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DESIGN AND ANALYSIS OF DUS SPECIAL TESTS

Document prepared by experts from the United Kingdom

INTRODUCTION

Applicants submitting varieties for distinctness, uniformity and stability (DUS) testing occasionally claim that special features, not routinely examined for in trials, differentiate their variety from others. When this situation arises, and the feature is one that can be measured, e.g. disease resistance, then registration authorities usually arrange for special tests to be conducted to validate the applicant's claim. This note sets out the statistical principles that apply in such cases and illustrate these with an example.

AIMS OF SPECIAL TESTS

The purpose of special tests may be broadly summarised as being to:

- provide conditions which fully express the delineating characteristics of the candidate and its close neighbours, particularly in respect of the special features;
- supply data which can be used as objective statistical evidence that:
  - the varieties are distinguishable on the basis of one or more characteristics;
  - the spread in the distribution of the characteristic from plant-to-plant of the candidate variety is not dissimilar from that of other varieties;
- ensure that the tests produce results that are likely to be confirmed if repeated;
- minimise experimental effort.

EXAMPLE

An example from a DUS special test illustrates the circumstances. In formal tests with standard DUS characters it was not possible to clearly distinguish a candidate variety (B) from an established variety (A). However, the applicant suggested a measurable feature, not routinely assessed, which it was believed distinguishes the two varieties. The authorities arranged to grow 60 plants of each of the varieties together in a glasshouse in order to evaluate the feature. The trial was repeated once and the results from the two trials are presented in Table 1.

Table 1: Data for a single character from a DUS special test with 2 varieties (A and B) tested in two trials (1 and 2) with 60 plants per variety per trial

Trial	**1**		**2**	
Variety	**A**	**B**	**A**	**B**
Plants				
1	61	56	21	53
2	35	42	37	56
3	40	30	31	45
4	49	42	18	43
5	29	44	24	51
6	37	29	35	52
7	44	34	38	53
8	25	57	35	51
9	40	48	34	35
10	48	26	35	48
11	30	37	30	39
12	40	30	27	51
13	28	42	42	26
14	37	41	27	45
15	16	40	37	30
16	31	53	39	53
17	51	21	17	41
18	51	53	41	52
19	35	52	38	51
20	38	41	35	36
21	45	55	19	28
22	51	26	37	53
23	39	60	22	43
24	28	34	25	41
25	30	30	33	31
26	30	22	11	60
27	59	50	46	44
28	38	37	3	33
29	61	38	22	30
30	38	29	30	37
31	60	51	26	31
32	48	40	42	52
33	39	54	32	42
34	55	32	27	48
35	38	48	30	40
36	41	49	32	50
37	47	44	11	50
38	45	39	42	44
39	37	41	45	42
40	44	41	21	52
41	43	64	58	39
42	35	36	21	53
43	27	31	43	40
44	45	46	40	24
45	26	39	48	47
46	44	36	20	47
47	50	38	36	42
48	35	24	32	37
49	33	71	38	42
50	34	58	26	33
51	56	25	25	61
52	34	54	36	46
53	39	38	50	34
54	41	38	21	57
55	20	41	47	55
56	37	57	38	40
57	38	26	31	25
58	60	59	28	45
59	50	42	18	59
60	24	45	25	37
Mean	40.1	41.8	31.3	43.8

ANALYSIS AND INTERPRETATION

Superficially, the results from the two trials appear to be contradictory, with a very small difference occurring between the varieties in the first trial and a clear difference apparent in the second trial.

However, if one digs more deeply, the results indicate that there are two sources of variety-by-environment variation operating. The first source applies within a glasshouse and represents the differences between plants within a relatively uniform environment. The second source of variation is that between trials and it relates to the manner in which the varieties respond to the changes in conditions from one sowing to the next.

Both sources of variation are important but the between-trials source is crucial for two reasons:

- it is usually much more costly to replicate trials than plants within trials, and so it is not easy to limit the effects of this source of variation through increased replication of trials;

- trial-to-trial variation determines how well the results will be replicated, if it proves necessary to repeat the trials. In other words, this source of variation is a key to the robustness of the tests.

The appropriate analysis in such a case is one that combines the data from the two trials and uses a statistical model that properly reflects the biological circumstances as well as the technical requirements. In this case the correct model is one which has as blocks the terms ‘Trials’ and ‘Plants-within-trials’.

The results of this combined-trials analysis are presented in Table 2. Here the test of variety differences is based on the variety means over trials and a standard error of difference that is derived from a combination of the two sources of variation. It is clear that in these data the difference between varieties A and B, in respect of the character, is not statistically significant.

It is also clear that two trials with two varieties provide insufficient 'degrees of freedom' to represent an effective discriminatory test. A reorganisation of the test is required, involving more independent sowings, and possibly more varieties, but with fewer plants per variety sown on each occasion. For example, a test with four sowings and four varieties might provide an LSD(p=0.05) of 12.2.

Table 2: Combined analysis of data from the two DUS special test trials

TABLE OF MEANS

	Variety			Within trial LSD (0.05)
Trial	A	B	Mean	
1	40.1	41.8	41.0	3.9
2	31.3	43.8	37.5	3.6
Mean	35.7	42.8		
SED	5.4			
DF	1			
LSD (0.05)	68.6			

ANALYSIS OF VARIANCE

Source of Variation	Mean square	Degrees of freedom
Trials	707	1
Varieties	2968	1
Varieties x trials	1760	1
Plants within varieties and trials	107	236

RECOMMENDED STATISTICAL PROCEDURE

Distinctness

The statistical procedures used for distinctness testing in such a case estimates the probability that the difference observed between candidate and close neighbour might have occurred by chance when there was no real difference between the varieties.

To obtain this probability in as effective a manner as possible requires:

- **Adequate replication:** The number of replicates of candidate and close neighbours should, where possible, give degrees of freedom for the significance test comparable with those generated when assessing standard characters; if this is not possible then a minimum of 20 degrees of freedom should be the aim.
- **Effective blocking:** In order to minimise the effects of extraneous sources of variation, the experimental units, i.e. plots, plants, pots, trays, sowings, from each replicate of the candidate and close neighbour, should be located together in the field or glasshouse and each replicate should be managed as one unit as far as possible.
- **Randomisation:** The plants or seeds of the candidate and its close neighbours should be assigned at random to the experimental units within each replicate.

- **Appropriate analysis:** An analysis of variance should be performed on the observations from the test. The analysis should include block terms for each of the major sources of variation in the test.
- **Significance test:** A t-test of the mean difference between the candidate and each of the close neighbours will, in general, form the basis of the significance test. The error term to be used will be specified by the analysis model as described above. The error term will usually be based on a contribution both from the candidate and close neighbours, unless the candidate is shown to be more variable, when an error term based on close neighbours should be used.
- **Significance level:** The appropriate critical probability standard to be applied will depend on the circumstances of the species and the judgement of the test centre, but in general, should be set at a similar level to that applied when assessing other characters. This level should be agreed before the test is done. Where there is more than one stratum of experimental variation in the test then the critical t-value will be a weighted mean of two or more t-values. For details see, for example, Cochran & Cox, Experimental Designs, 2nd Edition, Section 7.16.

Uniformity

Variation in the candidate variety's expression of the character from plant-to-plant should be checked by comparison with that of its close neighbours. A variance ratio (F-ratio) test can be used to compare the variance of the observations for the candidate with the variances for close neighbours. The appropriate probability level to be applied should be agreed in advance of the test.

SUMMARY

To assure the robustness of DUS test protocols it is essential that the procedures associated with tests for special features are of a similar rigour to those used for standard characters. This requires that appropriate experimental design and data analysis methods are applied in special tests.

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