	10
14	1201	301	134	77	50	35	26	20	16	13	11	10
15	1201	301	134	77	50	35	26	20	16	13	11	10
16	1201	301	134	77	50	35	26	20	16	13	11	10
17	1201	301	134	77	50	35	26	20	16	13	11	10
18	1201	301	134	77	50	35	26	20	16	13	11	10
19	1201	301	134	77	50	35	26	20	16	13	11	10
20	1201	301	134	76	50	35	26	20	16	13	11	10
21	1201	301	134	76	50	35	26	20	16	13	11	10
22	1201	301	134	76	50	35	26	20	16	13	11	10
23	1201	301	134	76	50	35	26	20	16	13	11	10
24	1201	301	134	76	50	35	26	20	16	13	11	10
25	1201	301	134	76	50	35	26	20	16	13	11	10

k	Smallest true difference (% of \bar{Y})					P<0.05						
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%
2	808	205	92	52	34	24	18	15	12	10	10 (8)	10 (7)
3	808	205	92	52	34	24	18	14	12	10	8	7
4	808	205	92	52	34	24	18	14	11	10	8	7
5	808	204	92	52	34	24	18	14	11	9	8	7
6	808	204	92	52	34	24	18	14	11	9	8	7
7	808	204	92	52	34	24	18	14	11	9	8	7
8	808	203	92	52	34	24	18	14	11	9	8	7
9	808	203	92	52	34	24	18	14	11	9	8	7
10	808	203	91	52	34	24	18	14	11	9	8	7
11	808	203	91	52	34	24	18	14	11	9	8	7
12	808	203	91	52	34	24	18	14	11	9	8	7
13	808	203	91	52	34	24	18	14	11	9	8	7
14	808	203	91	52	34	24	18	14	11	9	8	7
15	808	203	91	52	34	24	18	14	11	9	8	7
16	808	203	91	52	34	24	18	14	11	9	8	7
17	808	203	91	52	34	24	18	14	11	9	8	7
18	808	203	91	52	34	24	18	14	11	9	8	7
19	808	203	91	52	34	24	18	14	11	9	8	7
20	808	203	91	52	34	24	18	14	11	9	8	7
21	808	203	91	52	34	24	18	14	11	9	8	7
22	808	203	91	52	34	24	18	14	11	9	8	7
23	808	203	91	52	34	24	18	14	11	9	8	7
24	808	203	91	52	34	24	18	14	11	9	8	7
25	808	203	91	52	34	24	18	14	11	9	8	7

() : Values in parenthesis are the rejected calculated sample size (n_c) if v (degrees of freedom) < 18.
k = Number of treatments (mango varieties) to be compared

Table 8 Calculated sample sizes (n) required for statistical analysis of normalised values of radial vectors of mango (*Mangifera indica* L.) fruit measured at an angle of 315° relative to the orientation vector.

k	Smallest true difference (% of \bar{Y})					P<0.01 6%	7%	8%	9%	10%	11%	12%
	1%	2%	3%	4%	5%							
2	1172	299	134	76	49	35	26	21	17	14	13	11
3	1172	298	133	76	49	35	26	20	17	14	12	10
4	1172	297	133	76	49	34	26	20	16	14	12	10
5	1172	297	133	76	49	34	26	20	16	13	11	10
6	1172	294	133	76	49	34	25	20	16	13	11	10
7	1172	294	133	75	49	34	25	20	16	13	11	10
8	1172	294	133	75	49	34	25	20	16	13	11	9
9	1172	294	133	75	49	34	25	20	16	13	11	9
10	1172	294	133	75	49	34	25	20	16	13	11	9
11	1172	294	133	75	49	34	25	20	16	13	11	9
12	1172	294	131	75	49	34	25	20	16	13	11	9
13	1172	294	131	75	49	34	25	20	16	13	11	9
14	1172	294	131	75	49	34	25	20	16	13	11	9
15	1172	294	131	75	49	34	25	20	16	13	11	9
16	1172	294	131	75	49	34	25	20	16	13	11	9
17	1172	294	131	75	49	34	25	20	16	13	11	9
18	1172	294	131	75	49	34	25	20	16	13	11	9
19	1172	294	131	75	49	34	25	20	16	13	11	9
20	1172	294	131	75	49	34	25	20	16	13	11	9
21	1172	294	131	74	48	34	25	20	16	13	11	9
22	1172	294	131	74	48	34	25	20	16	13	11	9
23	1172	294	131	74	48	34	25	20	16	13	11	9
24	1172	294	131	74	48	34	25	20	16	13	11	9
25	1172	294	131	74	48	34	25	20	16	13	11	9

k	Smallest true difference (% of \bar{Y})					P<0.05 6%	7%	8%	9%	10%	11%	12%
	1%	2%	3%	4%	5%							
2	788	200	90	51	33	24	18	14	12	10	10 (8)	10 (7)
3	788	200	89	51	33	24	18	14	11	9	8	7
4	788	200	89	51	33	23	18	14	11	9	8	7
5	788	199	89	51	33	23	17	14	11	9	8	7
6	788	199	89	51	33	23	17	14	11	9	8	7
7	788	199	89	51	33	23	17	14	11	9	8	7
8	788	198	89	51	33	23	17	14	11	9	8	7
9	788	198	89	51	33	23	17	14	11	9	8	7
10	788	198	89	51	33	23	17	13	11	9	8	7
11	788	198	89	51	33	23	17	13	11	9	8	7
12	788	198	89	51	33	23	17	13	11	9	8	7
13	788	198	89	51	33	23	17	13	11	9	8	7
14	788	198	89	51	33	23	17	13	11	9	8	7
15	788	198	89	51	33	23	17	13	11	9	8	7
16	788	198	89	51	33	23	17	13	11	9	8	7
17	788	198	89	51	33	23	17	13	11	9	8	7
18	788	198	88	51	33	23	17	13	11	9	8	7
19	788	198	88	51	33	23	17	13	11	9	8	7
20	788	198	88	51	33	23	17	13	11	9	8	7
21	788	198	88	51	33	23	17	13	11	9	8	7
22	788	198	88	51	33	23	17	13	11	9	8	7
23	788	198	88	51	33	23	17	13	11	9	8	7
24	788	198	88	51	33	23	17	13	11	9	8	7
25	788	198	88	51	33	23	17	13	11	9	8	7

() : Values in parenthesis are the rejected calculated sample size (n_c) if v (degrees of freedom) < 18.
k = Number of treatments (mango varieties) to be compared

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