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UPOV

# INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

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INFLUENCE OF NUMBER OF PLANTS PER PLOT ON THE ASSESSMENT OF UNIFORMITY AND DISTINCTNESS FOR QUANTITATIVE CHARACTERISTICS IN RAPE SEED AND YELLOW MUSTARD

*Document prepared by experts from Denmark and Germany*

INTRODUCTION

1.      The purpose of this document is to show the influence of the number of plants per plot on uniformity and distinctness.  The Test Guidelines recommend the number of plants for the species which apply to all characteristics.  The Test Guidelines recommend the use of 60 plants (e.g. 3 times 20 plants) for assessment of distinctness and uniformity.  For some species the workload for the assessment of all quantitative characteristics on 60 plants is very high and crop experts are looking for possibilities to reduce this workload.

Trial data

Computations were made with DUS data of Germany and Denmark.

Germany:  Data of years 2002 to 2004 on rape seed.  There are two locations 'Scharnhorst' and 'Nossen'.

Denmark:  Data of years 1997 and 1998 on Yellow Mustard of location 'Tystofte'.
The numbers of reference and candidate varieties are given in Table 1.

Table 1: Locations, years and number of varieties

| Location | Year | Number of reference varieties | Number of candidate varieties (3 years) | Number of candidate varieties (2 years) | Total number of varieties |
|---|---|---|---|---|---|
| Scharnhorst (DE) | 2002, 2003, 2004 | 301 | 57 | 100 | 458 |
| Nossen (DE) | 2003, 2004* | 286 | - | 154 | 440 |
| Tystofte (DK) | 1997, 1998 | 52 | | 10 | 62 |

* data of year 2002 are missing in Nossen because of changing a trial station

2.     The most important quantitative characteristics, their characteristic in the UPOV Test Guidelines and the method of assessment are shown in Table 2.

Table 2:  Characteristics, UPOV number and type of assessment

| Characteristic | Char. no. | Assessment |
|---|---|---|
| Cotyledon: length | 2 | Image analysis |
| Cotyledon: width | 3 | Image analysis |
| Leaf: length | 8 | Measurement |
| Leaf: width | 9 | Measurement |
| Leaf: number of lobes | 6 | Count |
| Leaf: length of petiole | 10 | Measurement |
| Plant: total length including side branches | 17 | Measurement |
| Siliqua: length | 18 | Measurement |
| Siliqua: length of beak | 19 | Measurement |
| Siliqua: width | (M214)* | Measurement |
| Siliqua: length of peduncle | 20 | Measurement |
| Time of flowering | 11 | Count |

* (M214) is not a UPOV characteristic, it is the German number of a national characteristic

Methods

3.     To reduce the number of plants per plot, sub-samples of 60 plants were formed in different ways.  Table 3 shows the different cases, which depend on the number of plants per plot and number of replications used.

Table 3: Different sub-samples of 60 plants depending on number of plants per plot and number of replications

| Case | Number of plants per variety | Number of replication x number of single plants | Comment |
|---|---|---|---|
| A | 60 | 3 x 20 | Standard |
| B | 45 | 3 x 15 | First 15 plants |
| C | 40 | 2 x 20 | Replication 1 and 2 |
| D | 40 | 2 x 20 | Replication 1 and 3 |
| E | 30 | 3 x 10 | First 10 plants |
| F | 30 | 3 x 10 | Second 10 plants |

4.     To estimate the influence on assessment of uniformity COY-U criterion for all cases (case B to F) were calculated and compared to the standard calculation (case A).

5.     Additionally the influence on results of distinctness calculations (COY-D) was examined. This calculation was done by using data of reference varieties of the German trial station 'Scharnhorst' only.  LSD values were calculated for each case and all varieties were compared to each other.

Results

(a)    Uniformity assessment

6.    In Table 4, different results of uniformity assessments for case A and each of the other cases for each characteristic are shown (Comparison of results of case A and case B, of case A and case C and so on).

7.    The maximum number of different decisions between the standard case and the case with reduced number of plants is 4.9% for characteristic 18 'Siliqua: length'.  All Siliqua characteristics and the characteristic 'Time of flowering' have an increased percentage of different results.

8.    The 2-year results of the German trial station 'Nossen' also have an increased percentage of different results in comparison to the 3-year results of the German trial station 'Scharnhorst'.

9.    There is no tendency to an increasing percentage of different results by decreasing number of plants per plot if percentage of number of reference varieties is used as criterion. The results obtained from the Danish trial station 'Tystofte' are nearly the same as for the German trial stations.

Table 4:  Different uniformity results for each characteristic (percentage of number of reference varieties) for cases as described in Table 3

| UPOV No. | Scharnhorst* (3-year results) % | | | | | Nossen* (2-year results) % | | | | |
| | Case B 3x15 | Case C 2x20 | Case D 2x20 | Case E 3x10 | Case F 3x10 | Case B 3x15 | Case C 2x20 | Case D 2x20 | Case E 3x10 | Case F 3x10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0.0 | 0.3 | 0.3 | 0.3 | 0.0 | 1.4 | 1.8 | 2.1 | 1.8 | 2.1 |
| 3 | 1.0 | 1.7 | 1.0 | 1.0 | 1.0 | 3.5 | 3.9 | 4.2 | 3.2 | 4.9 |
| 8 | 1.3 | 1.0 | 1.0 | 1.3 | 1.3 | 1.8 | 1.4 | 3.2 | 1.4 | 2.1 |
| 9 | 0.7 | 0.7 | 0.7 | 1.0 | 0.7 | 2.8 | 2.1 | 1.8 | 2.5 | 2.1 |
| 6 | 1.7 | 1.0 | 1.0 | 1.3 | 1.3 | 1.4 | 1.8 | 1.8 | 1.8 | 1.1 |
| 10 | 1.0 | 1.3 | 1.0 | 1.0 | 1.0 | 1.4 | 1.4 | 1.8 | 1.4 | 1.4 |
| 17 | 1.3 | 0.3 | 1.0 | 1.3 | 1.0 | 1.4 | 2.5 | 2.5 | 1.4 | 1.8 |
| 18 | 3.3 | 2.3 | 4.0 | 3.3 | 2.7 | 4.9 | 3.5 | 2.5 | 3.9 | 2.8 |
| 19 | 2.0 | 1.7 | 1.7 | 1.7 | 2.0 | 2.1 | 1.8 | 1.8 | 2.1 | 4.2 |
| M214 | 2.0 | 2.7 | 2.0 | 1.7 | 3.0 | 1.8 | 1.4 | 2.1 | 2.5 | 2.5 |
| 20 | 0.7 | 1.7 | 1.7 | 1.0 | 1.7 | 3.2 | 3.5 | 2.1 | 2.8 | 3.2 |
| 11 | 2.7 | 1.7 | 1.3 | 3.3 | 3.0 | 2.8 | 2.8 | 2.1 | 3.5 | 3.5 |

*Number of reference varieties:  Scharnhorst = 301;  Nossen = 286

| UPOV No | Denmark* (2-year results) % | | | | |
| | Case B 3x15 | Case C 2x20 | Case D 2x20 | Case E 3x10 | Case F 3x10 |
|---|---|---|---|---|---|
| 2 | - | 3.8 | - | - | - |
| 3 | - | 3.8 | - | - | - |
| 8 | 0.0 | 0.0 | 0.0 | 1.9 | 0.0 |
| 9 | 1.9 | 3.8 | 1.9 | 1.9 | 1.9 |
| 6 | 0.0 | 1.9 | 0.0 | 1.9 | 1.9 |
| 10 | 0.0 | 1.9 | 3.8 | 0.0 | 1.9 |
| 17 | 0.0 | 3.8 | 0.0 | 1.9 | 3.8 |
| 18 | 0.0 | 1.9 | 0.0 | 1.9 | 0.0 |
| 19 | 0.0 | 3.8 | 3.8 | 0.0 | 1.9 |
| M214 | - | - | - | - | - |
| 20 | 0.0 | 0.0 | 3.8 | 1.9 | 0.0 |
| 11 | - | - | - | - | - |

* Number of reference varieties:  Denmark = 52

**Absolute number of changing decisions based on Case B to F compared to Case A
(Scharnhorst)**



**Absolute number of changing decisions based on Case B to F compared to Case A
(Nossen)**



**Absolute number of changing decisions based on Case B to F compared to Case A
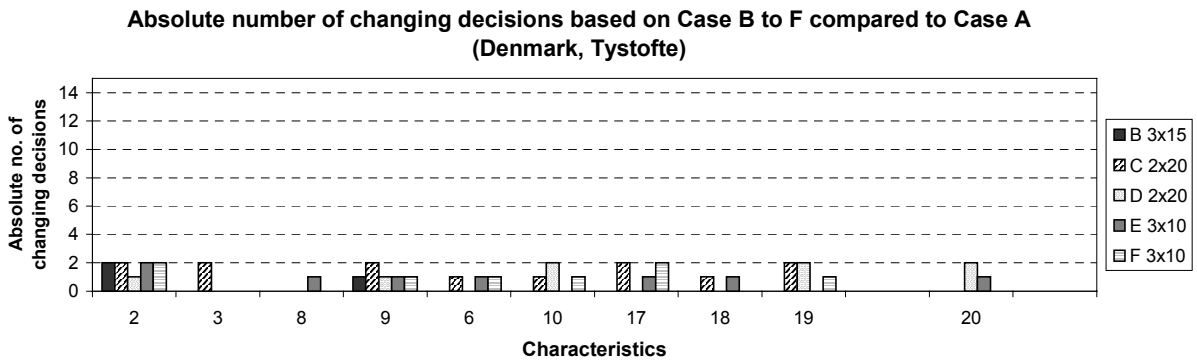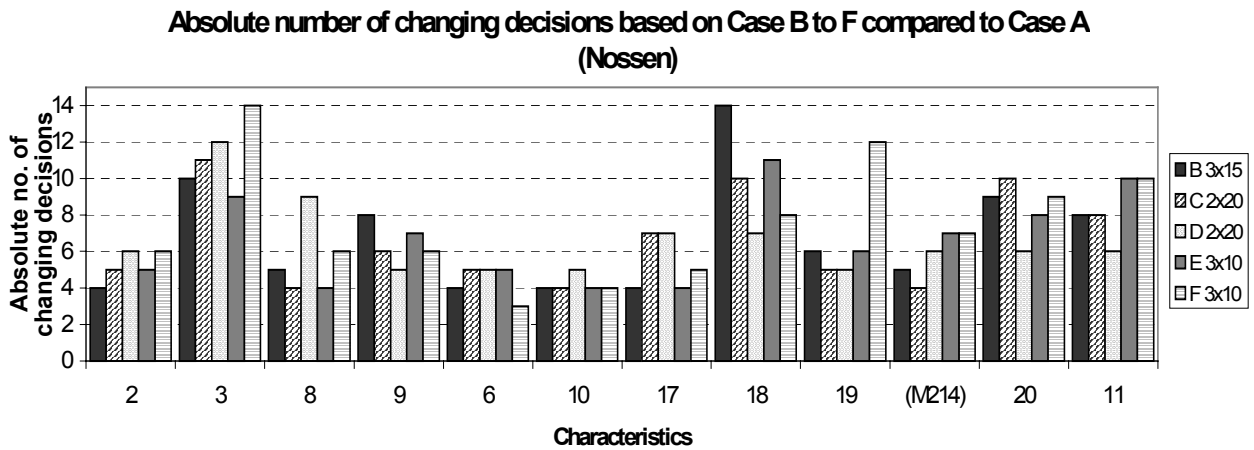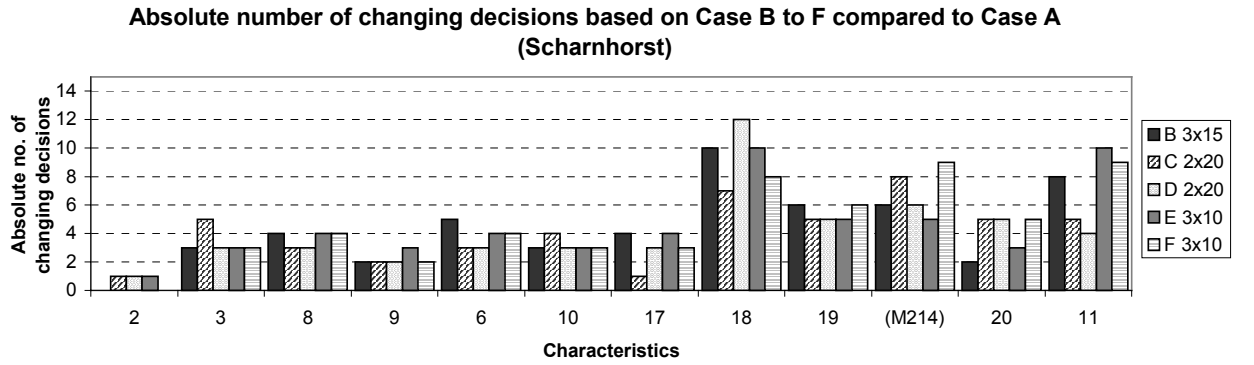(Denmark, Tystofte)**



Figure 1:  Absolute uniformity results of cases B to F with reduced number of plants (reference varieties only) for German trial stations 'Scharnhorst' and 'Nossen' and for Danish trial station 'Tystofte'.

10.    Further differentiation of results which differ by direction of changes (uniform to non-uniform and reverse) shows, at German location 'Scharnhorst', a tendency to increasing of uniformity decisions by reduced number of plants (Table 5).  This tendency is weaker for 2-years results at German location 'Nossen'.

11.    In Table 5, the actual situation (case A) is compared to a sub-sample (case B to F).  The sign '-/+' means that case A is compared to case B, for example.  The decision changes from non-uniform to uniform.  The sign '+/-' means that case A is compared to case B, for example.  The decision changes in the other direction from uniform to non-uniform.

12.    The number of reference varieties at trial station 'Tystofte' is smaller than at the other trial stations (52 instead of 301 or 286).

Table 5:  Differentiation of uniformity results which differ by direction of changes

| UPOV No. | Case B 3x15 | | Case C 2x20 | | Case D 2x20 | | Case E 3x10 | | Case F 3x10 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | -/+* | +/- | -/+ | +/- | -/+ | +/- | -/+ | +/- | -/+ | +/- |
| Scharnhorst (301 reference varieties, 3-year results) | | | | | | | | | | |
| 2 | | | | 1 | | 1 | | 1 | | |
| 3 | 3 | | 3 | 2 | 3 | | 3 | | 3 | |
| 8 | 3 | 1 | 3 | | 3 | | 3 | 1 | 3 | 1 |
| 9 | 2 | | 2 | | 2 | | 2 | 1 | 2 | |
| 6 | 3 | 2 | 2 | 1 | 2 | 1 | 3 | 1 | 1 | 3 |
| 10 | 3 | | 3 | 1 | 3 | | 3 | | 3 | |
| 17 | 3 | 1 | 1 | | 3 | | 3 | 1 | 3 | |
| 18 | 7 | 3 | 3 | 4 | 7 | 5 | 6 | 4 | 6 | 2 |
| 19 | 4 | 2 | 3 | 2 | 4 | 1 | 4 | 1 | 3 | 3 |
| M214 | 4 | 2 | 4 | 4 | 3 | 3 | 4 | 1 | 3 | 6 |
| 20 | 2 | | 4 | 1 | 5 | | 3 | | 4 | 1 |
| 11 | 5 | 3 | 5 | | 3 | 1 | 5 | 5 | 6 | 3 |
| Nossen (286 reference varieties, 2-year results) | | | | | | | | | | |
| 2 | 4 | | 5 | | 5 | 1 | 4 | 1 | 5 | 1 |
| 3 | 7 | 3 | 6 | 5 | 7 | 5 | 6 | 3 | 7 | 7 |
| 8 | 3 | 2 | 2 | 2 | 3 | 6 | 3 | 1 | 3 | 3 |
| 9 | 4 | 4 | 3 | 3 | 2 | 3 | 3 | 4 | 2 | 3 |
| 6 | 2 | 2 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 1 |
| 10 | 4 | | 4 | | 4 | 1 | 4 | | 4 | |
| 17 | 3 | 1 | 2 | 5 | 2 | 5 | 3 | 1 | 2 | 3 |
| 18 | 6 | 8 | 5 | 5 | 3 | 4 | 7 | 4 | 4 | 4 |
| 19 | 4 | 2 | 3 | 2 | 3 | 2 | 4 | 2 | 9 | 3 |
| M214 | 5 | | 2 | 2 | 5 | 1 | | | | 3 |
| 20 | 4 | *5* | 5 | 5 | 5 | 1 | 7 | 1 | 5 | 4 |
| 11 | 4 | 4 | 4 | 4 | 3 | 3 | 5 | 5 | 6 | 4 |
| Tystofte (52 reference varieties, 2-year results) | | | | | | | | | | |
| 2 | - | - | 1 | 1 | - | - | - | - | - | - |
| 3 | - | - | 1 | 1 | - | - | - | - | - | - |
| 8 | | | | | | | | 1 | | |
| 9 | 1 | | 1 | 1 | 1 | | 1 | | 1 | |
| 6 | | | | 1 | | | | 1 | | 1 |
| 10 | | | | 1 | | 2 | | | | 1 |
| 17 | | | 1 | 1 | | | | 1 | 1 | 1 |
| 18 | | | | 1 | | | | 1 | | |
| 19 | | | 1 | 1 | 1 | 1 | | | 1 | |
| M214 | - | - | - | - | - | - | - | - | - | - |
| 20 | | | | | 1 | 1 | | 1 | | |
| 11 | - | - | - | - | - | - | - | - | - | - |

* (standard sample / sub-sample;    + => uniform, - => non uniform

13.   A limit of allowed changes could be 1% for 3-year results as sum of two possibilities (-/+ and +/-) per comparison of different cases.  This is an assumption.  So for characteristics 18, 19, M214 and 11 (Siliqua characteristics and Time of flowering) reduction of the number of plants is impossible at location 'Scharnhorst'.

14.   There is no best case of reduction for this example.

(b)   Distinctness assessment

15.   The effect on distinctness assessment is described below.

16.   In Table 6 the actual situation (case A) is compared to a sub-sample (case B, C and E). The sign '-/+' means that case A is compared to case B, for example, and the decision changes from not distinct to distinct.  The sign '+/-' means that case A is compared to case B, for example, and the decision changes in the other direction, from distinct to not distinct.

17.   Further differentiation of results which differ by direction of changes (distinct to not distinct and vice versa) shows, at the German location 'Scharnhorst', a tendency to decreasing of the number of distinct variety pairs by reduced number of plants and reduced number of replications (Table 6).

Table 6:  Distinctness results of German trial station 'Scharnhorst' which differ by direction of changes for each characteristic (percentage of total number of variety pairs)

| UPOV No. | Scharnhorst (3-year results)* % | | | | | |
| | Case B 3x15 | | Case C 2x20 | | Case E 3x10 | |
| | +/-** | -/+ | +/- | -/+ | +/- | -/+ |
| 2 | 5.1 | **1.4** | 9.7 | **0.9** | 6.5 | **1.8** |
| 3 | 5.7 | **1.2** | 7.4 | **1.2** | 7.7 | **1.4** |
| 8 | 1.5 | **0.7** | 4.4 | **1.9** | 2.3 | **1.3** |
| 9 | 2.3 | **1.2** | 7.0 | **1.9** | 4.5 | **1.8** |
| 6 | 2.4 | **1.1** | 5.1 | **1.5** | 4.9 | **1.6** |
| 10 | 1.3 | **0.9** | 4.2 | **1.8** | 2.2 | **1.4** |
| 17 | 1.4 | **0.9** | 4.2 | **1.2** | 3.0 | **1.3** |
| 18 | 2.1 | **1.3** | 3.8 | **1.4** | 4.3 | **1.5** |
| 19 | 2.3 | **1.2** | 8.3 | **0.9** | 4.6 | **1.5** |
| M214 | 2.3 | **1.1** | 7.6 | **1.1** | 4.3 | **1.4** |
| 20 | 1.3 | **1.0** | 5.0 | **2.0** | 2.7 | **1.2** |
| 11 | 2.2 | **1.8** | 2.3 | **2.1** | 3.5 | **2.7** |

*   301 reference varieties => 45150 pair-wise comparisons
** (standard sample / sub-sample;   + => distinct, - => not distinct)

18.   A limit of allowed changes could be 5% as the sum of two possibilities (-/+ and +/-) per comparison of different cases.  This is an assumption.  So for characteristic 2 'Cotyledon: length' and characteristic 3 'Cotyledon: width' a reduction of the number of plants is

impossible.  Case C is the worst case for all characteristics except characteristic 11 'Time of flowering'.

19.   The best case is case B in comparison to case A, but the reduction of workload is very small.

20.   The results of Danish trial station 'Tystofte' do not differ greatly from results of German trial station 'Scharnhorst' (Table 7).  For some cases, different data structure (two replications instead of three) did not allow all calculations to be performed.

Table 7: Distinctness results of Danish trial station 'Tystofte' which differ by direction of changes for each characteristic (percentage of total number of variety pairs)

| UPOV No. | Tystofte (2-year results)* % | | | | | |
|---|---|---|---|---|---|---|
| | Case B 3x15 | | Case C 2x20 | | Case E 3x10 | |
| | +/-** | -/+ | +/- | -/+ | +/- | -/+ |
| 2 | 0.9 | **1.0** | - | - | - | - |
| 3 | 1.1 | **5.4** | - | - | - | - |
| 8 | 1.1 | **0.7** | 6.5 | **1.6** | 1.4 | **2.0** |
| 9 | 6.9 | **0.0** | 8.4 | **2.3** | 8.7 | **0.5** |
| 6 | 0.2 | **3.8** | 4.1 | **1.4** | 2.6 | **2.8** |
| 10 | 1.8 | **0.5** | 7.4 | **2.7** | 2.0 | **1.1** |
| 17 | 1.7 | **1.0** | 10.1 | **1.4** | 11.6 | **0.1** |
| 18 | 3.3 | **2.6** | 8.0 | **2.3** | 6.3 | **2.3** |
| 19 | 3.4 | **2.1** | 7.4 | **1.2** | 4.0 | **3.4** |
| M214 | - | - | - | - | - | - |
| 20 | 1.8 | **2.0** | 10.2 | **0.7** | 3.0 | **2.0** |
| 11 | - | - | - | - | - | - |

* 52 reference varieties => 1326 pair-wise comparisons
** (standard sample / sub-sample;   + => distinct, - => not distinct)

Conclusion

21.   The conclusion on these examples is that there are possibilities to reduce the number of plants, but there is no general rule for all characteristics.  Crop experts and statisticians need to look for effects on uniformity and distinctness simultaneously.

22.   For some characteristics a reduction to 45 plants is possible (e.g.: Plant: total length, UPOV characteristic number 17).  For other characteristics, a reduction is impossible (e.g.: Cotyledon: length and width, UPOV characteristic number 2 and 3) unless more deviating results can be tolerated.

23.   To obtain stable results it is necessary to include more growing cycles in the study.

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