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The influence of reduction of the number of measurements on decisions concerning distinctness of perennial ryegrass varieties

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SUMMARY

This paper discusses the problem of reduction of number of measurements in DUS (distinctness, uniformity and stability) trials on perennial ryegrass. The considerations are based on the results of DUS trials performed by the Research Centre for Cultivar Testing at the Experimental Station at Słupia Wielka in the years 2005–2009. It is shown that a reduction in the number of measurements from 60 to 30 will have very limited influence on decisions about the distinctness of ryegrass varieties.

Key words: DUS trials, number of measurements, perennial ryegrass

1. Introduction

Every new variety of any species, before being listed (e.g. in the National List) must prove its distinctness, uniformity and stability (DUS). Positive decisions (acceptance of variety) are usually taken after collecting the results of two or three years' trials performed at the same experimental point (experimental station). A negative decision can sometimes be taken after a one-year trial, if the variety is very far from fulfilling DUS requirements. A new (candidate) variety must be distinct from any other variety (variety of common knowledge) for at least one characteristic. The degree of uniformity of a candidate variety must be not worse than the average uniformity of all varieties used for comparison. The variety must be uniform for all characteristics used for distinctness purposes.

DUS trials on grass species are usually planned in a randomized complete block design (RCBD) in six replicates. From every plot the number of plants is chosen at random and necessary measurements are performed for all characteristics. According to UPOV (International Union for the Protection of New Varieties of Plants) Guidelines (see TG/4/8), in trials on perennial ryegrass from every plot a random sample of 10 plants is used for making measurements, giving in total 60 measurements for every variety. The list of compulsory and optional characteristics is also given in the UPOV Guidelines. Independently of the range of variation observed within particular characteristics, the same number of measurements is suggested in these Guidelines. On the other hand, as ANOVA is used as the main tool for single trial data, the number of measurements can influence the size of plot error only to a very limited degree as it is more dependent on the number of replicates. The number of measurements has a significant impact on the size of the sample error. The significance of differences among varieties is tested against plot error, so a reduction in the number of measurements is expected to have a rather limited influence on decisions concerning distinctness. To prove distinctness after collecting two or three years' data, the COYU method (combined over years distinctness; see Talbot [2000]) is used. In the COYU approach the distinctness of candidate varieties is tested against the mean square for variety by year interaction (MS_{VY}). This random variable is more dependent on the number of years and on the number of varieties than on the number of measurements of single plants. Hence also from the point of view of analysis of series of two or three years' results, it is expected that reduction of the number of measurements should have a limited influence on decisions about distinctness of varieties. To test this conjecture, the number of measurements in real DUS trials on perennial ryegrass was reduced by 50%. The analysis was performed for a full set of data and for a reduced subset, and decisions about distinctness were compared. The absolute and relative discriminating power of characteristics was also compared.

2. DATA

The results of a five-year series of trials (2005–2009) performed at the Variety Testing Experimental Station at Słupia Wielka on perennial ryegrass were the starting point of our considerations. All the trials were performed in an RCBD in six replicates. A random sample of 10 plants was taken from every plot and measurements performed. Hence for every characteristic there were 60 measurements per variety (each candidate variety and each variety of common knowledge – variety already registered in any country). The number of tested varieties varied from year to year (see Table 1). Nevertheless the number of varieties is large enough to ensure that the conclusions are independent of the tested sets of varieties.

Table 1. Number of candidate varieties and established varieties (varieties of common knowledge)

Year	Candidate	Established	In total
2005	24	67	91
2006	26	64	90
2007	25	63	88
2008	15	73	88
2009	14	97	111

The list of measured (quantitative) characteristics is given bellow. Among them are characteristics concerning the whole plant, flag leaf and inflorescence, namely:

Whole plant characteristics:

- natural height (coded here as RNWN),
- length of longest stem (ZDNZ)

Flag leaf characteristics:

- length (LFD)
- width (LFS)
- size (LFW)
- shape (ratio of length to width – LFK)

Inflorescence:

- length (KD)
- number of spikelets (KLK)
- density (KZ).

All the measurements were recorded at the proper stage of growth, as indicated in TG/4/8. The collected data were then analyzed using the method described below.

3. METHOD

The results of every single trial were analyzed twice using analysis of variance as a statistical tool. in the first analysis all the data were used, and in the second analysis only half of the measurements were used – from every plot 5 out of 10 measurements were chosen at random. So in fact for every variety and characteristic there were 60 measurements in the first analysis and 30 in the second. The mathematical model of observation was that for RCBD with more than one observation taken from every plot (see Oktaba, 1980):

$$y_{ijk} = \mu + r_i + t_j + e_{ij} + \eta_{ijk}$$

where y_{ijk} denotes the *k*-th measurement within the *i*-th block concerning the *j*-th variety, μ – overall mean, r_i – random effect of *i*-th block (*i*=1,2), t_j – fixed effect of *j*-th variety, e_{ij} – random effect of plot error, η_{ijk} – random effect of sample error.

All random variables are assumed to have independent normal distributions with expected values equal to zero and appropriate variances.

Analysis of variance was made for each characteristic step by step. After analysis of variance, the value of Fisher least significant difference (LSD) at level 0.05 for simple contrast between two variety effects was calculated and all pairs of varieties compared. It is worth noting that the LSD test is not a simultaneous test procedure, so the applied significance level is valid only for every single comparison. Instead of the LSD test one can use, for example, the simultaneous Tukey test of multiple comparisons; see Guenther [1964]. Here

the LSD-based procedure was applied as this procedure is officially promoted by the UPOV. Next the number of distinguished variety pairs was counted for every characteristic. The characteristic that distinguishes more pairs of varieties is considered as possessing higher absolute discriminating power. The characteristics were ranked according to their absolute discriminating power. The characteristics were also ranked according so-called relative discriminating power. The characteristic that distinguishes the largest (among all considered) number of variety pairs has the largest absolute and relative discriminating power. The second in line according to relative discriminating power is the characteristic that distinguishes the highest number of variety pairs but excluding all pairs already distinguished by the first characteristic, and so on.

4. RESULTS

All the methods described above were applied to the results of ryegrass trials performed at the variety testing experimental station at Słupia Wielka in the period from 2005 to 2009. The usefulness of characteristics was assessed by sums of ranks both for their absolute and relative discriminating power. The results for the full number of measurements are given in Table 2, and the results for a reduced number of measurements in Table 3. It is easy to notice that the three best characteristics according to absolute discriminating power are the same for full and reduced data (RNWW, LFS and KZ). Also according to the relative discriminating power the three leading characteristics are the same (RNWW, ZDNZ and KLK). It is clear that the total number of distinguished pairs of varieties must be lower for reduced data. To show this loss, in Table 4 the numbers of non-distinguished pairs of varieties are collected for particular years. It is easy to notice that the largest difference (87 pairs) appeared in year 2008, but it is only 2.3% of the total number of pairs. The smallest

Table 2. Absolute and relative number of distinguished pairs of ryegrass varieties for total number of measurements and ranks of characteristics – yearly results

	Characteristic	Year 2005				2006				2007				2008				2009				Sum of ranks	
		Absolute	Rank	Relative	Rank	Absolute	Rank	Relative	Rank	Absolute	Rank	Relative	Rank	Absolute	Rank	Relative	Rank	Absolute	Rank	Relative	Rank	Absolute	Relative
Plant	Natural height (RNWW)	2382	1	2382	1	1696	8	188	3	1810	2	679	2	2198	1	2198	1	4267	1	4267	1	13	8
	Length of longest stem (ZDNZ)	1794	8	119	4	2160	6	25	6	2426	1	2426	1	1905	3	121	4	2714	6	146	4	24	19
Flag leaf	Length (LFD)	1978	7	20	7	2199	4	5	8	945	5	7	9	1204	8	4	9	2171	9	6	9	33	42
	Width (LFS)	2283	3	15	8	1782	7	5	9	922	7	20	8	2126	2	845	2	3549	2	1011	2	21	29
	Size (LFW)	2355	2	895	2	1144	9	36	5	1038	4	113	4	1732	4	9	8	3277	4	34	7	23	26
	Shape (LFK)	870	9	41	6	2169	5	97	4	649	9	55	6	1397	7	69	6	2392	8	47	6	38	28
Inflorescence	Length (KD)	2249	5	8	9	2736	3	9	7	1163	3	173	3	1433	6	14	7	3231	5	12	8	22	34
	Number of spikelets (KLK)	1992	6	343	3	2740	2	722	2	923	6	71	5	1122	9	89	5	2654	7	261	3	30	18
	Density(KZ)	228	4	48	5	2794	1	2794	1	867	8	22	7	1593	5	239	3	3542	3	64	5	21	21
Number of tested varieties		91				90				88				88				111					
Number of pairs		4095				4005				3828				3828				6105					
Number of distinct pairs		3871				3881				3566				3588				5848					
Number of non-distinct pairs		224				124				262				240				257					

Table 3. Absolute and relative number of distinguished pairs of ryegrass varieties for reduced number of measurements and ranks of characteristics – yearly results

	Characteristic	Year 2005				2006				2007				2008				2009				Sum of ranks	
		Absolute	Rank	Relative	Rank	Absolute	Rank	Relative	Rank	Absolute	Rank	Relative	Rank	Absolute	Rank	Relative	Rank	Absolute	Rank	Relative	Rank	Absolute	Relative
Plant	Natural height (RNWW)	2176	2	866	2	1441	8	215	3	1592	2	618	2	1900	1	1900	1	4090	1	4090	1	14	9
	Length of longest stem (ZDNZ)	1592	8	139	4	2125	4	55	5	2382	1	2382	1	1762	3	141	4	2480	6	216	3	22	17
Flag leaf	Length (LFD)	1905	6	25	7	1994	5	117	4	932	7	8	9	970	8	86	7	1795	9	9	9	35	36
	Width (LFS)	2142	5	4	9	1640	7	37	6	1061	5	26	7	1868	2	884	2	3560	2	1116	2	21	29
	Size (LFW)	2264	1	2264	1	1033	9	15	8	1084	4	221	3	1485	4	9	9	2996	4	35	7	23	26
	Shape (LFK)	1001	9	83	5	1966	6	4	9	660	9	60	6	1185	7	91	5	2215	7	84	5	38	28
Inflorescence	Length (KD)	2174	3	18	8	2616	2	23	7	1096	3	16	8	1206	6	16	8	2862	5	41	6	22	34
	Number of spikelets (KLK)	1842	7	379	3	2404	3	742	2	860	8	77	5	902	9	74	6	2009	8	16	8	30	18
	Density(KZ)	2163	4	47	6	2639	1	2639	1	956	6	132	4	1464	5	300	3	3365	3	155	4	21	21
Number of tested varieties		91				90				88				88				111					
Number of pairs		4095				4005				3828				3828				6105					
Number of distinct pairs		3825				3847				3540				3501				5762					
Number of non-distinct pairs		270				158				288				327				343					

Table 4. Total numbers of pairs and numbers of non-distinguished pairs – yearly results

Year	Total number of pairs	Number of non-distinguished pairs		
		whole data	reduced data	difference
2005	4095	224	270	46
2006	4005	124	158	34
2007	3828	262	288	26
2008	3828	240	327	87
2009	6105	257	343	86
In total	21861	1107	1386	279

difference – 26 pairs – was observed for the year 2007, being only 0.67% of the total number of pairs of varieties. For the full data, the difference was 279 pairs, i.e. 1.28% of all pairs.

5. COMMENTS AND CONCLUSIONS

A decision to accept or reject a new (candidate) variety is usually taken after analyzing two or three years' trial data. But if the candidate variety is extremely non-uniform (and non-distinct from other varieties) a rejection decision can be taken after one year. It has been shown that reducing the number of measurements from 60 to 30 has a very limited impact on the decision concerning distinctness of varieties within a single (one-year) trial. This means that a reduced number of measurements is large enough for estimation of the mean square of trial error (differences among varieties are tested against that error). It can be expected that the influence on such decisions in a multi-year series of trials would be even smaller, as in that case differences among varieties are tested against mean square for variety-by-year interaction, which is more dependent on the number of years than on the number of measurements. A detailed investigation of this phenomenon will be contained in our subsequent paper. There was also observed a lack of substantial influence of reduction in numbers of measurements on the discriminating power of characteristics. It is interesting to ask how far one can go with such reduction of sample size (number of measurements) within plots. The answer to this question can be

found in a book by Elandt [1964], who suggested using such sample size as to ensure that the size of sample error is smaller than the size of plot error. In our reduced data this was always the case, so we have not yet reached the risky boundary of minimal sample size. It means also that the number of degrees of freedom associated with the estimate of variance of sample error was sufficiently large.

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