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REVISION OF DOCUMENT TGP/8:
PART II: TECHNIQUES USED IN DUS EXAMINATION
New Section 11 – Examining DUS in Bulk Samples

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. It was also agreed that the example of sugar beet should be replaced by a crop for which there were UPOV Test Guidelines (see document TC/48/22 "Report on Conclusions" paragraph 55).

3. The Annex to this document contains the proposed text for New Section 11 – Examining DUS in Bulk Samples, prepared by Mr. Kristian Kristensen (Denmark).

[Annex follows]

ANNEX

TGP/8/1: PART II: NEW SECTION 11: EXAMINING DUS IN BULK SAMPLES

Introduction

The term “bulk sampling” is here used for the process of merging some or all individual plants or part of plants before recording the expression of the characteristics. Bulking is usually only applied where the measurement of the characteristic is very expensive or very difficult to obtain for each individual plant. Some examples are: erucic acid in seed of Oilseed rape (TG/36/6 Corr.) which is usually based on a seed sample sent in by the applicant and thus there will be no possibility to have values for individual plants. Another example is the thousand seed mass of Pea (TG/7/10), which is usually bases on a bulked sample, e.g. 3 by 100 seeds from each replicate.

There are different degrees of bulking ranging from: 1) merging of pairs of plants, 2) merging 3 or 4 up to all plants within a plot and 3) merging all plants for each the variety. The degree of bulking may play an important role for the efficiency of the tests and may exclude even exclude some tests.

Consequences of bulking for DUS examination

The consequences of bulking will be more serious when testing for uniformity than when testing for distinctness.

Testing for Uniformity

If the test for uniformity is based on the number of off-types any bulking may completely mask the off-types as now only the mean the characteristic over the bulked plants can be evaluated.

For many continuous variables uniformity is tested using the COYU method which is based on logarithm of the standard deviation of individual plants within each plot. For this method the effect of moderate bulking is mainly caused by a decrease in the number of degrees of freedom and thereby larger uncertainty on the logarithm of the standard deviations. Moderate bulking (bulking pairs of plants) will in most cases decrease the power of tests. Further bulking, up to having only two bulked samples per plot will further decrease the power of the tests which means that the degree of non-uniformity must be much higher for it to be detected – about 3-4 times higher if 30 plants from each of two blocks were bulked into 2 groups of 15 plants for each of the two blocks before the recording was made. These calculations assume that equal amount of material were bulked from each plant. If that is not done the effect of bulking is expected to be larger.

In general, if all plants in a plot are bulked such that only a single sample is available for each plot, it becomes in general impossible to calculate the within plot variability and in such cases no tests for uniformity can be performed. In rare cases, where non-uniformity maybe judged from values that can only be found in mixtures, non-uniformity may be detected even where a single bulk sample for each plot is used. For example, in the characteristic “erucic acid” in oil seed rape, values between 2% and 45% can only arise because of a lack of uniformity. However this only applies in certain special cases and even here the non-uniformity may only show up under certain circumstances.

Bulking across plots have the consequences that that part of the between plot (and block) variation will be included in the estimated standard deviation between bulks. If this variation is relatively large then this will tend to mask any differences in uniformity between varieties. In addition some noise may also be added because the ratio of material from the different plots may vary from bulk to bulk. Finally the assumptions for the present recommend method, COYU, may not be fulfilled in such cases. Therefore it is recommended only to bulk within plots.

Testing for distinctness

The effect of bulking will usually decrease the power of the distinctness much less than for the uniformity test – and may in some cases result in an ignorable small decrease in power. The reason for this is that both the COYD method and the 2×1% method are based on means (per year and variety for COYD method and per year, block and variety for the 2×1% method). Therefore, the only loss of precision here is the increase in variability caused by fewer measurements. The uncertainty caused by the measurement is usually much smaller than the uncertainty caused by other sources such as plant, soil and climate. If the uncertainty

caused by the measurement is very small (relatively to other sources of variation) it is thus expected that the decrease in power will be ignorable as long as there are at least one bulked sample per year and variety for the COYD method and one bulked sample per year, block and variety for the 2×1% method. Also here it is assumed that equal amount of material were bulked from each plant. If that is not the case the effect of bulking may not be as small as described here.

Examples

Erucic acid in seed of Oilseed rape (TG/36/6 Corr.). For these data there is only one or two observation per variety and thus no possibility for statistics analysis. In Denmark the results of the analysis is converted to one of two states: erucic acid absent (1) or erucic acid present (9). No statistical analysis is carried out on this characteristic and the characteristic is only used for describing new varieties and thus no tests for distinctness or uniformity are carried out.

Thousand seed mass of fodder peas (TG/7/10). The data for a selected subset of varieties in Germany 2010 and 2011 are shown in table 1. For each replicate there are 3 recorded values each based on 100 seeds. The seeds are taken as a random sample from a bulk sample for each variety in each replicate, and the 100 seeds in each sample may represent up to 100 plants. Data from trials in Germany in 2010 and 2011 were used for the following simulation, but, for practical reasons only the first 20 varieties were used here.

The means across replicates for each variety in each year may be used for testing distinctness by the COYD method and the results show that variety Q was significant different from variety E and M at the 1% level of significance. As the measurement error in determining the thousand seed weight is expected to be small compared to the other sources of random variation the effect of bulking is expected to be small when testing for distinctness. For the data shown here it can be verified that the effect is small by comparing the actual standard error on a difference between two varieties with the theoretical minimum value that could be obtained if measurements on 30 plants had been used instead of 3 samples in each replicate. The actual standard error was 11.53 and the theoretical minimum value was 11.46. So for this example the effect of bulking on the tests for distinctness using COYD is very small, but for other variables, crops or growing conditions the effect of bulking may be larger.

Table 1 Thousand seed mass for 20 fodder peas varieties for three bulked samples in two replicates in each of two years (variety Q is a candidate variety, while the other are reference varieties)

Year	2010						2011					
Rep	1			2			1			2		
Variety\Sample	a	b	c	a	b	c	a	b	c	a	b	c
A	238	236	235	229	228	230	289	290	297	291	294	289
B	241	241	240	236	236	235	294	290	295	296	295	291
C	238	233	232	234	228	235	258	261	258	248	247	245
D	250	247	245	250	251	246	315	313	317	306	310	311
E	215	218	220	216	216	218	250	252	251	259	256	263
F	225	221	219	239	228	233	283	278	274	279	274	276
G	250	252	246	249	248	245	248	241	244	266	258	258
H	267	271	264	262	263	265	325	325	323	327	329	325
I	235	238	243	237	238	237	289	292	293	289	292	288
J	234	238	236	225	231	230	265	269	266	268	269	263
K	261	261	263	270	266	270	311	312	318	309	316	313
L	246	241	239	260	259	259	288	282	284	300	294	302
M	223	223	221	205	201	204	250	254	250	261	259	262
N	231	224	229	219	223	222	269	275	267	272	269	270
O	259	267	261	259	257	260	338	332	330	346	340	342
P	251	252	248	250	248	256	307	305	304	302	301	296
Q	242	239	238	237	243	245	307	305	304	308	315	311
R	270	262	261	259	262	258	317	318	314	314	322	321
S	255	263	253	263	258	267	318	308	314	310	315	311
T	242	244	241	242	240	246	293	285	289	299	291	293

The pooled standard deviations within each year can be used to test for uniformity using the COYU method. The COYU analyses has shown that the standard deviation for variety Q was slightly larger than the mean of standard varieties for all reference varieties, but far from being significant (P>50%). However, the power of the test is much lower than if no bulking had been performed. There are three reasons for this:

1. There are only 3 values available in each of two replicates and thus only 4 degrees of freedom (3-1 in replicate one and 3-1 in replicate two) for estimating the standard deviation instead of 58 degrees of freedom if the recordings had been made for each of 30 varieties in each replicate.

2. Each sample will contain observations from several plants which will tend to mask the differences between individual plants.

3. Because of the sampling method applied here seeds from many of the plants may very likely be represented in more samples (or even all samples) from the same replicate. This will make the differences between sample means small because a possible outlying plant will influence all samples where it occurs in the same direction and thus tend to mask the effect of outlying plants.

Remark: In current practice in Germany, the assessment of distinctness is based on the mean values for each variety. Individual replications are not considered for further calculations. There is no test for uniformity in this characteristic.

[End of Annex and of document]