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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
Geneva

TECHNICAL COMMITTEE

Fifty-First Session
Geneva, March 23 to 25, 2015

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

Document prepared by the Office of the Union

1. The purpose of this document is to report on developments concerning document TGP/8: Part II: Techniques Used in DUS Examination, New Section: “Examining Characteristics Using Image Analysis”.

2. The following abbreviations are used in this document:

TC:	Technical Committee
TC-EDC:	Enlarged Editorial Committee
TWA:	Technical Working Party for Agricultural Crops
TWC:	Technical Working Party on Automation and Computer Programs
TWF:	Technical Working Party for Fruit Crops
TWO:	Technical Working Party for Ornamental Plants and Forest Trees
TWPs:	Technical Working Parties
TWV:	Technical Working Party for Vegetables

BACKGROUND

3. The background to this matter is provided in document TC/50/27 “Revision of document TGP/8: Part II: Selected Techniques Used in DUS Examination, New Section: Examining Characteristics Using Image Analysis”.

DEVELOPMENTS IN 2014

Technical Committee

4. The TC, at its fiftieth session, held in Geneva, from April 7 to 9, 2014, considered document TC/50/27 “Revision of document TGP/8: Part II: Selected Techniques Used in DUS Examination, New Section: Examining Characteristics Using Image Analysis”.

5. The TC agreed to the redrafting of the proposed text, by an expert from the European Union, into a standard TGP style of impersonal speech and to add the following introduction to the proposed text, as set out in document TC/50/27, paragraph 9 (see document TC/50/36 “Report on the Conclusions”, paragraph 63), as follows:

"1. Introduction
"Characteristics which may be examined by image analysis should also be able to be examined by visual observation and/or manual measurement, as appropriate. Explanations for observing such characteristics, including where appropriate explanations in Test Guidelines, should ensure that the characteristic is explained in terms which would enable the characteristic to be understood and examined by all DUS experts."

"2. Combined characteristics
"2.1 The General Introduction (document TG/1/3, Chapter 4, Section 4) states that:

'4.6.3 <u>Combined Characteristics</u>

'4.6.3.1 A combined characteristic is a simple combination of a small number of characteristics. Provided the combination is biologically meaningful, characteristics that are assessed separately may subsequently be combined, for example the ratio of length to width, to produce such a combined characteristic. Combined characteristics must be examined for distinctness, uniformity and stability to the same extent as other characteristics. In some cases, these combined characteristics are examined by means of techniques, such as Image Analysis. In these cases, the methods for appropriate examination of DUS are specified in document TGP/12, 'Special Characteristics'.'

"2.2 Thus, the General Introduction clarifies that the use of image analysis is one possible method for examining characteristics which fulfill the basic requirements for use in DUS testing (see document TG/1/3, Chapter 4.2), which includes the need for the uniformity and stability of such characteristics to be examined. With regard to combined characteristics, the General Introduction also explains that such characteristics should be biologically meaningful."

<u>Technical Working Parties</u>

6. At their sessions in 2014, the TWO, TWF, TWC, TWV and TWA considered documents TWO/47/20, TWF/45/20, TWC/32/20, TWV/48/20, TWV/48/20 Add. and TWA/43/20 "Revision of document TGP/8: Part II: Selected Techniques Used in DUS Examination, New Section: Examining Characteristics Using Image Analysis", respectively.

7. The TWO, TWF, TWC, TWV and the TWA noted the proposal from the expert from the European Union to prepare a new draft for New Section "Examining Characteristics Using Image Analysis" for inclusion in document TGP/8 for consideration by the TC and the TWPs at their sessions in 2015 (see documents TWO/47/28 "Report", paragraph 55, TWF/45/32 "Report", paragraph 46, TWC/32/23 "Report", paragraph 41, TWV/48/43 "Report", paragraph 56 and TWA/43/27 "Report", paragraph 51).

8. The TWO and TWC agreed to request the drafter to consider including typical examples of characteristics that could be assessed by image analysis, such as leaf area and length / width of grain (see documents TWO/47/28, paragraph 56 and TWC/32/23, paragraph 42).

9. The TWC noted that experiences on the use of image analysis would be presented to the TWV (see document TWC/32/23, paragraph 43).

10. The TWV received presentations from experts from Czech Republic, France, Netherlands and United Kingdom on their use of image analysis for DUS examination, as reproduced in annexes to document TWV/48/20 Add. "Addendum to Revision of Document TGP/8: Part II: Selected Techniques used in DUS Examination, New Section: Examining Characteristics using Image Analysis" (see document TWV/48/43, paragraph 57).

11. The TWV agreed that some of the software currently used for Image Analysis should be mentioned in UPOV/INF/22 "Software and equipment used by members of the Union" (see document TWV/48/43, paragraph 58).

12. The TWV agreed that experts from Czech Republic, France, the Netherlands, Poland and the United Kingdom would help the drafter of the European Union in the preparation of a new draft for consideration by the TC and the TWPs at their sessions in 2015 (see document TWV/48/43, paragraph 59).

13. The TWA agreed on the importance of precise definition of characteristics to be assessed using image analysis (see document TWA/43/27, paragraph 49).

14. The TWA noted the use of image analysis: in Australia, for measurement of leaf length and width in ornamental plants; in Denmark, for measurement of petals, cotyledons and siliquas in oilseed rape and length of ears and awns in barley; in the United Kingdom, for measurement of petals, cotyledons and siliquas in oilseed rape, and various characteristics in sugar beet and field beans; and in France for the assessment of cotyledons in oilseed rape (see document TWA/43/27, paragraph 50).

15. A new draft, proposed by an expert from the European Union, is reproduced in the Annex to this document.

Enlarged Editorial Committee

16. The TC-EDC, at its meeting held in Geneva, on January 7 and 8, 2015, considered document TC-EDC/Jan15/11 "Revision of document TGP/8: Part II: Techniques Used in DUS Examination, New Section: Examining Characteristics Using Image Analysis" and made the following recommendations:

Annex, Introduction and paragraph 19	The TC-EDC noted the contradiction between the Introduction and paragraph 19 of the draft guidance and recommended to delete paragraph 19
Annex, paragraph 5	to add "in cases where image analysis is automated" at the end of the first sentence
Annex, paragraph 14	to delete heading above paragraph
Annex, paragraph 18	to read "RHS colour chart"
Annex, paragraph 22	to read "…possible to use it for a wider range of standard UPOV characteristics in future."

17. The TC is invited to consider the proposed draft guideline on "Examining Characteristics Using Image Analysis", as presented in the Annex to this document, in conjunction with the comments made by TC-EDC at its meeting in 2015, as set out in paragraph 16.

[Annex follows]

ANNEX

Document prepared by experts from the European Union

EXAMINING CHARACTERISTICS USING IMAGE ANALYSIS

INTRODUCTION

1. Section III of document TGP/12/1 Draft 7 "Special Characteristics" reads:

> *"Characteristics which may be examined by image analysis should also be able to be examined by visual observation and/or manual measurement, as appropriate. Explanations for observing such characteristics, including where appropriate explanations in Test Guidelines, should ensure that the characteristic is explained in terms which would enable the characteristic to be understood and examined by all DUS experts."*

2. Combined characteristics

2.1 The General Introduction (document TG/1/3, Chapter 4, Section 4) states that:

> *'4.6.3 Combined Characteristics*
>
> *'4.6.3.1 A combined characteristic is a simple combination of a small number of characteristics. Provided the combination is biologically meaningful, characteristics that are assessed separately may subsequently be combined, for example the ratio of length to width, to produce such a combined characteristic. Combined characteristics must be examined for distinctness, uniformity and stability to the same extent as other characteristics. In some cases, these combined characteristics are examined by means of techniques, such as Image Analysis. In these cases, the methods for appropriate examination of DUS are specified in document TGP/12, 'Special Characteristics'.'*

"2.2 Thus, the General Introduction clarifies that the use of image analysis is one possible method for examining characteristics which fulfill the basic requirements for use in DUS testing (see document TG/1/3, Chapter 4.2), which includes the need for the uniformity and stability of such characteristics to be examined. With regard to combined characteristics, the General Introduction also explains that such characteristics should be biologically meaningful."

~~1.~~3. 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.~~4. 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.

IMAGE RECORDING: CALIBRATION AND STANDARDIZATION

~~3.~~5. 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.

~~5.~~6. Size calibration is necessary. Since the measure unit in pictures is the pixel, a relation needs to be established between the pixels on the image and millimeters~~: 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.

~~4.~~7. Illumination calibration is also necessary: 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 automatically. 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.

~~5.~~8. 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.

9. It should be ensured ~~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.

10. 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.

11. 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 which should consequently sufficiently be warmed up~~, so let them burn about 15 minutes~~ before starting the recordings. If fluorescent tubes are used, it should ~~check~~ regularly be verified that ~~if~~ they ~~still~~ have more or less the same intensity/color, as they may change rather rapidly with age. ~~You can use the c~~Calibration charts can be used to this purpose ~~for notification~~.

12.10.Especially when recording shiny objects like apples or certain flowers, you need to be aware of specular reflection needs to be taken into account. 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.

13.11.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

14.12.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.

15.13.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.

16.14. 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.

17.15.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 ration ratio between length and width.

18.16. 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 colour charts.

ANALYSIS OF NON STANDARD CHARACTERISTICS

19.17.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

20.~~18.~~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.

21.~~19.~~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.

22.~~20.~~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.

[End of Annex and of document]