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REVISION OF DOCUMENT TGP/8:
PART II: TECHNIQUES USED IN DUS EXAMINATION
New Section – Statistical Methods for Visually Observed Characteristics

Document prepared by an expert from Denmark

BACKGROUND

1. The Technical Committee (TC), at its forty-eighth session, held in Geneva from March 26 to 28, 2012, considered the revision of document TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability" on the basis of document TC/48/19 Rev. The TC noted that new drafts of relevant sections would need to be prepared by April 26, 2012, in order that the sections could be included in the draft to be considered by the TWPs at their sessions in 2012 (see document TC/48/22 "Report on Conclusions" paragraph 49).

2. The TC, at its forty-eighth session, agreed that the section should be redrafted with assistance from DUS experts in Denmark in order to focus on guidance for DUS examiners and should replace detailed statistical models with a general reference to suitable statistical methods. The TC agreed that the examples based on sugar beet should be replaced by a crop for which there are Test Guidelines and that the example for wheat should be replaced by a realistic example, such as could be found in Hemp or Spinach. The TC also agreed that the TWC should explore the consequences of the decisions for DUS examination, because the method is a test for differences in the distribution (both location and dispersion). It also agreed that the consequences of excluding certain varieties from the test, where there were insufficient numbers in some cells, should be further investigated (see document TC/48/22 "Report on Conclusions" paragraph 61).

3. This document contains a proposed text for a new section "Statistical methods for visually observed characteristics" of document TGP/8.

PROPOSED TEXT FOR: TGP/8/1: PART II: STATISTICAL METHODS FOR VISUALLY OBSERVED CHARACTERISTICS

THE COMBINED OVER-YEARS METHOD FOR NOMINAL CHARACTERISTICS

Summary of requirements for application of the method

The method is appropriate to use for assessing distinctness of varieties where:

- The characteristic is nominal and recorded for individual plants (usually recorded visually)
- There are some differences between plants
- The observations are made over at least two years or growing cycles on a single location
- There should be at least 20 degrees of freedom for estimating the random variety-by-year interaction term.
- The expected number of plants for each combination of variety and note should be at least one – and for most of the combinations the number should be at least 5.

Summary

The method can be considered as an alternative to the χ^2-test for independence in a contingency table. The χ^2-test only takes the variation caused by random sampling into account and may thus be too liberal if additional sources of variation are present. The combined over-years method for nominal characteristics takes other sources of variation into account by including a random variety-by-year interaction term (as for the COYD method described in TGP/8/1 Part II: 3). The inclusion of the random effect is expected to decrease the number of distinct pairs of varieties compared to the χ^2-test for independence, but to better ensure that the decisions are consistent over coming years. The method is based on a generalisation of the traditional analyses of variance and regression methods for normally distributed data, which are called "generalized linear mixed models". A detailed description of the method – using other examples of data may be found in Kristensen (2011).

The combined over-years method for nominal characteristics involves

- Calculating the number of plants for each note for each variety in each of the two or three years of trials, which results in a 3-way table (see the example)
- Analyse the data using appropriate software
- Compare each candidate to the reference varieties and the other candidates at the appropriate level of significance to see which varieties the candidate is distinct from
- Check if the variety-by-year interaction term for distinct pairs is considerably larger than the average for all variety pairs

Example

For demonstration a subset of varieties from a DUS experiment with sugar beets was chosen. The notes for hypocotyl colour (Table 1) were analysed. Because some varieties had notes with zero plants in both years, there were difficulties in meeting the requirements mentioned above. Therefore, the varieties *M*, *N*, *O*, *Q*, *R*, *S* and *V* were excluded from the analyses shown here.

The estimated percent of plants in each note for each variety are shown in table 2.

Treating varieties *A* and *B* as candidates and the remaining varieties *C, D, …, U,* as reference varieties, the F-values and the P-values for testing the hypothesis of no difference between candidate and reference varieties were calculated. The F-values and the P-values are shown in Table 3. The F_3-values and their significances are also shown in Table 3.

Using the 1% level of significance as a decision rule for comparing the candidates with the reference varieties, we found that candidate *A* was distinct from 7 of the other varieties, while candidate *B* was distinct from 5 of the other varieties. The largest F_3-values were found for the variety pairs *B-K* and *A-K*. This seemed to be caused mainly by variety *K*, which had many green and no red hypocotyls in year 1, but few green and many red hypocotyls in year 2.

Table 1. Number of individuals with each note for hypocotyl colours for some varieties in sugar beets

Variety	Colour							
	1 Green		2 White		3-5 Red[1]		7 Orange	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
A	30	21	9	1	15	25	46	53
B	5	9	9	5	48	46	38	40
C	0	3	17	12	31	35	52	50
D	1	0	7	8	71	77	21	15
E	0	3	5	0	80	72	20	25
F	30	28	0	4	30	30	40	38
G	33	25	12	2	16	24	39	49
H	72	76	2	4	3	2	23	18
I	3	2	4	2	37	29	56	67
J	82	82	2	0	7	5	9	13
K	52	7	16	33	0	44	32	16
L	50	37	17	9	5	12	28	42
M	0	0	12	2	58	56	30	42
N	0	0	9	8	74	69	17	23
O	0	0	12	10	58	65	30	25
P	25	22	0	10	17	11	58	57
Q	0	0	0	10	65	64	35	26
R	0	0	0	0	75	55	25	45
S	0	0	6	1	53	61	41	38
T	83	92	5	1	3	1	9	6
U	54	30	12	13	3	4	31	53
V	0	0	6	18	71	63	23	19

[1)] Sum of three different reddish colours (pink, red and dark red)

Table 2. Estimated percent of plants for each note of each variety

Variety	Colour			
	1 Green	2 White	3-5 Red	7 Orange
A	25.8	3.9	19.8	50.5
B	7.0	6.8	47.2	39.1
C	1.5	14.3	33.0	51.1
D	0.5	7.5	74.2	17.8
E	1.5	1.8	74.7	22.0
F	29.1	1.7	30.1	39.2
G	29.5	5.6	20.1	44.8
H	74.1	2.9	2.5	20.5
I	2.5	2.9	33.0	61.6
J	82.2	0.9	6.0	11.0
K	27.7	29.3	14.0	29.0
L	44.0	12.7	8.0	35.2
P	23.9	3.4	14.1	58.7
Q	88.0	2.5	2.0	7.5
U	41.7	12.8	3.5	42.0

Table 3. Differences and F_3 values together with P-values for relevant pairs of varieties

Variety	Candidate A				Candidate B			
	F	$P_{dif.}$	F_3	P_{F3}	F	$P_{dif.}$	F_3	P_{F3}
A	-	-	-	-	2.34	0.1157	0.50	0.6855
B	2.34	0.1157	0.50	0.6855	-	-	-	-
C	5.70	0.0062	0.57	0.5829	2.06	0.1432	0.02	0.9826
D	6.29	0.0033	0.50	0.6485	2.05	0.1404	0.42	0.7800
E	5.40	0.0063	0.41	0.6601	1.35	0.2866	0.19	0.8542
F	0.52	0.6757	1.20	0.2671	3.20	0.0522	0.50	0.7097
G	0.16	0.9224	0.01	0.9976	2.79	0.0786	0.46	0.7701
H	6.91	0.0036	0.94	0.4998	14.33	<.0001	0.15	0.9024
I	5.44	0.0073	0.24	0.7018	2.27	0.1143	0.24	0.9500
J	10.36	0.0004	0.19	0.8365	17.65	<.0001	0.18	0.9506
K	2.19	0.1361	3.17	0.0405	4.54	0.0189	4.31	0.0071
L	2.02	0.1621	0.11	0.9719	6.55	0.0051	0.64	0.7790
P	0.21	0.8896	1.79	0.0934	2.67	0.0847	0.92	0.4270
T	13.62	<.0001	0.65	0.7695	21.42	<.0001	0.05	0.9946
U	2.34	0.1202	0.52	0.7387	7.38	0.0027	1.18	0.8181

In order to get an indication of whether the varieties left in the present analysis the analyses may be performed using another method (Laplace's maximum likelihood) instead of the present method (a kind of residual maximum likelihood). However it should be noted that using maximum likelihood may give too many significant results, but this method is much to prefer compared to a χ^2-test for independence in a contingency table. As an example variety *M* would then be estimated to have M 0, 6.2, 57.6 and 36.2 per cent plants with note green, white, red and orange, respectively. Variety *O* would – using this method – be significant different form variety *A* (P=0.0014) but not significant different from variety *B* (P=0.7224). From this it is judged that variety *A* and *M* would be distinct as the P-values is well below 0.01 and that variety *B* and *M* are not significant as the P-values for proposed method allways will be larger than for the altenative maximum likelihood method.

The F_4values for each variety in the analysis of the hypocotyl colours are shown in Figure 1. The largest F_4 value was found for variety *K*. The value seemed to be extremely large and an explanation for the unusual result should be sought.

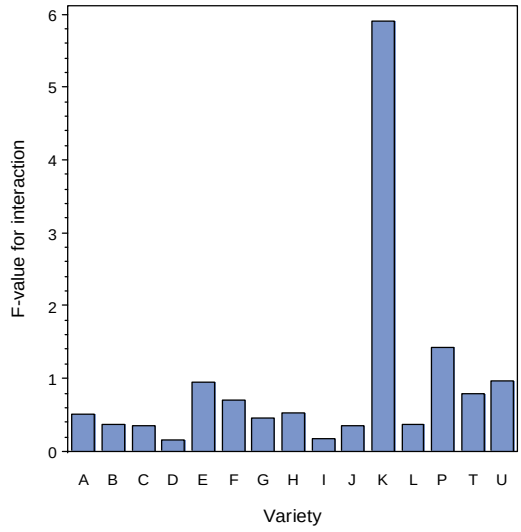

Figure 1: F_4-values for each variety's contribution to the interaction for nominal characteristic hypocotyl colour

THE COMBINED OVER-YEARS METHOD FOR ORDINAL CHARACTERISTICS

Summary of requirements for application of the method

The method is appropriate to use for assessing distinctness of varieties where:

- The characteristic is ordinal and recorded for individual plants (usually recorded visually)
- There are some differences between plants
- The observations are made over at least two years or growing cycles on a single location
- There should be at least 20 degrees of freedom for estimating the random variety-by-year interaction term.
- The distribution of the characteristic should be unimodal, i.e. notes with large number of plants should occur next to each other, zeros at one or both ends of the scale should not cause problems as long as most varieties have plants that fall in different notes
- The total number of plants for each variety should not be too low, at least 5 times the number of notes the variety covers

Summary

The method can be considered as an alternative to the χ^2-test for independence in a contingency table. The χ^2-test only takes the variation caused by random sampling into account and may thus be too liberal if additional sources of variation are present. Also the χ^2-test does not take the ordering of the notes into account. The combined over-years method for ordinal characteristics takes other sources of variation into account by including a random variety-by-year interaction term (as for the COYD method described in TGP/8/1 Part II: 3).It takes the ordering of notes into account by using a cumulative function over the ordered notes. The inclusion of the random effect is expected to decrease the number of distinct pairs of varieties compared to the χ^2-test for independence, but to better ensure that the decisions are consistent over coming years. Taking the ordering of notes into account is expected to increase the power of the test and thus to increase the number of distinct pairs.

The method is based on a generalisation of the traditional analyses of variance and regression methods for normally distributed data, which are called “generalized linear mixed models”. A general description of the method may be found in Agresti (2002) and a more specific description – using other examples of data may be found in Kristensen (2011).

The combined over-years method for nominal characteristics involves

- Calculating the number of plants for each note for each variety in each of the two or three years of trials, which results in a 3-way table (see the example)
- Analyse the data using appropriate software
- Compare each candidate to the reference varieties and the other candidates at the appropriate level of significance to see which varieties the candidate is distinct from
- Check if the variety-by-year interaction term for distinct pairs is considerably larger than the average for all variety pairs

Example

For demonstration a subset of varieties from a DUS experiment with carrots in France was chosen. The notes for root tip (when fully developed) (Table 4) were analysed (Characteristic 13 of TG/49/8). In most cases 60 plants were recorded in each year.

The estimated percent of plants in each note for each variety are shown in Table 5.

Table 4. Number of individual plants with each note for note on root tip for some varieties in carrots

Variety	Note					
	1 blunt		2 slightly pointed		3 strongly pointed	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
A	15	50	31	9	14	1
B	26	52	4	8	0	0
C	30	43	29	17	1	0
D	55	53	5	7	0	0
E	43	54	15	6	2	0
F	0	1	3	24	57	35
G	43	52	16	8	1	0
H	16	29	30	28	13	2
I	39	55	17	5	4	0

Table 5. Estimated percent of plants for each note of each variety

Variety	Note		
	1 blunt	2 slightly pointed	3 strongly pointed
A	52.4	42.3	5.3
B	86.1	12.9	1.0
C	62.8	33.7	3.5
D	90.1	9.2	0.7
E	82.6	16.1	1.3
F	1.3	16.4	82.3
G	80.5	18.0	1.5
H	35.3	54.6	10.1
I	81.0	17.6	1.4

Treating varieties *A* and *B* as candidates and the remaining varieties *C, D,…, I* as reference varieties, the F-values and the P-values for testing the hypothesis of no difference between candidate and reference varieties were calculated. The F-values and the P-values are shown in Table 6. The F_3-values and their significances are also shown in Table 6.

For the data shown here candidate *A* and *B* could both be separated from 1 of the other varieties (variety *F*) when using a 1% level of significance. The F_3 values were not significantly larger than 1 for any of the tested variety pairs shown in table 6. The largest F_3 was found for the variety pair *A*-*D*. The second largest F_3 was found for the variety pair *A*-*B*.

Table 6. Differences and F_3 values together with P-values for relevant pairs of varieties

Variety	Candidate A				Candidate B			
	Difference	$P_{Difference}$	F_3	P_{F3}	Difference	$P_{Difference}$	F_3	P_{F3}
A	-	-	-	-	1.73	0.0485	3.90	0.0836
B	-1.73	0.0485	3.90	0.0836	-	-	-	-
C	-0.43	0.5593	2.30	0.1675	1.30	0.1158	0.21	0.6591
D	-2.11	0.0214	4.97	0.0563	-0.38	0.6373	0.06	0.8060
E	-1.46	0.0764	1.46	0.2610	0.27	0.7342	0.59	0.4655
F	4.42	0.0003	0.18	0.6846	6.15	<.0001	2.42	0.1586
G	-1.33	0.1007	2.11	0.1848	0.41	0.6050	0.28	0.6139
H	0.70	0.3434	1.56	0.2477	2.43	0.0109	0.53	0.4868
I	-1.36	0.0966	0.71	0.4226	0.38	0.6340	1.28	0.2909

The F_4 values for each variety in the analysis of root tip on carrots are shown in Figure 2. It is seen that only three varieties have a value larger than 1. The largest F_4 is found for variety *A*. It is also seen that variety *E* and *H* has a very low interaction with year indicating that their response to year is very close to the mean reaction for all varieties.

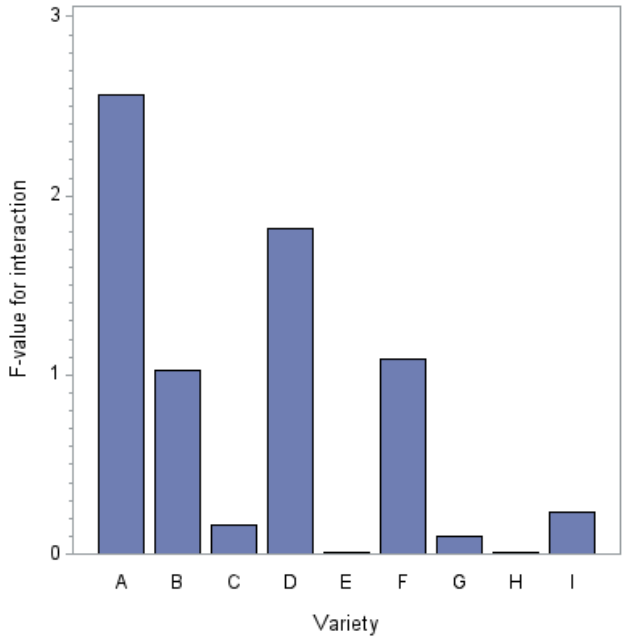

Figure 2 F_4-values for each variety's contribution to the interaction for ordinal characteristic toot tip of carrots

THE COMBINED OVER-YEARS METHOD FOR BINOMIAL CHARACTERISTICS

Summary of requirements for application of the method

The method is appropriate to use for assessing distinctness of varieties where:

- The characteristic is recorded for individual plants (usually recorded visually) using a scale with only 2 levels (such as present/absent or similar)
- There are some differences between plants
- The observations are made over at least two years or growing cycles on a single location
- There should be at least 20 degrees of freedom for estimating the random variety-by-year interaction term.
- The expected number of plants for each combination of variety and note should be at least one – and for most of the combinations the number should be at least 5.

Summary

The method can be considered as an alternative to the χ^2-test for independence in a contingency table. The χ^2-test only takes the variation caused by random sampling into account and may thus be too liberal if additional sources of variation are present. The combined over-years method for ordinal characteristics take other sources of variation into account by including a random variety-by-year interaction term (as for the COYD method described in TGP/8/1 Part II: 3). The inclusion of the random effect is expected to decrease the number of distinct pairs of varieties compared to the χ^2-test for independence, but to better ensure that the decisions are consistent over coming years.

The method is based on generalisation of the traditional analyses of variance and regression methods for normally distributed data, which are called “generalized linear mixed models”.

The combined over-years method for binomial characteristics involves

- Calculating the number of plants for each note for each variety in each of the two or three years of trials, which results in a 3-way table
- Analyse the data using appropriate software
- Compare each candidate to the reference varieties and the other candidates at the appropriate level of significance to see which varieties the candidate is distinct from
- Check if the variety-by-year interaction term for distinct pairs is considerably larger than the average for all variety pairs

Example

The proportion of plants with cyanid glucoside (Characteristic 4 in TG/38/7) was measured for some white clover varieties in Northern Ireland in each of 3 years. The variable was recorded as absent or present. In this example only 20 varieties are used and variety 1 and 2 are considered as candidates, while the remaining varieties are considered as references. The data are shown in Table 7.

Table 7. Number of plants without and with cyanid glucoside in 20 white clover varieties in each of 3 years

	Year 1		Year 2		Year 3	
Variety	Absent	Present	Absent	Present	Absent	Present
1	31	29	22	38	17	43
2	40	20	42	18	41	19
3	50	10	52	8	55	5
4	42	18	40	20	34	26
5	37	23	42	18	37	23
6	51	9	49	11	52	8
7	30	30	25	35	26	34
8	37	23	31	29	30	30
9	27	33	27	33	25	35
10	48	12	47	13	43	17
11	40	20	40	20	32	28
12	18	42	13	47	12	48
13	10	50	12	48	5	55
14	41	19	46	14	45	15
15	58	2	55	5	58	2
16	7	53	10	50	11	49
17	25	35	22	38	20	40
18	48	12	54	6	52	8
19	20	40	20	40	23	37
20	57	3	54	6	55	5

The analysis showed that for these data there was no interaction between variety and year, which means that the variance component for year by variety was estimated to be zero and thus all variation in the data could be explained by sampling variation. The F-test for comparing the varieties was 36.67 with a P-value less than 0.01%, so there were clearly some differences among the varieties.

More specifically the analysis showed that candidate variety 1 was significantly different from 12 of the reference varieties at the 1% level (Table 8) whereas candidate variety 2 was significantly different from 11 of the reference varieties. Also the two candidate varieties were significantly different at the 1% level (Table 8).

As there was no interaction between variety and year, all F_3 and F_4 values are estimated to be zero for these data. Therefore, they are not shown here.

Table 8. Estimated percent of plants with cyanid glucoside for each variety and comparison of each variety with the candidate varieties 1 and 2 using F-tests

	Estimated	Candidate 1		Candidate 2	
Variety	percent	F	P	F	P
1	61.1			30.45	<.0001
2	31.6	30.45	<.0001		
3	12.7	77.01	<.0001	17.58	0.0002
4	35.5	23.05	<.0001	0.61	0.4395
5	35.5	23.05	<.0001	0.61	0.4395
6	15.5	70.09	<.0001	12.54	0.0011
7	55.0	1.38	0.2473	19.58	<.0001
8	45.5	8.69	0.0054	7.27	0.0104
9	56.1	0.93	0.3414	21.39	<.0001
10	23.3	49.59	<.0001	3.12	0.0853
11	37.8	19.27	<.0001	1.48	0.2309
12	76.1	9.28	0.0042	66.21	<.0001
13	85.0	24.61	<.0001	90.68	<.0001
14	26.6	41.43	<.0001	1.09	0.3034
15	5.0	82.34	<.0001	33.21	<.0001
16	84.5	23.44	<.0001	89.25	<.0001
17	62.8	0.11	0.7463	33.81	<.0001
18	14.4	72.95	<.0001	14.45	0.0005
19	65.0	0.58	0.4492	38.53	<.0001
20	7.8	84.99	<.0001	28.18	<.0001

COMMON TO ALL THREE METHODS

Software

The procedure *GLIMMIX* of *SAS* (SAS Institute Inc., 2010) can be used to estimate the parameters of the generalised linear mixed model, and the data-step facilities (and/or the procedure *IML*) of the same package can be used for the remaining calculations. However, similar facilities may be found in other statistical packages, thus the *glmer*() function of the package *lme4* of R can do the binomial analysis provided that there are more than one observations for each combination of variety and year.

Final note

In the case where are only two notes, the methods for nominal and ordinal scaled characteristics both become identical as they reduce to the same binomial method: meaning that both methods can be applied to binomially distributed data.

References and literature

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