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**THE POSSIBILITY OF REDUCING THE NUMBER OF ASSESSED PLANTS FOR
QUANTITATIVE CHARACTERISTICS FOR REFERENCE VARIETIES**

Document prepared by experts from Denmark and Germany

Introduction

1. For many crops it is common to make observations on 50-60 plants in each growing season. However, for some characteristics the variability between plants within the same plot may be very small compared to the variability between plots or between years. For such characteristics, the discrimination power of the COY-D method may be very insensitive to the number of plants observed in each plot. In this paper we present a method that can be used to calculate the number of plants to be observed if a small reduction of the discrimination power can be accepted.

Method

2. The method used is based on the calculation of LSD-values. In order to calculate the LSD-value for different numbers of plants the variance components for: (a) variety×year, (b) plot within year and block, and (c) plant within plot has to be available. These values are then used to set up a formula for calculating the number of plants to assess each year if the LSD value can be accepted to increase slightly. We choose to calculate the number of plants to assess if the LSD value could be accepted to increase by 1 %, 5 % or 10 %.

3. The details can be described as follows: as a start, the LSD-value is calculated for a comparison of observing the recommended 50-60 plants per growing season. This value is called LSD_a . Given the variance component the LSD-value can be calculated as:

$$LSD_a = t * \sqrt{\frac{2}{y} \sigma_{y \times v}^2 + \frac{2}{yr} \sigma_{plot}^2 + \frac{1}{yn} \sigma_{plant}^2 + \frac{1}{ym} \sigma_{plant}^2}$$

where

$t = 99.5\%$ fractile in the t - distribution

$y =$ number of years

$r =$ number of replication (complete blocks) in each year

$n =$ number of observed plants per year for the candidate variety

$m =$ number of observed plants per year for the reference variety

$\sigma_{y \times v}^2 =$ variance component for year by variety

$\sigma_{plot}^2 =$ variance component for plots within year and block

$\sigma_{plant}^2 =$ variance component for plants within plots

4. Now we have to determine the minimum value of m that would increase the LSD-value by a certain percentage, i.e. the value of m that would yield an LSD-value of $c \times LSD_a$ (where c is the constant that makes the LSD-value increase by 1 %, 5 % or 10 %). Solving for this we get:

$$m = \left\{ \frac{y c^2 LSD_a^2}{t^2 \sigma_{plant}^2} - 2 \frac{\sigma_{y \times v}^2}{\sigma_{plant}^2} - \frac{2}{r} \frac{\sigma_{plot}^2}{\sigma_{plant}^2} - \frac{1}{n} \right\}^{-1}$$

As we cannot assess a fraction of a plant, we have to round up the value of m to the nearest integer that is a multiple of the number of replicates.

5. The variance components were estimated by the method REML (see e.g. Searle et al., 1992) using the following model:

$$Y_{yrvp} = \mu + \alpha_v + A_y + B_{yr} + C_{yv} + D_{yrv} + E_{yrvp}$$

where

Y_{yrvp} = Assessed value for variety v on plant p in replicate (block) r in year y

μ and α are systematic effects for the mean and varieties

A , B , C , D and E are random effects. It is assumed that all are i.i.d. normally distributed variables with mean 0 and variances σ_{year}^2 , $\sigma_{replicate}^2$, $\sigma_{year \times variety}^2$, σ_{plot}^2 and σ_{plant}^2 , respectively

Examples

6. The examples are based on data with Yellow Mustard (YM) and Winter Oil Seed Rape (WOR). The data are summarised in Table 1.

Table 1: Summary of data

Location	Crop	Years	Number of			
			Varieties	Replicates	Plants	Characteristics
Tystofte, DK	YM	1997-1998	62	3	20	13
Scharnhorst, DE	WOR	2002-2004	595	3	20	12
Nossen, DE	WOR	2003-2004	301	3	20	12

7. At the time of observation, no Test Guidelines were available for Yellow Mustard, so the Test Guidelines for Rape Seed were used. First we show the estimated variance components and the LSD-value (based on COY-D) that would result if all 3×20 observations were taken each year (Table 2). The three variance components show all contributions to the variance for comparing different varieties, but the importance of the individual components varies from characteristic to characteristic and also to some degree from trial location to trial location. For some characteristics, the variability caused by plant to plant variation played a very important role, i.e. for the characteristic “6 Leaf: number of lobes” the plant to plant variability accounts for about 80% to 90% of the components whereas for the characteristic “8 Leaf: length (blade and petiole)” the plant to plant variation only accounts for about 40% to 50% of the components.

Table 2a: Range, variance components and LSD when using all 60 observations per year in 2 years in Denmark

UPOV characteristic number ^{a)} and name	Range ^{b)}	Variance components			LSD _a
		y×v	Plot	Plant	
2 Cotyledon: length	4.9	0.19	0.38	1.73	1.53
3 Cotyledon: width	7.1	0.18	1.04	6.28	2.06
6 Leaf: number of lobes	3.2	0.02	0.06	0.69	0.59
8 Leaf: length (blade and petiole)	65.4	33.44	219.85	161.47	27.20
9 Leaf: width	41.0	0.65	49.48	127.04	11.41
10 Leaf (6.): length of petiole	46.0	0.00	125.09	174.09	17.36
13 Flower: length of petals	2.8	0.10	0.24	0.58	1.13
14 Flower: width of petals	2.4	0.06	0.10	0.39	0.84
16 Plant: height at full flowering	60.5	24.48	49.93	9.92	16.71
17 Plant: total length including side branches	64.3	26.09	53.30	135.55	17.66
18 Siliqua: length	13.4	0.61	1.44	13.53	2.99
19 Siliqua: length of beak	8.0	0.39	0.63	6.99	2.20
20 Siliqua: length of peduncle	5.9	0.19	0.40	3.47	1.61

^{a)} according to UPOV Test Guidelines TG/36/6.

^{b)} range between means of varieties.

Table 2b: Range, variance components and LSD when using all 60 observations per year in 3 years for the location Scharnhorst

UPOV characteristic number ^{a)} and name	Range ^{b)}	Variance components			LSD _a
		y×v	Plot	Plant	
2 Cotyledon: length	5.9	0.11	0.78	1.91	1.35
3 Cotyledon: width	8.9	0.49	1.33	4.77	2.14
6 Leaf: number of lobes	8.2	0.08	0.15	1.21	0.82
8 Leaf: length (blade and petiole)	122.8	209.92	246.26	469.98	36.76
9 Leaf: width	35.2	17.19	25.78	125.11	11.21
10 Leaf (6.): length of petiole	97.6	131.54	145.21	311.33	28.88
11 Time of flowering	23.4	0.94	0.28	6.08	2.26
17 Plant: total length including side branches	93.6	17.08	21.40	67.76	10.69
18 Siliqua: length	28.4	3.10	3.24	28.69	4.58
19 Siliqua: length of beak	7.7	0.07	0.43	2.92	1.08
20 Siliqua: length of peduncle	16.9	0.28	1.52	5.72	2.00
00 Siliqua: width	2.3	0.01	0.02	0.09	0.27

^{a)} according to UPOV Test Guidelines TG/36/6.

^{b)} range between means of varieties.

Table 2c: Range, variance components and LSD when using all 60 assessments per year in 2 years for the location Nossen

UPOV characteristic number ^{a)} and name	Range ^{b)}	Variance components			LSD _a
		y×v	Plot	Plant	
2 Cotyledon: length	7.3	0.31	7.04	16.32	1.41
3 Cotyledon: width	11.2	2.55	12.27	47.19	2.24
6 Leaf: number of lobes	5.6	0.06	0.14	0.85	0.91
8 Leaf: length (blade and petiole)	119.6	113.13	176.86	213.90	34.46
9 Leaf: width	51.1	8.74	37.40	82.25	12.35
10 Leaf (6.): length of petiole	81.2	50.71	118.65	133.33	25.00
11 Time of flowering	24.3	0.43	0.14	3.30	1.90
17 Plant: total length including side branches	113.2	29.92	17.25	52.70	15.72
18 Siliqua: length	40.0	2.08	6.76	20.40	5.62
19 Siliqua: length of beak	10.5	0.16	0.34	2.52	1.47
20 Siliqua: length of peduncle	16.7	0.45	0.71	4.81	2.28
00 Siliqua: width	2.2	0.01	0.02	0.13	0.34

^{a)} according to UPOV Test Guidelines TG/36/6.

^{b)} range between means of varieties.

Table 3a: Number of plants to assess for different levels of acceptable increase in the LSD-value (Denmark)

UPOV characteristic number ^{a)} and name	Level of increase		
	1%	5%	10%
2 Cotyledon: length	40.5	17.4	10.0
3 Cotyledon: width	48.3	26.9	17.0
6 Leaf: number of lobes	50.8	31.2	20.8
8 Leaf: length (blade and petiole)	22.8	6.4	3.3
9 Leaf: width	43.9	20.9	12.4
10 Leaf (6.): length of petiole	37.1	14.5	8.0
13 Flower: length of petals	33.7	12.1	6.6
14 Flower: width of petals	36.5	14.0	7.8
16 Plant: height at full flowering	5.4	1.1	0.6
17 Plant: total length including side branches	33.0	11.6	6.3
18 Siliqua: length	48.6	27.3	17.4
19 Siliqua: length of beak	48.1	26.6	16.8
20 Siliqua: length of peduncle	47.4	25.5	15.9

^{a)} according to UPOV Test Guidelines TG/36/6.

8. In Table 3, the minimum number of plants to be observed on the reference varieties if the different levels of acceptable increase in LSD-values may not exceed 1%, 5% or 10%. UPOV characteristics 6 and 19 were found to be most sensitive to a reduction in the number of observed plants, but other characteristics, 3, 18, 20 and the German characteristic “Siliqua width” were found to be rather sensitive to a reduction in the number of observed plants. The reason for this is that a very large part of the recorded variability for these characters seemed to be caused by plant to plant variation (Table 2). On the other end of the scale, UPOV characteristic 16 was the characteristic found to be least sensitive to reduction in the number

of observed plants. For this characteristic, the LSD would be increased by only 1% if the number of recorded plants was reduced from 60 to 6, i.e. from 20 plants per plot to 2 plants per plot. If the number of plants per plot was reduced to just 1 plant per plot (3 plants per year) the LSD-value for this characteristic would increase by less than 5%. This is the characteristic that is most sensitive to variety×year interaction and plot to plot variation. When judging the effect of accepting the LSD-value to be increased, it may be valuable to compare the increase to the range of expression for the character. For this characteristic a 5% increase means an increase from e.g. 100 cm to 105 cm, about 5 cm, for a characteristic where the difference between the smallest and highest variety is 65 cm. Also characteristics 8 and 10 were rather insensitive to the number of observed plants on all locations.

Table 3b: Number of plants to observe for different levels of acceptable increase in the LSD-value for the location Scharnhorst

UPOV characteristic number ^{a)} and name	Level of increase		
	1%	5%	10%
2 Cotyledon: length	39.8	16.7	9.5
3 Cotyledon: width	39.7	16.6	9.4
6 Leaf: number of lobes	46.4	24.1	14.8
8 Leaf: length (blade and petiole)	23.6	6.8	3.5
9 Leaf: width	39.0	16.0	9.1
10 Leaf (6.): length of petiole	24.6	7.2	3.8
11 Time of flowering	41.4	18.2	10.5
17 Plant: total length including side branches	31.5	10.7	5.8
18 Siliqua: length	43.1	20.0	11.8
19 Siliqua: length of beak	49.4	28.6	18.5
20 Siliqua: length of peduncle	43.7	20.7	12.3
00 Siliqua: width	41.4	18.3	10.6

^{a)} according to UPOV Test Guidelines TG/36/6.

Table 3c: Number of plants to observe for different levels of acceptable increase in the LSD-value for the location Nossen

UPOV characteristic number ^{a)} and name	Level of increase		
	1%	5%	10%
2 Cotyledon: length	41.9	18.7	10.9
3 Cotyledon: width	43.5	20.4	12.1
6 Leaf: number of lobes	44.6	21.7	13.0
8 Leaf: length (blade and petiole)	20.1	5.4	2.8
9 Leaf: width	36.1	13.7	7.6
10 Leaf (6.): length of petiole	22.4	6.3	3.2
11 Time of flowering	43.1	20.0	11.8
17 Plant: total length including side branches	22.4	6.3	3.2
18 Siliqua: length	38.7	15.7	8.9
19 Siliqua: length of beak	45.9	23.4	14.3
20 Siliqua: length of peduncle	43.3	20.2	11.9
00 Siliqua: width	45.1	22.4	13.5

^{a)} according to UPOV Test Guidelines TG/36/6.

9. The calculations shown are valid only if the variance components for the coming trials will be the same as in the trials used for estimating the variance components. As this probably will not be the case it is recommended not to reduce the number of plants as much as indicated by the shown calculations. A strategy that most probably would increase the LSD-value by less 1 % could be to:

1. continue to observe all 60 plants for characteristics observed on cotyledons, siliquas, number of lobes lobes and time of flowering;
2. reduce the number of observations for total plant length, leaf, and petal measurements from 60 to 45 or from 60 to 30;
3. reduce the number of observations for plant height at full flowering from 60 to 15.

Remarks

10. The calculations show that for some characteristics the number of observations may be decreased without increasing the LSD-value by more than 1%. If it can be accepted to increase the LSD-value by a larger percentage, e.g. accept a 5% increase, then the number of observations may be reduced to less than 30 plants for almost all characteristics.

11. For the assessment of uniformity of candidate varieties, it might be beneficial to reduce the number of plants for reference varieties only. In that case 50-60 plants should still be observed in each growing season for the candidate varieties.

12. It is expected that reduction of the number of observed plants for reference varieties will only have a small effect on the tests for uniformity as long as all 50-60 plants are observed for candidate varieties. This is because in COY-U the uncertainty used for comparing the variability of the individual candidate varieties is the average variability of the relevant reference varieties. So, if this mean includes many reference varieties, the variance of the difference will only change marginally as the component for the candidate will be dominating. However, this means that the present COY-U method may have to be adapted in order to handle different number of observations in reference and candidate varieties.

13. The results from the 3 locations seem to be in good accordance with each other as characteristics for which the LSD-values are sensitive to the number of plants are almost the same and the characteristics where the LSD-values are relatively insensitive to the number of plants are almost the same.

14. All calculations have been done in a SAS-macro using the procedures mixed, summary and the data-step facilities of SAS.

References

Searle, S.R., Casella, G. & McCulloch, C.E. 1992. Variance Components. John Wiley and Sons Inc. New York

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