



TC-EDC/Jan14/15

ORIGINAL: English

DATE: December 13, 2013

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

ENLARGED EDITORIAL COMMITTEE**Geneva, January 8 and 9, 2014**

REVISION OF DOCUMENT TGP/8: PART II: SELECTED TECHNIQUES USED IN DUS EXAMINATION,
NEW SECTION 12: EXAMINING CHARACTERISTICS USING IMAGE ANALYSIS

Document prepared by experts from the Netherlands and the European Union

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1. The purpose of this document is to report on the development of document TGP/8: Part II: Techniques Used in DUS Examination, New Section: "Examining Characteristics Using Image Analysis".
2. The Technical Committee (TC), at its forty-eighth session, held in Geneva, from March 26 to 28, 2012, considered the proposal for New Section 12: "Examining Characteristics Using Image Analysis" in document TGP/8. The TC agreed that Subsection 12.1 "Introduction" of New Section: "Examining Characteristics Using Image Analysis" should be reworded to explain that image analysis would be an alternative method for observing a characteristic, rather than a principal method for observing a characteristic. The TC agreed that the Technical Working Party on Automation and Computer Programs (TWC) should develop Subsection 12.3 "Guidance on the use of image analysis" and agreed that a new section should be prepared on the basis of the discussion on documents TWC/29/19 "Image Analysis for DUS in the United Kingdom", TWC/29/21 "The Use of Image Tool in Measurements of Grain Length of Rye (*Secale cereale* L.), TWC/29/27 "Image Analysis in the Czech Republic" and TWC/29/29 "Image Analysis in the Netherlands". The drafters would be experts from Netherlands (first drafter), Czech Republic, Finland and the United Kingdom (see document TC/48/22 "Report on the Conclusions", paragraphs 56 to 58).
3. The TWC, at its thirtieth session, held in Chisinau, Republic of Moldova, from June 26 to June 29, 2012, agreed that a draft for New Section - Examining Characteristics Using Image Analysis for document TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability" should be prepared, by an expert from the Netherlands in collaboration with an expert from the European Union, for the TWP sessions in 2013 (see document TWC/30/41 "Report", paragraph 80).
4. The TC, at its forty-ninth session, held in Geneva, from March 18 to 20, 2013, noted the plans for the development of a New Section: "Examining Characteristics Using Image Analysis" for inclusion in document TGP/8, Part II: Techniques Used in DUS Examination, as set out in paragraphs 8 and 9 of document TC/49/33 "Revision of document TGP/8: Part II: Techniques Used in DUS Examination, New Section: Examining Characteristics Using Image Analysis".
5. The experts from the Netherlands and the European Union responsible for drafting the new section proposed that the first draft be presented only to the TWC in 2013.
6. The TWC, at its thirty-first session, in Seoul, from June 4 to 7, 2013, considered the draft of the new section "Examining Characteristics Using Image Analysis" for inclusion in document TGP/8, as contained in Annex to document TWC/31/20 Add. as presented by an expert from the European Union by electronic means. The TWC agreed that the expert from the European Union should revise the text to provide guidance on the use of the method with suitable language for inclusion in document TGP/8, to be presented to the TWPs at their sessions in 2014 (see document TWC/31/32 "Report", paragraph 81).

7. The Annex to this document provides the proposed text for “New Section: Examining Characteristics Using Image Analysis”, as presented by Mr. Gerrie van der Heijden (the Netherlands) and Mr. Jean Maison (European Union) at the thirty-first session of the TWC. The revisions to the text made by Mr. Jean Maison (European Union) following the consideration by the TWC (see paragraph, above) are indicated by highlighting and strikethrough for deletions and highlighting and underlining for additions.

8. *The TC-EDC is invited to note the information in this document to be presented to the TC and propose any improvements to the document in that regard.*

[Annex follows]

ANNEX

EXAMINING CHARACTERISTICS USING IMAGE ANALYSIS

INTRODUCTION

1. Image analysis is the extraction of information (e.g. plant measurements) from (digital) images by means of a computer. Image analysis is used in plant variety testing to help in the assessment of plant characteristics. It can be regarded as an intelligent measurement device (advanced ruler). This document aims to give guidance when using image analysis in plant variety testing.

2. Image analysis can be used in a fully automated or semi-automated way. When fully automated, the expert just records images of plant parts with a camera or scanner and the computer automatically calculates relevant characteristics without human interference. In a semi-automated way, the computer shows the images on a screen and a user can interact with the software to measure specific plant parts, e.g. by clicking with a mouse.

~~3. UPOV sent an image analysis questionnaire to all member states in 2012 on the use of image analysis. The results of this questionnaire are reproduced in document TWC/31/20 (see document TWC/31/20 "Revision of document TGP/8: Part II: Techniques used in DUS Examination, New Section: Examining Characteristics Using Image Analysis", paragraphs 3 and 4). Image analysis is used in more than 10 member states on a routine basis to measure a range of characteristics regarding size, shape, color and patterns of plant parts. The most often used characteristics are the size and shape of seeds.~~

IMAGE RECORDING: CALIBRATION AND STANDARDIZATION

~~3. 4.~~ An important aspect to consider when recording and analyzing digital images is standardization and calibration. Standardization is done by using as much as possible the same setup (illumination, camera, camera-settings, lens, perspective, and object-camera distance) for every recording. It is important to check that the recordings are done according to a prescribed protocol, as the software may depend on it. For example, pods may have to be orientated horizontally in the images, with the beaks pointing to the left. Calibration of the system is needed to make the recording as much as possible independent of any varying conditions by correcting for the variations, e.g. in size or color.

~~4. 5.~~ Size calibration: if we want to assess the length of e.g. a seed, we need to know the size of a pixel (picture element in a digital image) in the real world (e.g. mm/pixel), as the computer measures every object in an image in pixels. A standard way to perform this calibration is to include a ruler in every recorded image, at the same distance from the camera as the plant part being recorded. In that case the user can relate the size of the ruler to the number of pixels, and make the calibration manually. A preferred way is to use an object of standard dimensions, e.g. a coin, which can automatically be analyzed with the software and then used for an implicit size calibration. A coin also allows checking if pixels are square (i.e. if the aspect ratio of every pixel is 1:1). In all cases, the object should be sufficiently close to the calibration object and sufficiently far from the camera, to minimize the effect of varying magnification with distance. Alternatively a telecentric lens could be used to minimize this effect.

~~5. 6.~~ Illumination calibration: an object has to be segmented from the background in the image. An often used and very simple way to do this, is to use thresholding: a pixel with a (grey) value above a certain threshold is considered an object pixel and below the threshold a background pixel (or vice versa). If the illumination is not constant, it may occur that the segmentation is not optimal for every image and that part of the pixels are assigned to the wrong class (object/background), even if the threshold value is determined automatic. This may result in erroneous measurements. It is therefore advisable to check the segmentation results by having a quick look at the segmented binary images.

~~6. 7.~~ In many situations only a silhouette/contour of the plant material is necessary, e.g. for size and shape. In these cases it is often advisable to use a background illumination, e.g. a light box. This will increase the contrast between the background and the object, and make the segmentation result much less dependent on the threshold value.

~~7. 8.~~ Check that the lighting is homogenously distributed over the image. Darker parts in the image may result in a wrong segmentation and hence lead to incorrect and incomparable measures, especially when multiple objects are recorded in the same image.

8. 9. For colors and (variegation or blush) patterns on the plant part, it is essential that the illumination is done correctly and checked regularly, preferably for every image. In that case illumination calibration can be done by recording (part of) a standard color chart in the image. Special algorithms are available to correct for color changes due to differing illumination conditions, but in many situations this correction causes some loss of precision.



9. 40. The light source is of large influence on the observed color in the image. Especially for color, the type of light source is important. In many cases, lamp color and intensity change during warming up of the lamps, so let them burn about 15 minutes before starting the recordings. If fluorescent tubes are used, check regularly if they still have more or less the same intensity/color, as they may change rather rapidly with age. You can use the calibration chart for notification.

10. 44. Especially when recording shiny objects like apples or certain flowers, you need to be aware of specular reflection. Objects with specular spots cannot be measured reliably. In such cases, attention should be paid to uniform and indirect illumination, using special light tents as shown below.



11. 42. Both (color) cameras and scanners can be used for image recording. The choice is dependent on the application and the preference of the user. Other more advanced systems, such as 3D cameras or hyperspectral cameras are not yet used in standard plant variety testing.

ANALYSIS OF STANDARD UPOV CHARACTERISTICS

12. 43. In general image analysis is used to automate the measurement of characteristics described in the guidelines of UPOV. In that case the aim is to replace a hand measurement by a computer measurement. This requires an additional calibration in addition to the image recording calibration. The measurements can then be checked with manual measurements for consistency, e.g. by a scatterplot of hand versus computer measurement with a regression line and the line $y=x$.

13. 44. In some cases, image analysis requires a more precise and mathematical definition of the characteristic than is required for human experts. E.g. the length of the pod can be redefined as the length of the medial axis of the pod, excluding the stem. In such cases, there is a special need to check for differences

in behavior for different genotypes (bias). The measurement for some genotypes may be exactly the same, whereas for others a systematic difference may be present. A nice example is for determining the bulb height in onions (van der Heijden, Vossepoel and Polder, 1996), where the top of the bulb was defined as the bending point of the shoulder. As long as such a change or refinement of the definition of a characteristic is known and accounted for, this is not a problem. In general, it is advisable to consult the crop experts for redefining a characteristic and check with UPOV if a minor modification of the guideline might be necessary.

14. 45. In some cases the object consists of different parts which have to be measured separately, e.g. the pod, beak and stem of a pod of French bean. This requires a special algorithm to separate the different parts (distinguish stem and beak from the pod) and this has to be tested extensively on a large number of genotypes in the reference collection, to be sure that the implementation is robust over the entire range of expressions.

15. 46. Shape characteristics can also be measured with image analysis, but in general it will be restricted to characteristics already in the guideline, e.g. by defining the shape as the ~~ratio~~ ratio between length and width.

16. 47. Although color is a standard UPOV characteristic, and could be measured by image analysis, it is not used often. ~~Color measurements by image analysis are described in document TWC/24/15 "Image Analysis of Ornamentals, with Emphasis to Rose and Alstroemeria".~~ In most cases, crop experts still rely on visual observation with RHS color charts.

ANALYSIS OF NON STANDARD CHARACTERISTICS

17. 48. In addition to standard characteristics, image analysis offers the possibility to assess more complex characteristics which could be more difficult to observe visually or to measure.. E.g. the total shape distribution of an onion can be described by storing the onion width along the different positions of the length axis, the ground coverage of foliage could be observed more precisely than with a visual observation, disease resistance could be assessed in measuring the area of infection on a leaf or the curvature of the perimeter of leaves could help assessing the fineness of foliage.

CONCLUSIONS

18. 49. Image analysis is used for measurements and to automate, at least partially, the assessment of characteristics. It requires a good and precise definition of the characteristic, computerization using existing or home-made software, a good preparation of samples, checking with existing procedures, careful calibration and standardization. It often necessitates therefore an investment which can only be profitable versus hand assessment of characteristics if it concerns a significant number of measurements or measurements which are difficult and time consuming to assess by the examiner. In case of organs of a small size, seed size for example, image analysis will be more precise and more reliable.

19. 20. Image analysis offers the possibility to store information: images can be recorded and analyzed at a later stage in order to avoid peaks of work and they can be retrieved at a later stage to compare varieties for example in case of doubt.

20. 24. Today it is mainly used for size and shape features but with the development of techniques, it will be possible to use it for a wider range of characteristics in future.

REFERENCES

van der Heijden, G., A. M. Vossepoel & G. Polder (1996) Measuring onion cultivars with image analysis using inflection points. *Euphytica*, 87, 19-31.