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Full Length Research Paper

Optimal number of observation, treatment and replication in field experiments

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Experimental recognostic trial was carried out in spring barley agrophytocenoses, evaluating data standard error (SE) and mean accuracy (MA%) depending on arranged elementary trial plots, treatment and replication number, their model size. The received experimental data are applicable carrying out all agronomical and biological field trials. Trial field was divided into 222 elementary plots of 13.44 m^2. The trial objective was agrophytocenoses of spring barely *Roland*. Observation number increasing from 2 to 6, trial data accuracy demonstrably increases, while increasing it from 6 to 10 accuracy increases more slowly, and increasing the number of observations from 10 to 30, accuracy almost does not change. Enlarging plot size from 13.44 to 134.4 m^2, the accuracy of experimental data increased. However, experimental field was homogeneous enough. Increasing replication number, trial SE regularly decreases independently of the arranged treatment number in the trial. Modelling trial with 40 m^2 size plots with 2 to 3 replications, data accuracy reaches 10.2 to 6.2%, so trials can be acknowledged as inaccurate. Increasing replication number from 4 to 6, data accuracy decreases from 4.7 to 2.8%. Increasing replication number to 10, trial accuracy increases to 2.0%. Further increasing replication number from 10 to 37, data accuracy progressively increases from 1.5 to 1.6%. Therefore, carrying out field trials in moderate plots relating to 40 m^2, experiments should not have less than 3 to 4 replications. Optimal number of treatments, according to SE and accuracy evaluation, is between 4 and 7. Then the highest accuracy of experimental data is reached.

Key words: Elementary plot, number of observation, replication, treatment, recognostic field trial, standard error, data accuracy.

INTRODUCTION

Field experiments have been designed to account spatial structures since the inception of randomized complete block designs by R. A. Fisher (Legendre et al., 2004). Statistical methods are used to assist scientists in interpreting data, which are, by the very nature of biological systems, variable. For this reason, the use of statistics has become an indispensable part of the process of research (Onofri et al., 2010). Agronomic field trials conventionally utilized classical randomized block design. In order to avoid influences of spatial variability, replicated treatment plots should be established as homogeneous as possible. A widely accepted assumption was that existing field soil variability could be compensated by a large number of replicated plots (Davis, 1986). Many sites used for field trials exhibit a spatially-dependent variance structure in those nearby observations that are autocorrelated. This may affect treatment comparisons made at unequal distances (van Es and van Es, 1993). It is recommended, before a new experiment that was laid out and conducted particularly where previous plots had been treated with different treatment levels to eliminate the previous treatment effects. The common practice was to crop the site

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uniformly for one or more years (Cassel et al., 2000). Researches must secure the representative number of experiment treatment replications (Plant, 2007). In the frequent case of normally distributed data sets, the measure of variability should be either the standard deviation (SD), or the standard error (SE) of the estimate mean, etc. (Onofri et al., 2010). In many experimental designs, replication occurs at numerous levels. Experiments may be replicated within plots, and plots may be replicated in locations, and locations may be replicated in space (Ramirez et al., 2000). Researchers should clearly designate the unit of replication and the number of replicate samples in the experiment (Andow, 2003). Therefore, experiments may need replicates (Onofri et al., 2010), while their optimal number can be under discussion. The experimental data accuracy indicates SE that is essentially influenced by the number of observation or replication (Доспехов, 1979; Stancevičius and Arvasas, 1981; Зайцев, 1984). Sampling mean is exact enough when its error does not exceed 5%, estimate when its error varies from 5 to 10% and inaccurate when its error exceeds 10% (Stancevičius and Arvasas, 1981).

The objective of the research was recognostic field trial experimental data accuracy change in dependence of model plot size, number of observations, treatments and replications.

The aim of this work was to evaluate impact of observation, plot number on data mean accuracy (MA%) and SE; to establish optimal number of treatments and replications carrying out field trials seeking for exact and reliable experimental results.

The received experimental data are applicable carrying out all agronomical and biological field trials.

MATERIALS AND METHODS

Experiment was conducted during 2000 at the Experimental Station of Lithuanian University of Agriculture (Aleksandras Stulginskis University). Data of recognostic field trial were evaluated estimating SE and MA% depending on elementary plot number, its model size, model number of treatments and replications. Agrophytocenoses of spring barley variety *Roland* was grown on trial field. Agrophytocenoses is a community encompassing agricultural plants and weeds (Lazauskas and Pilipavičius 2004; Pilipavičius et al., 2011).

Experimental field was divided into 222 elementary plots, which size was 13.44 m^2 (Figure 1). Spring barley was harvested at hard stage of maturity. Yield data were evaluated by the dispersion analysis establishing SE, MA% (Tarakanovas, 1997, 1999) and dependences between variables were determined by the correlation - regression analysis (SPSS Science, 2000). Field model plot data according to observation/plot number were evaluated applying Equations 1 and 2.

Field model plot data SE was calculated by the Equation 1 and mean data accuracy by the Equation 2.

$$SE = \pm\sqrt{\frac{\sum (x-\bar{x})^2}{n*(n-1)}} \quad (1)$$

$$MA\% = \frac{SE}{\bar{x}} * 100 \quad (2)$$

Where: *SE*, Standard error (spring barley grain yield mean error, t ha^{-1}); *MA%*, mean accuracy (%); ∑, sum; x, data, spring barley grain yield, t ha^{-1}; $\bar{x}$, mean, spring barley grain yield, t ha^{-1}; $\sum (x-\bar{x})^2$, square sum of differences, t ha^{-1}; n, number of observations/plots; 100, coefficient (%).

Modelling number and size of observations/plots, treatments and replications were appealed to initial factual elementary plot size 13.44 m^2. Modelled plot size constitutive to 40.32 m^2 and 134.4 m^2 by the model did not exceed factual size of experimental field. Spring barley grain yield data (Figure 1) for statistical estimation were taken randomly utilising mathematical model on MS EXEL basis.

RESULTS AND DISCUSSION

Experimental field was divided into 222 elementary plots of 13.44 m^2 (Figure 1). Agrophytocenoses of spring barley *Roland* was taken as the research object. In elementary plots of recognostic trial factual grain yield of spring barley was established, which data were used estimating influence of number of observations/plots to SE and mean accuracy, for calculation of optimal number of treatments and replications and for evaluation of importance of field plot size change to experiment data quality.

Grain yield of spring barley from 222 elementary plots was grouped into 8 groups with 0.5 kg per plot distance. Distribution of spring barley productivity, soil fertility, showed data set deflection to right (Figure 2).

Influence of observation/plot number on standard error and data mean accuracy

Experimental data SE and mean accuracy MA% essentially depended on observation number and plot size (Figures 3 to 8). Increasing number of observations at given plot size 13.44 m^2 (Figure 3) and 134.4 m^2 (Figure 4), level of SE regularly decreased and data MA% increased, that is, the percentage value of MA% decreased. Preserving steady number of observations and increasing size of model plot from 13.44 to 134.4 m^2, SE decreased and data MA% increased (MA% decreased) (Figures 3 to 4). However, it should be paid attention to the fact, that experimental field was homogeneous enough (Figures 1 to 2). On the other hand, modelling different plot size (13.44 and 134.4 m^2), factual boundaries of the experimental field were not exceeded, that is, plot size increasing 10 times, number of replications accordingly were decreased 10 times.

Spring barley productivity data SE and MA% variation were subjected to correlation-regression analyses. It confirmed negative non-linear dependence of observations number on SE and data MA%. Increasing number

4.72	4.68	4.60	5.60	6.18	5.68	5.68	5.58	5.00	5.90
4.18	5.12	5.48	4.98	4.54	5.80	4.72	4.60	4.80	5.22
4.14	4.26	4.10	3.58	4.26	4.56	4.48	4.68	3.58	4.16
3.80	5.24	4.02	4.04	4.81	4.74	5.36	4.14	5.30	4.58
4.00	5.36	3.74	4.68	4.24	4.80	5.44	5.48	5.42	4.52
4.82	5.32	4.48	4.38	5.02	5.16	5.48	5.52	5.78	5.02
4.18	5.18	4.16	4.54	4.66	5.04	4.32	5.06	5.06	4.74
5.38	5.44	4.76	4.76	4.72	5.18	5.66	5.72	5.24	5.14
4.10	5.66	3.82	4.44	4.94	5.32	5.24	5.06	5.66	5.38
4.38	4.04	4.72	5.08	5.20	5.20	6.10	5.40	5.46	5.62
4.26	4.88	3.98	4.76	4.62	5.14	5.18	5.76	5.50	4.74
4.70	5.62	4.82	4.84	4.76	4.40	5.18	5.28	4.96	5.70
3.34	4.98	4.86	5.20	5.46	5.24	5.48	6.02	5.90	5.84
3.70	5.06	3.94	5.30	5.08	5.24	5.54	5.60	5.58	5.20
	3.98	4.00	4.70	5.36	4.98	5.68	5.60	5.32	5.36
	2.92	3.90	4.38	4.94	4.36	4.52	5.22	4.10	5.00
		4.54	5.08	5.42	4.96	4.92	6.06	5.18	4.58
		4.70	4.76	4.78	4.72	5.54	5.04	4.88	2.98
		5.28	5.44	4.62	4.74	5.40	5.20	5.46	3.16
			4.74	4.28	4.42	4.06	4.22	4.66	3.24
			5.76	4.46	4.20	4.16	4.24	4.96	3.42
			4.88	4.28	5.12	5.06	4.18	5.14	3.46
				4.66	4.68	5.48	4.56	5.58	4.60
				4.14	4.92	5.28	4.76	4.58	4.80
				4.76	5.10	4.50	4.62	5.92	5.02
									4.82

2.51-3.00	4.51-5.00
3.01-3.50	5.01-5.50
3.51-4.00	5.51-6.00
4.01-4.50	6.01-6.50

Figure 1. Distribution of 222 elementary plot productivity growing spring barley in recognostic field trial. Spring barley grain yield, kilograms per elementary plot of 13.44 m^2.

of observations – number of evaluation sites – experimental data SE and MA% decreased (decreasing MA% data are recognised more accurately) at plot size13.44 m^2 (Figures 5 to 6) and at plot size134.4 m^2 (Figures 7 to 8). MA% calculation directly belongs on SE and data mean ratio (Equation 2), therefore, the correlation-regression analysis between those both variables was not evaluated. Other statistical index, coefficient of variation (CV), mathematically is close to MA than to SE. When CV is to 10%, variation of the experimental indication is small, from 10 to 20% - average and from 20% can be acknowledged as high. Practically, in experimental data evaluation CV is used less often than SE. Hence, statistical index of SE was major in our experiment. On the other hand, SE does not give possibility of comparison of separate experiments with different or even similar variables. To compare separate experiments and their accuracy it was used statistical index of MA%. Respectively, it is originated a possibility to draw a parallel between separate experiments and check their accuracy. Increasing number of observations/replications or number of

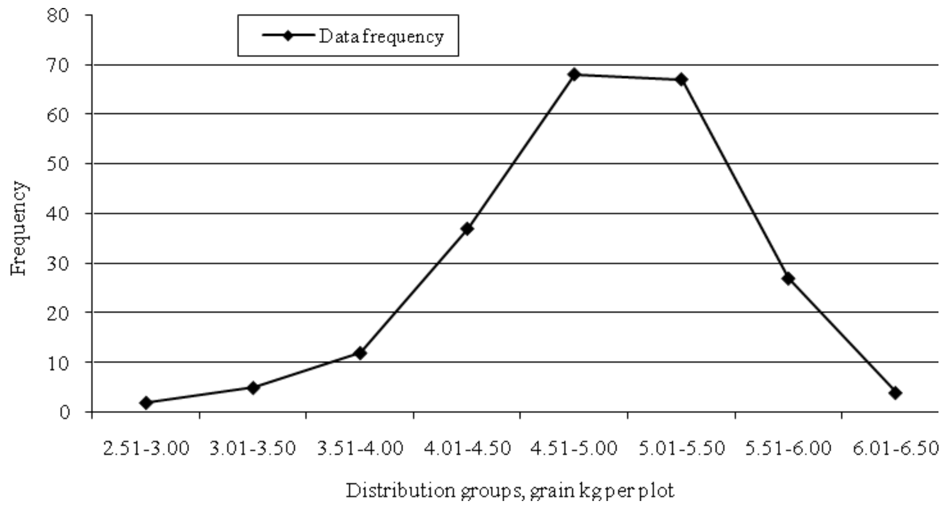

Figure 2. Spring barley productivity data set distribution of recognostic field trial.

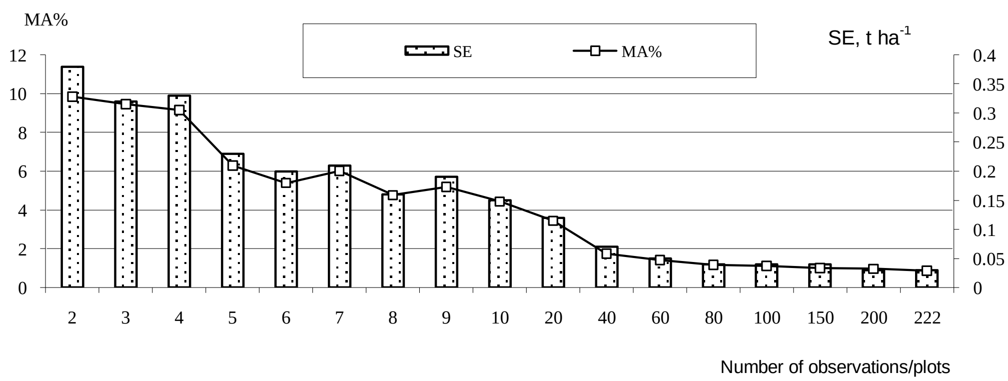

Figure 3. Spring barley productivity data SE and mean accuracy, MA% changing observation/plot number (plot size 13.44 m^2).

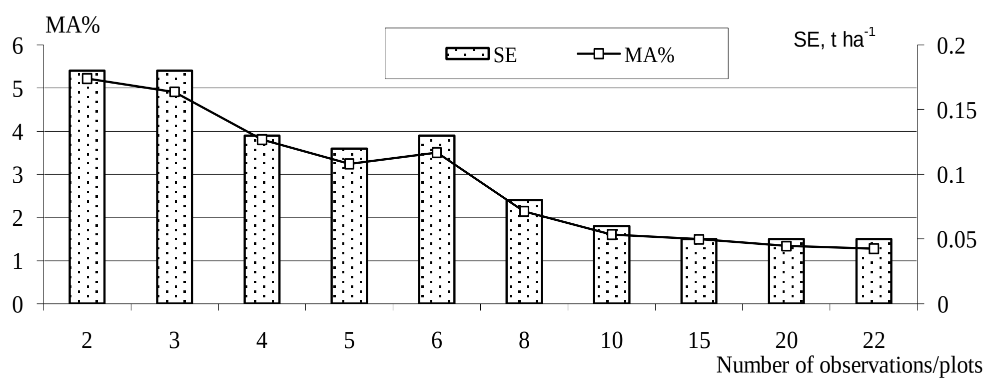

Figure 4. Spring barley productivity data SE and mean accuracy, MA% changing observation/plot number (plot size134.4 m^2).

number of evaluation sites from 2 to 4 - 6, experimental data accuracy essentially increased. Increasing number of observations/replications from 4 - 6, to 10, increase of data accuracy moderated and from 10 to 30 observations/replications increase of data accuracy became hardly visible (Figures 3 to 8).

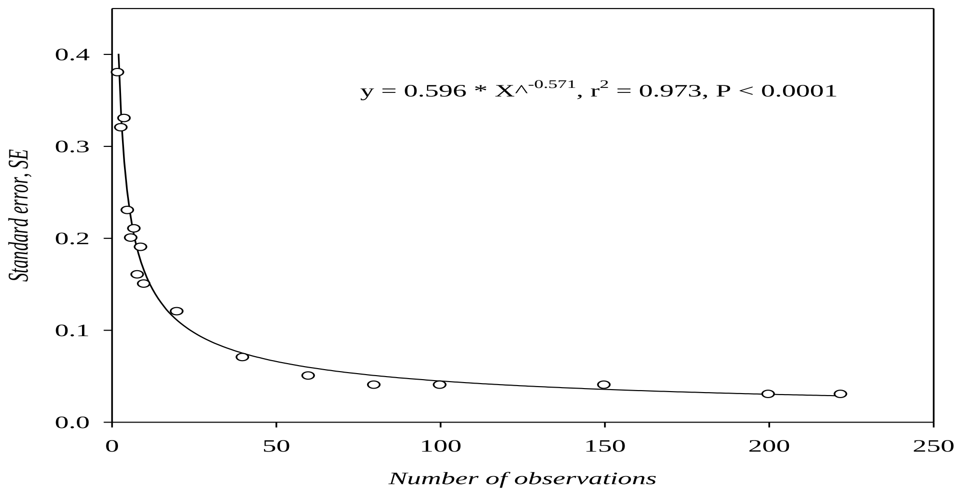

Figure 5. SE variation dependence on observation number when plot size is 13.44 m^2.

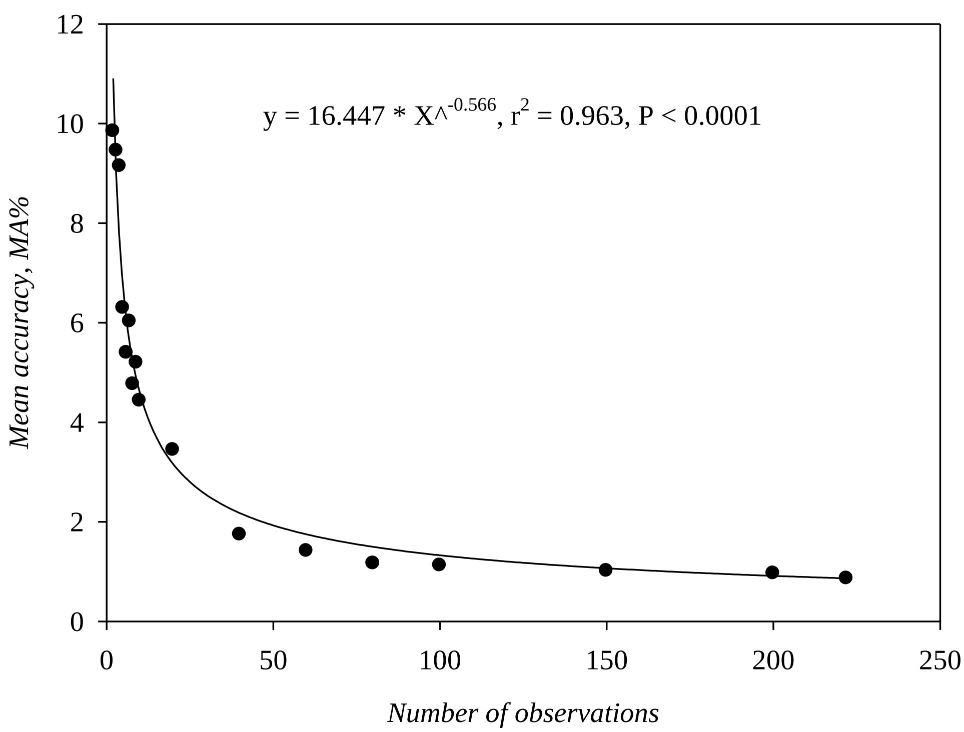

Figure 6. Data mean accuracy, MA% variation dependence on observation number when plot size is 13.44 m^2.

Optimal number of treatments and replications in field trials

Carrying out field trials conventionally, number of treatments is foreseen in advance that totally covers aim and tasks of particular experiment. However, number of experimental treatments can influence the accuracy of experimental data. Model plot size was calculated to 40.32 m^2. On recognostic experiment basis, organising random plot distribution to different number of treatments and their replications were established. Increasing treatment number from 2 to 5 having steady number of replications, has tendency to decrease SE of experimental data. Increasing treatment number from 6 to 7 SE varied to increase or decrease while from 8 model treatments SE

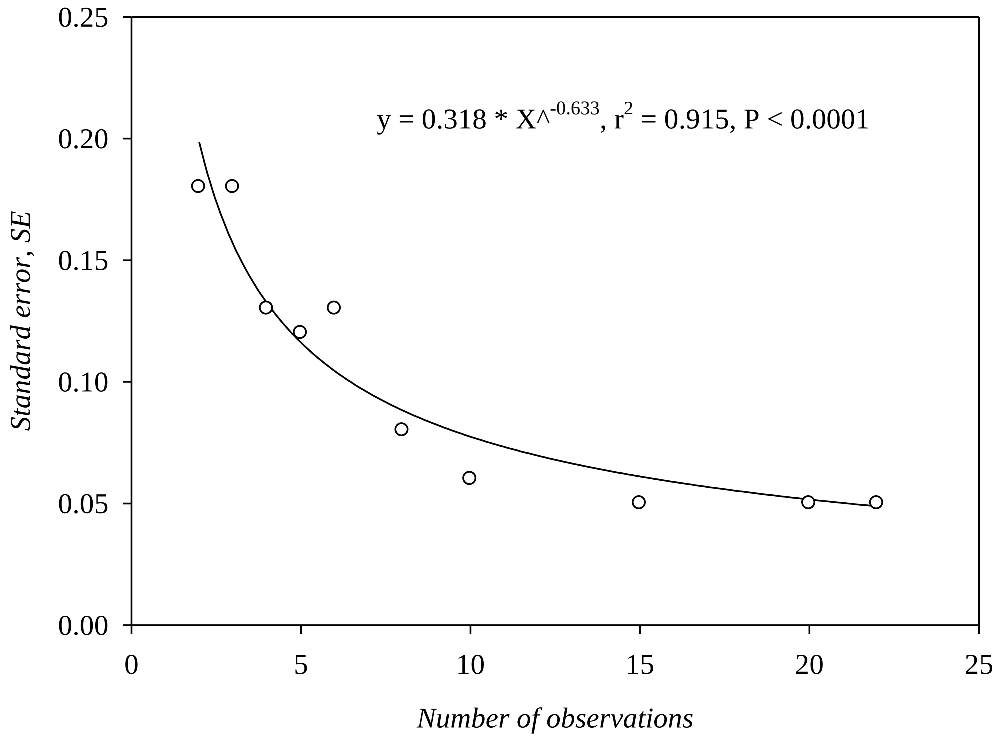

Figure 7. SE variation dependence on observation number when plot size is 134.4 m^2.

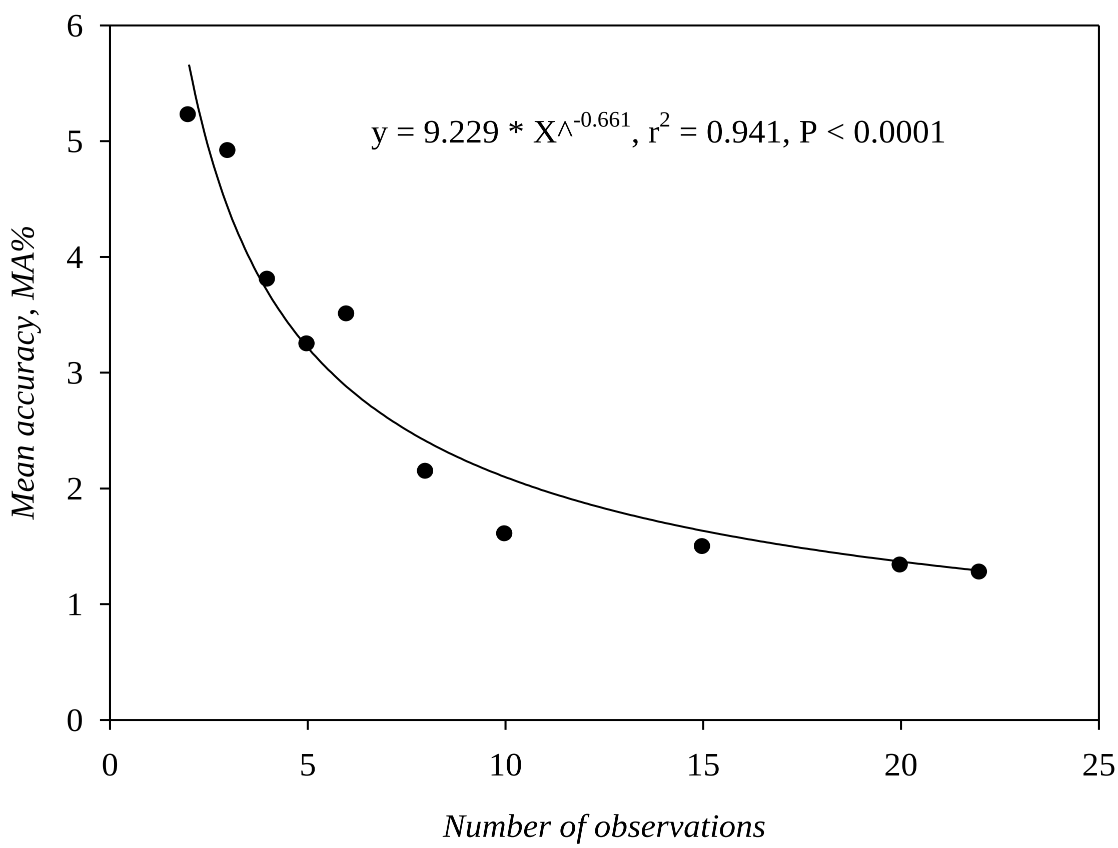

Figure 8. Data mean accuracy, MA% variation dependence on observation number when plot size is 134.4 m^2.

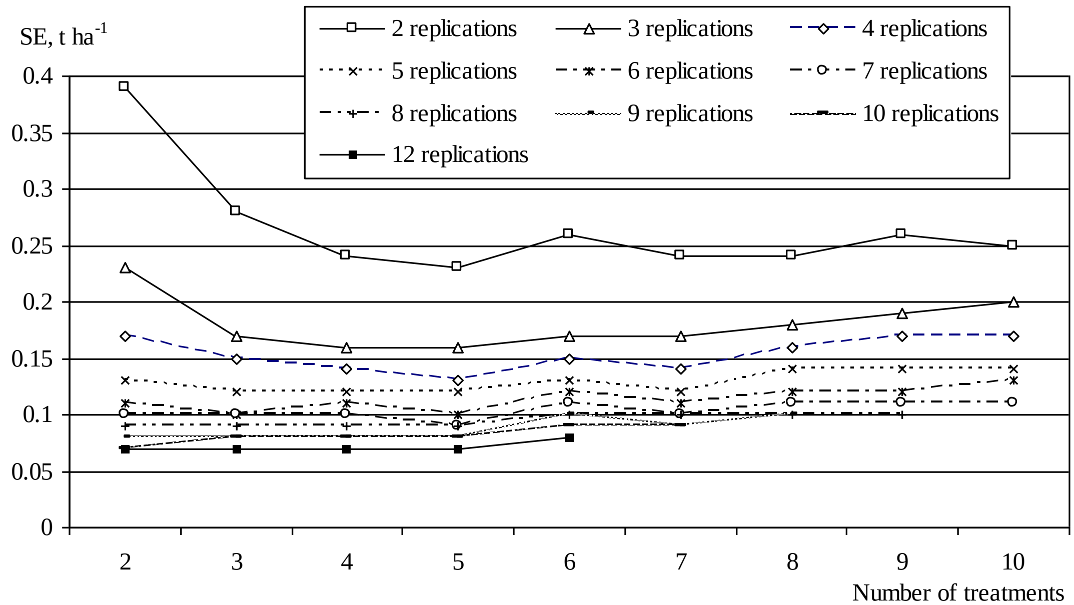

Figure 9. Influence of treatment number on spring barley grain yield experiment data SE, model plot size 40.32 m^2.

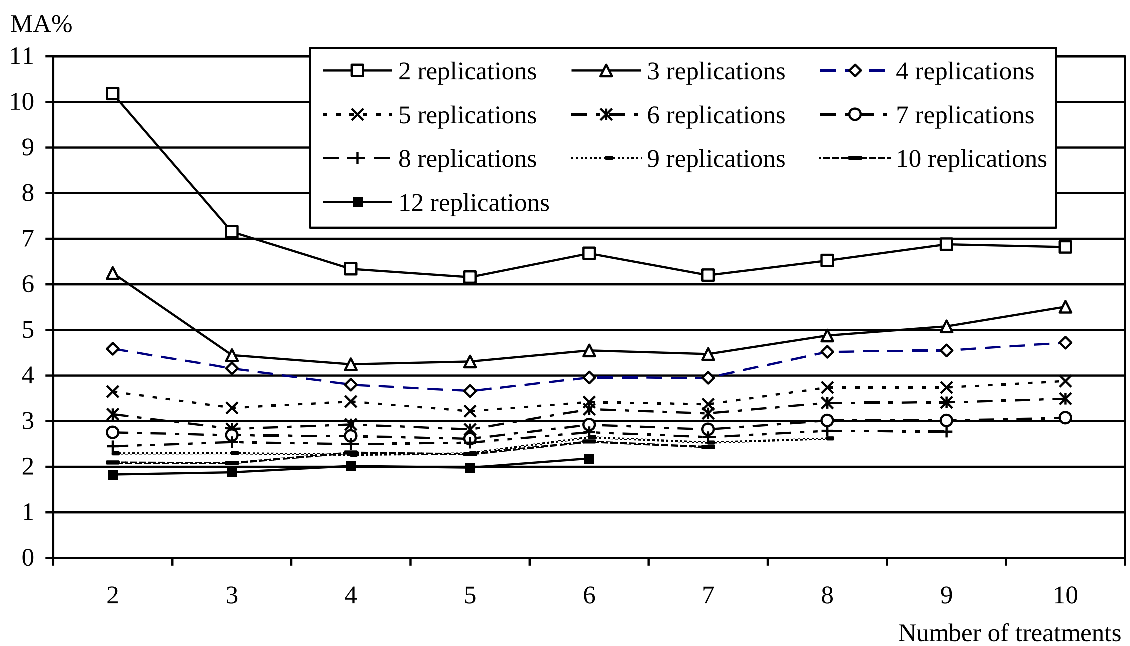

Figure 10. Influence of treatment number on spring barley grain yield experiment data mean accuracy, MA%, model plot size 40.32 m^2.

slightly started to increase (Figures 9 to 10).

Experiment with higher number of treatments theoretically can increase accuracy of research data, because it regularly increases degrees of freedom. Hence, rising treatment number in experiment covers bigger area and increase of experimental plot area accordingly increases variation of soil productivity (Figure 1) and distance between model comparison treatments. Therefore, it is complicated to select for experiment equal or similar productivity plots. It

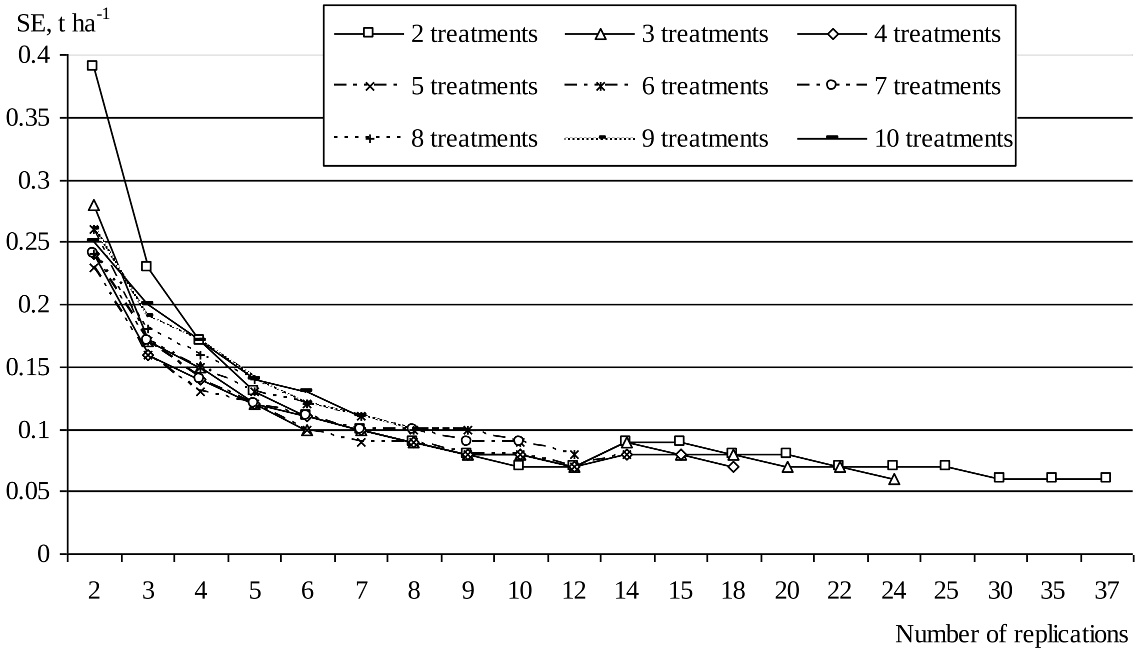

Figure 11. Influence of replication number on spring barley grain yield experiment data SE changing number of treatments from 2 to 10, model plot size 40.32 m^2.

causes decrease of experimental data accuracy. Hence, treatment number in experiment should rise limitedly and safely. Arranging more treatments in experiment arise demand of non-standard methods utilisation. Low number of treatments is issue of no less importance. Having low number of treatments (2 to 3), it is obligatory to increase number of replications seeking for correct and accurate evaluation of experimental data. Accuracy of experimental data can increase in correct way when it is composed sufficient number of observations. According to findings of our recognostic experiment, optimal number of treatments in trials should vary between 4 and 7 concerning to SE and MA% of experimental data (Figures 9 to 10).

Researchers in agricultural sciences practically use different number of the trial treatment replications: 3 (Nsarellah et al., 2011; Kolo and Umaru, 2012), 4 (Šarauskis et al., 2010; Vasileva and Ilieva, 2011; Kazerani, 2012), 5 (Mlakar et al., 2012), 6 (Gnepe et al., 2011) etc. Consequently, increase of number of treatment replications leads to experimental input increase. Though, research data purchaser is imperative to receive statistically reliable and secure research data with marginal inputs. Different number of experiment treatment replications selection adequately influenced field trial data accuracy and reliability. Replication of treatments in space allowed to cover bigger field plot of soil productivity variation. It leads to receiving more accurate and reliable means of experimental data. Increasing number of replications, SE of data regularly decreased and accuracy increased irrespectively of treatment number in trial. Particularly, SE soundly decreased and MA% decreased to 4.7 to 2.8% according to number of treatments, replication number increasing from 2 to 4 - 6. Increasing number of replications from 4 - 6, to 9 - 10, experimental data SE started to decrease moderately and MA% increased to 2.0% and modelling experiment with more than 10 replications, experimental data SE almost did not change (MA% increased from 1.5 to 1.6%). While experiment data accuracy with 2 to 3 replications was just from 10 to 6.2% (Figures 11 to 12), it means that correctness of such research data use could be under query.

Because of experimental data accuracy and reliability demand, field trial should be utilised at least with 3 replications expecting low variability of experimental indication, optimal number of treatment replications according to our experiment data could be 4 and 5 to 6 could give tangible accuracy of experimental data. Increasing number of replications, change of soil productivity can be eliminated, especially when soil productivity change regularly, data SE can be decreased and data MA% increased. The importance of the number of sites or observations are described and shown in Figures 3 to 8.

It is important to recognise the difference between a true replicate and a pseudoreplicate. When the

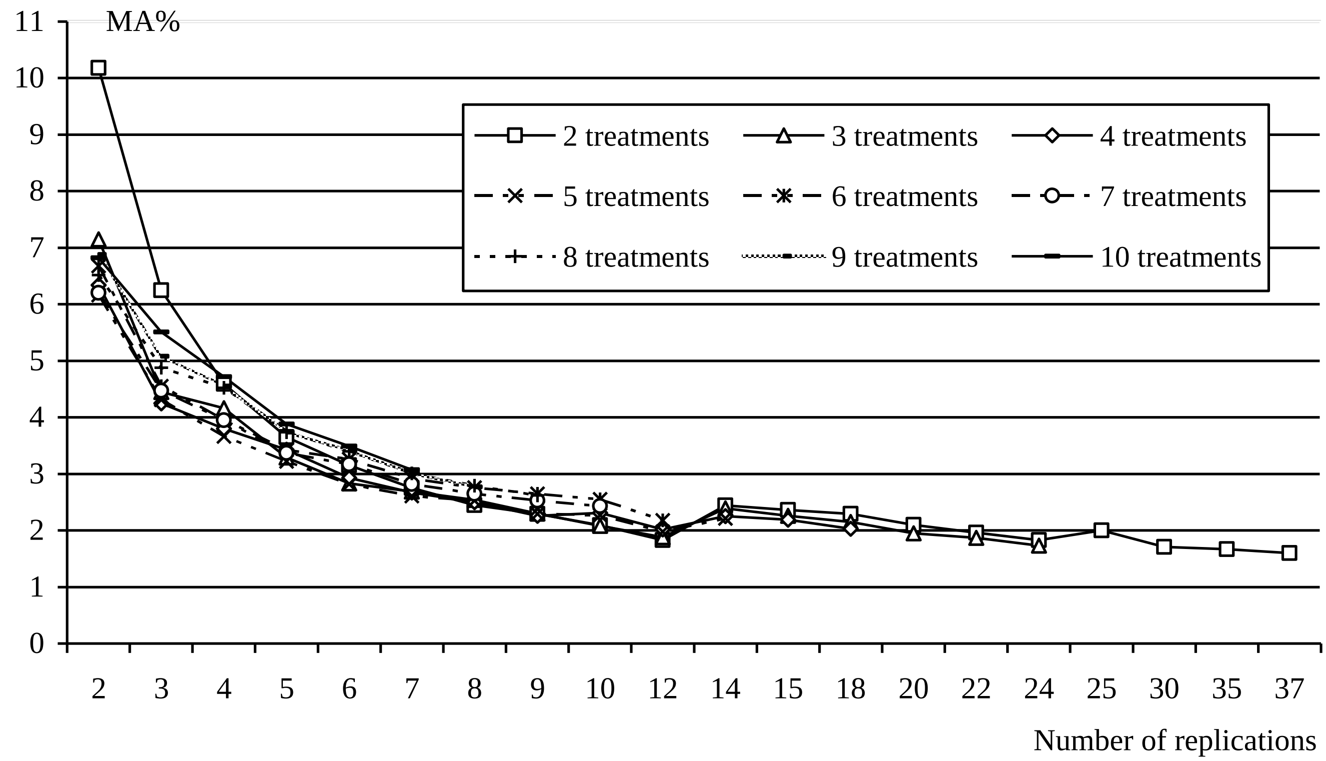

Figure 12. Influence of replication number on spring barley grain yield experiment data mean accuracy, MA%, model plot size 40.32 m^2.

randomisation process to allocate the treatment is applied to several independent experimental units, there are true replicates. This must be clearly distinguished from pseudoreplication (sub-sampling), where several measurements are taken on a single sample and thus they are not independent, because they share the same sample. If there are no true replicates, no matter how often the unit is sub-sampled (Onofri et al., 2010). Pseudoreplication should never be mistaken for true replication, even in the case of laboratory experiments (Morrison and Morris, 2000).

Summarised results of this study showed that (1) increasing number of observations from 2 to 6, experimental data accuracy increased essentially, number of observations increasing from 6 to 10 – increase of data accuracy moderated and from 10 to 30 observations data accuracy increased very slightly; (2) obligatory number of experimental treatment replications is 3 to 4; (3) seeking for tangible accuracy of experimental data number of experimental treatment replications is 5 to 6; (4) optimal number of treatments is between 4 and 7; (5) agronomical and biological field experiment optimally should be carried out with 4 to 7 treatments arranged in 3 to 6 replications, according to SE and experimental data accuracy evaluation.

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