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THE INFLUENCE OF REDUCTION IN MEASURED PLANTS NUMBER ON DISTINCTNESS OF AMENITY VARIETIES OF PERENNIAL RYEGRASS (*LOLLIUM PERENNE* L.) TESTED IN DUS TRIALS

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Summary

In a paper the problem of number of measurements in DUS (distinctness, uniformity and stability) trials on amenity varieties of perennial ryegrass is discussed. The results of five trials performed in the period 2005-2009 at Variety Testing Experimental Station at Słupia Wielka are used. The results are analyzed twice using standard procedure for this type of trials. In the first analysis all data were used whereas in the second analysis data were randomly excluded, then the analysis was performed on 50% of original data. Finally, the decisions about distinctness in these analyses were compared.

Keywords and phrases: amenity varieties, DUS trials, number of measurements, perennial ryegrass

Classification AMS 2010: 62K99, 62P10

1. Introduction

Each new variety (of each crop) before its registration (e.g. in National List of Varieties, or European Common Catalogue) has to show that it is distinct

from all other varieties, has to be sufficiently uniform and has to be stable (must fulfill DUS requirements). In Poland DUS trials are conducted by The Research Centre for Cultivar Testing. Positive decisions (registration candidate as variety) are usually taken after two or three years of trialing. Trials for every crop are performed each year at one (the same) experimental station. An early negative decision can sometimes be taken after one year when variety is very far from fulfilling DUS requirements. Trials on all grasses are planned in randomized complete block design in six replicates. The measurements are performed for ten randomly chosen plants from each plots, so finally there are 60 measurements for each characteristic, independently of the variability between plants. The list of compulsory and optional characteristics for countries associated with UPOV (International Union for the Protection of New Varieties of Plants) can be found in so-called TG (Technical Guidelines) documents issued by this international organization. For perennial ryegrass all basic requirements are given in TGP/4/8 (2006). Candidate variety must be distinct from all other (commonly cultivated) known varieties. Significant difference for one characteristic fulfills this requirement. Variety must be sufficiently uniform for all characteristics used in testing distinctness. For self-fertilizing crops distinctness can be often revealed visually. For cross-fertilizing crop (e.g. for grasses) distinctness is usually verified with use biometrical measurements and statistical methods. In single trials (results from one location and year) the pair of varieties is declared distinct if (for at least one characteristic) the difference between them is larger than the least significant difference (LSD) at appropriate significance level. The LSD values are calculated with use the mean square for plot error in randomized complete block design with six replicates and more than one measurements within each plot. For important (from economical point of view) crops apart of DUS trials, additional VCU (value for cultivation and use) experiments are also conducted. So candidate variety must fulfill not only DUS requirements but also VCU requirements. Both DUS and VCU trials are expensive and long lasting. Therefore within UPOV (see e.g. document TWC/26/17 and TWC/29/26) and also within particular countries some attempts of optimizing are undertaken, see Meyer, Laidig and Drobek (2011) in Germany and Pilarczyk and Kowalczyk (2011) in Poland. In this paper interest is focused on assessment how decisions about distinctness of amenity varieties of perennial ryegrass (*Lollium perenne* L.*)* would change after reduction of measurements number for 50%.

2. Trial data

The results of DUS trials on amenity varieties of perennial ryegrass conducted it the period from 2005 to 2009 by The Research Centre for Cultivar

Testing at Variety Testing Experimental Station at Słupia Wielka form basis for all considerations. All trials were established in randomized complete block design. Six replicates were applied and 10 plants were measured from each plots giving finally 60 measurements for every characteristic (for every established and candidate variety). Total numbers of tested varieties in years from 2005 to 2009 were respectively 90, 89, 87, 87 and 110. The following characteristics concerning the whole plant, leaf and flower were observed (see TGP/4/8):

Plant:

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

Flag leaf characteristics:

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

Inflorescence:

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

All the observations (measurements) were performed at the proper stage of growth, as indicated in TGP/4/8. The data were analysed using described below methods.

3. Method

The analysis of variance was used as the main statistical tool according to the linear ANOVA model for data regarding a trait obtained from one-factor experiment (trial) planned in randomized complete block design when more than one observation is present for each experimental unit being a plot (see Elandt, 1964 or Oktaba, 1980). Next, the Fisher least significant difference (LSD) for $\alpha = 0.05$ significance level was applied for testing significance of trait mean differences between pairs of varieties. It is worth to mention that applied test is not a multiple comparison (simultaneous) test procedure so applied significance level concern every single comparison but overall significance level in not known. Such procedure was applied as it is common technique used in all UPOV member countries in interpretation of single trial data. Next the number of distinct and not distinct pairs of varieties was counted for every characteristic. The absolute discriminating power of every characteristic is defined by the number of pairs of varieties declared distinct. The characteristic that

distinguishes the highest number of pairs has the highest discriminating power. All considered characteristics were ranked according to their absolute discriminating power. The characteristic that distinguishes the highest number of pairs has also the highest relative discriminating power. Next in turn according to the relative discriminating power is such characteristics that distinguishes the highest number of pairs but after excluding all pairs already declared distinct by characteristics with higher relative discriminating power. All characteristics were also ranked according to their relative discriminating power. Such approach allows to assess usefulness of characteristics in DUS investigations. All mentioned calculations were performed independently for each year at first for complete set of data (60 measurements), and also for randomly reduced for 50% set of data (measurements of only 5out of 10 plants taken from every plot). Finally the comparisons of discriminating power of characteristics was performed for these two different numbers of measurements.

4. Results

The overall list of decisions on distinctness of varieties made on the basis of complete and reduced set of data are given in table 1 and table 2, respectively. Comparison of figures (sums of ranks) from these tables shows that for full and reduced data, the three characteristics possessing the highest absolute discriminating power are exactly the same (RNWW, LFS and KZ). The same phenomenon is observed for relative discriminating power. In the later case, the three best characteristics are RNWW, ZDNZ and KLK both for full and reduced data. It means that usefulness of characteristics from that point of view is exactly the same for these two different numbers of measurements. In table 3 the numbers of distinct and indistinct pairs of varieties in succeeding years are given. In the considered years there were 1331 and 1870 indistinct pairs for full and reduced data respectively. The difference is 539 pairs. It is only 4,24% off all 12700 considered pairs. It is interesting to observe that for all years but year 2007 the differences were rather meaningless.

5. Discussion and conclusions

Performed analysis of multiyear DUS data concerning amenity varieties of perennial ryegrass showed that discriminating power of characteristics (ability of distinguishing varieties) changes only a bit when number of measurements is reduced from 60 to 30 keeping the same number of replicates. For reduced

number of measurements, the number of not distinct varieties is – as expected – slightly larger. It is the result of commonly known fact that the variance of average is reversely proportional to the number of measurements. For analyzed set of data the number of indistinct pairs of varieties increased for about 4% after reducing numbers of measurements for half. It is worth to mention that final decision concerning distinctness (lack of distinctness) for majority of candidate varieties is taken after collecting two or thee years results. But in the latter case the differences among varieties are tested against the mean square for variety-by-year interaction (in yearly analyses against experimental error). The mean square for that interaction depends more on the number of years than on the number of measurements (and replicates). So it is expected that reduction of number of measurements will have smaller influence on distinctness decisions in series of DUS trials than that observed in single (one year) trials. It will be considered in our next paper. Similar considerations concerning the influence of number of plants on assessment of uniformity of candidate varieties in series of trials can be found in a paper by Kristensen and Meyer (2006). They showed that in DUS trials on rape and yellow mustard there is some room for changing the number of measurements, at least for some of observed characteristics. Our conclusions are in line with conclusions presented in that paper. The same pair of authors, Meyer and Kristensen (2008) considered also the consequences of reduction of number of measurements not for candidate varieties but for so-called reference set varieties. Slightly similar consideration can be also found in a papers by Pilarczyk and Zawieja (2001). They considered more general problem of optimal relationship among number of years, replicates and measurements in DUS trials. In a paper by Roberts (2007) the problem of decreasing the size of reference set is considered.

Table 1 Absolute and relative numbers of distinguished pairs of amenity varieties (*Perennial ryegrass* L.) revealed on the basis of full set of data and ranks of characteristics

Characteristic		Year																				Sum of ranks	
		2005				2006				2007				2008				2009					
		Absolute number of pairs	Rank	Relative number of pairs	Rank	Absolute number of pairs	Rank	Relative number of pairs	Rank	Absolute number of pairs	Rank	Relative number of pairs	Rank	Absolute number of pairs	Rank	Relative number of pairs	Rank	Absolute number of pairs	Rank	Relative number of pairs	Rank	Absolute	Relative
Plant	RNWW	891	1	891	1	1253	1	1253	1	1326	3	2	3	802	4	140	4	2058	1	2058	1	**10**	**10**
	ZDNZ	296	8	50	6	1227	2	578	2	1212	4	0	6,5	743	7	238	3	1582	6	36	6	37	**23.5**
Flag leaf	LFD	507	6	38	7	573	7	12	7	23	6	0	6,5	618	9	27	7	1322	7	3	9	35	36.5
	LFS	529	5	20	9	737	6	38	6	0	8,5	0	6,5	1014	1	1014	1	1753	2	50	5	22.5	27.5
	LFW	634	3	215	3	785	4	244	3	5	7	0	6,5	834	3	4	9	1676	4	519	2	**21**	**23.5**
	LFK	255	9	69	5	563	8	2	9	1966	2	31	2	728	8	54	6	643	9	21	7	36	29
Inflores-cence	KD	336	7	35	8	783	5	8	8	2176	1	2176	1	754	6	7	8	1661	5	11	8	24	33
	KLK	684	2	411	2	977	3	114	4	416	5	0	6,5	872	2	441	2	1751	3	184	3	**15**	**17.5**
	KZ	610	4	150	4	534	9	49	5	0	8,5	0	6,5	795	5	68	5	1062	8	108	4	34.5	24.5
No.of varietes		73				72				67				66				80					
No.of pairs				2628				2556				2211				2145				3160			
No.of disting. pairs				1879				2298				2209				1993				2990			
No. of non-disting. pairs				749				258				2				152				170			

Table 2. Absolute and relative numbers of distinguished pairs of amenity varieties (*Perennial ryegrass* L.) revealed on the basis of reduced set of data and ranks of characteristics

Characteristic		Year																				Sum of ranks	
		2005				2006				2007				2008				2009					
		Absolute number of pairs	Rank	Relative number of pairs	Rank	Absolute number of pairs	Rank	Relative number of pairs	Rank	Absolute number of pairs	Rank	Relative number of pairs	Rank	Absolute number of pairs	Rank	Relative number of pairs	Rank	Absolute number of pairs	Rank	Relative number of pairs	Rank	Absolute	Relative
Plant	RNWW	867	1	867	1	1083	2	532	2	1160	2	504	2	692	5	250	3	1948	1	1948	1	**11**	**9**
	ZDNZ	275	8	61	5	1162	1	1162	1	1191	1	1191	1	631	7	56	6	1549	5	206	3	22	**16**
Flag leaf	LFD	408	6	22	8	532	7	14	8	851	3	128	3	459	9	7	9	1311	7	9	9	35	36.5
	LFS	427	5	17	9	498	8	6	9	570	7	4	8	855	1	855	1	1551	4	14	8	25	35
	LFW	638	2	428	2	749	5	267	3	611	5	7	7	635	6	26	7	1638	2	569	2	**20**	**21**
	LFK	210	9	47	7	503	7	36	6	574	6	24	5	615	8	75	5	657	9	42	6	39	29
Inflores-cence	KD	315	7	54	6	732	6	61	5	785	4	58	4	696	4	21	8	1533	6	18	7	27	30
	KLK	624	3	111	4	853	3	127	4	145	8	16	6	771	2	134	4	1589	3	52	5	**19**	**23**
	KZ	512	4	214	3	469	9	19	7	117	9	2	9	739	3	466	2	952	8	103	4	33	25
No.of varietes		73				72				67				66				80					
No.of pairs				2628				2556				2211				2145				3160			
No.of disting. pairs				1821				2224				1934				1890				2961			
No. of non-disting. pairs				807				332				277				255				199			

Table 3. Numbers of indistinct pairs of varieties – yearly results

Years	Total numer of pairs	Numer of indistinct pairs	
		complete data	reduced data
2005	2628	749	807
2006	2556	258	332
2007	2211	2	277
2008	2145	152	255
2009	3160	170	199
In total	12700	1331	1870

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