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IMAGE ANALYSIS IN DUS TESTING IN NIAB

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IMAGE ANALYSIS IN DUS TESTING AT NIAB (U.K.).

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Introduction

1. Much DUS testing involves the observation and recording of morphological characters of plants. These observations may be in the form of visual "scores", or may require measurements of some kind. There are various measurements that can be made, but in many species there are UPOV characteristics based on the size and/or shape of organs such as seeds, cotyledons, leaflets, leaves, and pods. UPOV Test Guidelines do not generally prescribe how these measurements are to be made.

2. Image analysis (also known as machine vision or automatic measurement), IA, has been in use at NIAB since the mid-1980s and has been used directly for DUS testing in the UK since the early 1990s. There are several potential advantages to the use of IA:

- More accurate measurements
- More precise measurements
- More consistent (reproducible) measurements
- Reduction of risks (lower operator errors)
- Secures expertise
- Reduction of costs
- Provides opportunity to develop new characteristics, e.g. features which the crop expert can recognise, but has previously been unable to describe or quantify adequately
- Image databases can be used for management of reference collections
- Image databases can be used for other purposes (e.g. verification of identity, research)

3. The IA systems developed and applied at NIAB, for both agricultural and ornamental crops, have enabled all of these advantages to be realised, albeit to differing degrees in various species. In many cases, what were previously lengthy and tedious manual processes have been automated, which has enabled increased numbers of varieties to be analysed with no increase in resources and has produced benefits in improving the discrimination between varieties. In addition, experience has shown that ratios (cf. "Combined Characteristics", TG/1/3, 4.6.3) give less variable data than simple measurements and can minimise difficulties with the provenance of material or other environmental interactions, as they reduce the influence of absolute size.

4. This paper will summarise some of the current uses of IA at NIAB, concentrate in more detail on the approaches taken in oilseed rape, and outline some possible future developments.

Current applications

5. In the context of agricultural crop DUS testing, IA is currently used at NIAB in field beans, linseed and oilseed rape. IA systems and databases are also in development for chrysanthemums, and shortly will be investigated for application to roses.

<u>Field beans (Vicia faba L.)</u>

6. The use of IA in field bean DUS testing is well established in the UK, and Table 1 summarises the characteristics currently recorded using IA in field beans:

Table 1 – Current Use of IA for DUS Testing of Field Beans in the UK.

UPOV TG/8/6 Char. No.	**U.K. Char. No.**	**Characteristic**
4 *	32	Leaflet: length
5 *	33	Leaflet: width
6	34	Leaflet: widest point to base
	93	Leaflet: size
	92	Leaflet: leaf area/length x width
	94	Leaflet: width/length
	95	Leaflet: base to widest point/length
	96	Leaflet: basal angle
	97	Leaflet: top angle
15*	42	Pod: length without beak
16	43	Pod: median width (from suture to suture)

7. It is evident that this represents a mixture of characteristics, some included in the current UPOV Test Guidelines (e.g. numbers 4, 5, 6, where IA provides an automated, accurate and precise method of measurement) and others that have been found to be valuable in the UK. The national characteristics are both straightforward measurements (e.g. 95) and calculated or derived characters (e.g. 94). IA is particularly useful in instances where there are visual differences between varieties which are difficult to assess manually, e.g. areas, or ratios. It should be noted that uniformity as well as distinctness is assessed for all characteristics when required.

<u>Linseed (Linum usitatissimum L.)</u>

8. In linseed, DUS applications of IA in the UK have concentrated on examining the shape of the seeds, which can be seen by crop experts to vary in different varieties but which has proved difficult to describe. The characteristics used are summarised in Table 2.

Table 2 – IA of Seed Characteristics in Linseed.

U.K. Char. No.	**Characteristic**
81	Seed area
82	Seed width
83	Seed length
U.K. Char. No.	**Characteristic**
84	Seed base to nearest wide point
85	Seed base to furthest wide point
86	Seed: Distance from base to mean wide point
87	Seed: Aspect ratio
88	A measure of how rectangular the seed appears
89	The aspect ratio of the distal half of the seed i.e. below the wide point
90	The aspect ratio of the narrowing portion of the seed at the distal end of the seed.
91	Seed: The relative position of the near wide point in relation to the length
92	Seed: The relative position of the far wide point in relation to the length
93	Seed: The relative position of the mean wide point in relation to the length

9. None of these are UPOV Test Guidelines characteristics – currently, the seed characteristics in the Test Guidelines are Seed: weight per 1000 seeds (which is a measure of seed size to some degree) and Seed: color (14). The first five of the characteristics in Table 2 (81-85) are all simple measurements, whereas the remainder are derived characteristics which describe more or less complex shape attributes, and thus could be considered to have a biological meaning. Research has shown that the most useful characteristics in terms of discrimination for D purposes are 82, 83, 87 and 89. The measure of discriminating power, the F1 statistics, for all of seed characteristics are much higher than for the current UPOV Test Guidelines characteristics, whilst the lambda values (a measure of repeatability or consistency over tests) are equivalent to or less than those for the UPOV Test Guidelines characteristics. Again, where used for D, varieties have to be sufficiently uniform for the seed characteristics. More recently, it has been shown that the use of IA to assess shape characteristics in submitted seed samples could provide a means of reducing the unit costs of linseed DUS testing.

Oilseed rape (*Brassica napus*)

10. Oilseed rape (OSR) represents probably the best developed and applied system of IA currently in use for DUS testing purposes at NIAB. Table 3 lists the characteristics that are recorded using IA, both routinely (i.e. on all varieties) and only in special cases (i.e. where D

has otherwise proved difficult to establish). To date, these are all features measured on the cotyledons. Uniformity is always also assessed where necessary. An explanation of the nature of the measurements is provided in Figure 1.

Table 3 – IA of Oilseed Rape for DUS purposes in the UK.

A. Currently used (recorded on all varieties)

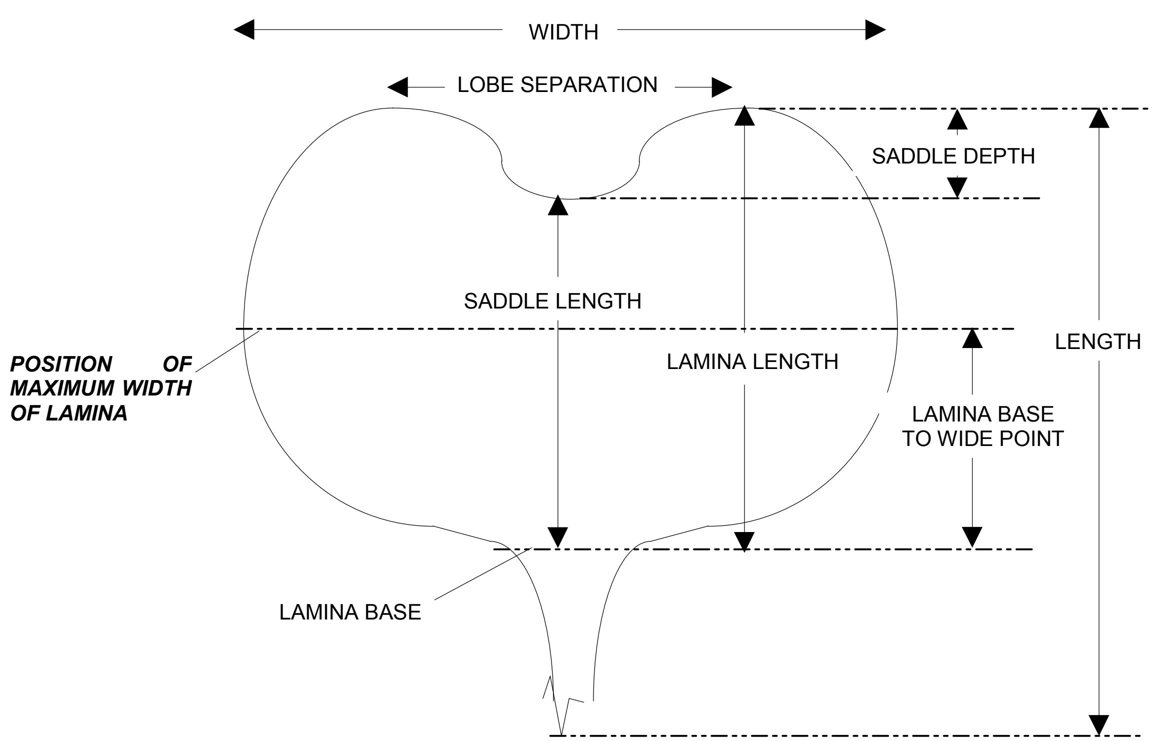
UPOV TG/36/6 Char. No.	**U.K. Char. No.**	**Characteristic**	**Sample size for assessment**	**Method of assessment**	**States of expression**
3	10	Cotyledon: width at widest point	40 plants per variety: 20 plants per replicate.	Measured IA	1 to 9 very narrow to very wide
2	12	Cotyledon: Length	40 plants per variety: 20 plants per replicate.	Measured IA	1 to 9 very short to very long
	13	Cotyledon: width/length Ratio	40 plants per variety: 20 plants per replicate.	Calculated IA	1 to 9 very small to very large

B. Additional (national characteristics, only used if necessary)

UPOV TG/36/6 Char. No.	**U.K. Char. No.**	**Characteristic**	**Sample size for assessment**	**Method of assessment**	**States of expression**
	15	Cotyledon: lobe separation	40 plants per variety: 20 plants per replicate	Measured Image Analysis	1 to 9 very small to very large
	16	Cotyledon: lamina base to wide point (lbtwp)	40 plants per variety: 20 plants per replicate	Measured Image Analysis	1 to 9 very small to very large
	17	Cotyledon: lamina length	40 plants per variety: 20 plants per replicate.	Measured Image Analysis	1 to 9 very short to very long
	70	Cotyledon: lobe separation/ width ratio	40 plants per variety: 20 plants per replicate.	Measured Image Analysis	1 to 9 very small to very large
	71	Cotyledon: lobe separation/ lamina length ratio	40 plants per variety: 20 plants per replicate.	Measured Image Analysis	1 to 9 very small to very large

UPOV TG/36/6 Char. No.	U.K. Char. No.	Characteristic	Sample size for assessment	Method of assessment	States of expression
	72	Cotyledon: saddle length/lamina length ratio	40 plants per variety: 20 plants per replicate.	Measured Image Analysis	1 to 9 very small to very large
	73	Cotyledon: lbtwp/lamina length ratio	40 plants per variety: 20 plants per replicate.	Measured Image Analysis	1 to 9 very small to very large
	74	Cotyledon: lbtwp/width ratio	40 plants per variety: 20 plants per replicate.	Measured Image Analysis	1 to 9 very small to very large
	75	Cotyledon: lobe separation/ saddle depth ratio	40 plants per variety: 20 plants per replicate.	Measured Image Analysis	1 to 9 very small to very large
	76	Cotyledon: (saddle length lbtwp)/ lamina length ratio	40 plants per variety: 20 plants per replicate.	Measured Image Analysis	1 to 9 very small to very large

Figure 1 - Diagrammatic representation of an oilseed rape cotyledon illustrating the parameters measured by IA.



11. The cotyledon characteristics, both routine and additional, have proven to be very useful for OSR DUS testing purposes in the UK. They provide some of the highest levels of discrimination between varieties (as measured by F1 statistics) and show levels of consistency within and between years (lambda values) that are comparable to other DUS characteristics in OSR.

12. Recent work has enabled considerable improvements to be made to the IA system used for OSR work at NIAB. Briefly, the operational approach is as follows:

Method

Cotyledons are gathered from seed sown in controlled conditions. The larger of the two cotyledons from any given seedling should be used. Damaged or deformed cotyledons should not be used. The cotyledons are laid out evenly on an acetate sheet, with the upper side of the cotyledon placed face down and with all the hypocotyls pointing downwards. The plot number (identifier) is written in the top left-hand corner and the sample is left for at least an hour until the cotyledons lie flat. A maximum of 20 cotyledons should be placed on one acetate.

Imaging

For each crop application, specific camera height, lighting and reference objects are required:

COTYLEDONS-	
CAMERA SETTING	: 1.0 (AGC)
REFERENCE OBJECT	: 90mm BLACK CIRCLE ACETATE(300 pixels by 300 pixels)
DIFFUSERS	: TWO
HEIGHT @ TOP POINT	: 53.4 CM
LENS	: CAMISCAR
LAYOUT	: :20 LEAVES ‘FACE DOWN’ IN ROWS (5 * 4)

System

The system consists of a fixed mounted camera attached to a “riser” stand. This allows the camera to be moved to the required distance from the object. A light box is placed underneath and opaque diffusers can be placed on top of the light box, controlling the amount of reflected light into the camera. There are two monitors, which allow the user to operate the program on one monitor and to handle the data (e.g. with Excel or Word) on the other monitor. The GLI software (below) has a “dongle” attached to a serial port in the back of the camera, which acts as a licensing tool. A PCI- or AGP-compatible frame-grabber transfers the image from the camera into the vision processors or PC memory, for processing.

Global Lab/Image and other software

The current version of the DUS image analysis program used at NIAB makes use of the Global Lab/Image (GLI) software library. GLI is used mainly for its interface to the frame grabber hardware, and for a few basic image processing functions, such as individual object boundary extraction and area measurement. Other software is custom-written and consists of a program called DUS ImageAPP.exe. This is a Windows-style program with a menu-driven system allowing the user to select different crops and measures to analyse, as required.

The camera captures the image, storing length and width measurements in individual plot files. These files are given the file extension (.ia), and the program will automatically calculate derived characters from these data files. These files are stored as (.der) files. Also, the program will store each individual plot file as a bitmap. These bitmaps can be re-analysed at any point, so no data are lost.

The program will make an audible “beep” if there are problems with the plot image. The image should contain 20 spaced objects - if there less than 20, the user is warned and provided with a message stating the number of objects. The program will also notify the user if objects have been placed too close to the edge of the field of view of the camera.

Future prospects

13. The system outlined above can be used to measure OSR cotyledons, linseed seeds and bean leaves and pods. However, given the way that it has been written, it could in principle be applied to almost any plant material that can be imaged. Thus the developments of the IA facilities at NIAB have increased the prospects of further improvements in DUS testing technology. There are for instance a range of currently recorded characteristics in OSR (both UPOV and national) that could be amenable to more automated measurement – Table 4 illustrates these, many of which are being examined in on-going research. Other features of linseed, such as characteristics of the bolls, could also be investigated, as well as other measured characters in beans, such as flower length (UPOV Guideline Char. No. 7).

Table 4 - Possibilities for Future IA Applications in Oilseed Rape (characteristics currently recorded manually).

UPOV TG/36/6 Char. No.	**U.K. Char. No.**	**Characteristic**
13	52	Flower: length of petals
14	53	Flower: width of petals
	54	Petal width/length ratio
8	26	Leaf: length (blade and petiole)
9	27	Leaf: width (widest point)
10	28	Varieties with lobed leaves Leaf: length of petiole
	92	Silique: length/ Number of seeds
	93	Silique: pod length/pedicel length ratio
	94	Silique: pod length/beak length ratio
	61	Silique: width at widest point
20	62	Silique: length of peduncle
18	63	Silique: length (between peduncle and beak)
19	64	Silique: length of beak

14. Other developments include the possible use of IA in the field, e.g. to take measurements of, e.g. leaves, whilst they are attached to the plant rather than having to pick them and bring them in to the laboratory. There are clearly practical difficulties with such an approach, but advantages as well which make this an intriguing prospect.

Conclusions

15. IA is crucial to the DUS testing operations in certain crops in the UK and confers all of the potential advantages identified above.

16. The UK strategy has been to concentrate on automating the recording of existing characteristics, whilst at the same time deriving new and meaningful characters. The imaging technology and computing power now available increase the opportunities for the uses of IA in the DUS testing context.

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