P L A N T   B R E E D I N G   A N D   S E E D   S C I E N C E
Volume 45 Number 1 2001

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SPATIAL VARIABILITY AND EFFICIENCY OF TREATMENT MEAN COMPARISONS IN AN EXPERIMENT WITH FODDER PEA USING MODERN STATISTICAL METHODS

ABSTRACTS

It is typical of breeding experimentation to conduct experiments on large breeding material tested on small plots with a limited number of replications. Under such conditions, observations made on adjacent plots are biased by the effect of autocorrelation and fertility trends. The actual treatment effects can be masked and the capability of the breeder to detect true treatment differences is impaired.

This paper deals with the problem of the estimation of effects of spatial variability and their impact on the efficiency of treatment comparisons. The considerations are based on the results from a breeding experiment with 25 treatments of fodder pea arranged according to the partially balanced incomplete block design (IBD) with 4 replications.

Plant height and seed yield were analysed with the conventional statistical method ANOVA, the nearest neighbour analysis (NNA) and kriging. Eventually, the efficiency of the neoclassical methods relative to the completely randomised design (CRD) and randomised block design (RBD) was calculated.

The estimation of the treatment effect on plant height was accomplished most efficiently with the NNA, whereas the efficiency of the alternative methods in the estimation of seed yield was comparable to the efficiency of the RBD.

Key words: spatial variability, ANOVA, ANCOVA, NNA, kriging, relative efficiency.

INTRODUCTION

The primary aim of plant breeding is to obtain and improve genotypes of crops. Breeding programmes start with the selection of initial plant material, which is then improved through certain breeding techniques. Efficient testing of new breeding material is an essential methodological problem. On the one hand, the breeder must assure that his decision on the selection of objects for further breeding work is unquestionable, on the other hand any exclusion of undesirable objects should be burdened with the smallest possible error margin.

Communicated by Ludwik Spiss

Testing of newly obtained breeding material is conducted under conditions of a field experiment. Due to a short supply of seeds at the initial steps of a breeding programme, testing experiments are conducted on microplots, on which a large number of entries are tested with a relatively low number of replications.

Under such conditions, observations from adjacent plots are frequently correlated. Effects of autocorrelation in conjunction with spatial effects related to soil variability influence the estimated experimental error, and consequently the capacity of the experiment to demonstrate the true entry effects. Biased entry effects lead to erroneous decisions, which may destroy or delay the outcome of the breeder's work. Also, cost of research increases.

Recently, researchers investigating field experimentation methods have tried to create a new approach to data analysis in which spatial variability is given more importance, a new approach that will be supplementary or alternative to the traditional analysis of variance for a given experimental design. The new type of analyses incorporates additional information on the location of an entry on a field, which constitutes a starting point for further correction of the value of a trait observed on the plot. Of the new approaches, the following deserve our attention: methods of analyses of trends in soil fertility, Papadakis's method called the nearest neighbour method (NNA) and kriging (Bartlett 1978, Krige 1966, Matheron 1963, 1971, Papadakis 1937)

The objective of the present study was (i) to present and discuss some data analysis methods which include information on spatial variability of the experimental field; (ii) to evaluate spatial variability of the experimental field on which two traits of 25 entries (genotypes) of fodder pea were tested in terms of soil acidity and nutrient availability (iii) to compare the efficiency of different data analysis methods.

METHODS

The study was based on the results obtained from a field experiment with fodder pea (*Pisum sativum* L.) conducted in 1998. The experiment was located on a field of the Experimental Station of the Olsztyn University of Agriculture and Technology in Tomaszkowo. A partially balanced square lattice design with 25 entries in 4 replications was applied in the experiment.

Prior to the establishment of the experiment, soil samples were taken to make chemical analyses on soil acidity and content of available nutrients (P_2O_5, K_2O, Mg). A total of 98 samples, including 50 samples for the pea experiment and 48 samples from the adjacent experiment with a yellow lupine experiment, were taken from the part of the experimental field covered with the two experiments: one with pea and the other with yellow lupine. A 4 m × 6 m measuring net was applied. The plot size of the experiment was 1.5 × 3m. Spring wheat was used as an intercrop between the plots. The mean height of plants was recorded on plots at harvest, after which the plants were threshed and the seeds were weighed.

Statistical analysis of the results was composed of the analysis of variance, for completely randomised design (CRD), randomised block design (RBD), incomplete block design (IBD) as well as the analysis of covariance with concomitant variables determined according to Papadakis's method (NNA) (Papadakis 1937, Bartlett 1978) and kriging (Gołaszewski 1997, Webster and Oliver 1990). Efficiency of the considered methods of data analysis was determined (Steel and Torrie 1980).

Below you will find a presentation of some methodological aspects related to the application of relatively new procedure like the NNA and kriging methods to the analysis of experimental results.

Papadakis's method - NNA

The method elaborated by Papadakis (1937) is also known as the Nearest Neighbour Analysis (NNA). According to Brownie *et al.* (1993), the term „Neighbour Analysis" stands for all types of analyses based on the information from adjacent (neighbour) plots in the estimation of spatial variability.

The approach suggested by Papadakis is intuitive and, although raising many doubts of the statistical nature, it is commonly applied in methodological studies (Kempton and Howes 1981, Wilkinson *et al.* 1983). In essence, this is a covariance method with an „untypical" concomitant variable. Untypical in the sense that it is determined on the data for a dependent variable.

Generally, the method consists in the 'removal' of entry variability from plot values, followed by the determination of the means of residuals from the adjacent plots, which are used as a concomitant variable in the analysis of covariance. For the purpose of this study, one variant of the method suggested by Bartlett (1978) was applied. The concomitant variable is determined by the iterative process, which helps stabilise the object mean and square error mean in covariance analysis. The calculation algorithm (i. e. the first differences) used in this paper can be sketched as follows:

1. Setting up the data according to the design on a field.
2. Determination of residuals of the trait measured on each plot from the entry means.
3. Determination of a concomitant variable for each plot minus the entry variability, which is the mean of diversions from the adjacent plots.
4. Correction of the entry means according to the neighbours.
5. Points 2-4 repeated - subsequent iterations.
6. Covariance analyses with concomitant variables from subsequent iterations.

Kriging

Kriging allows the researcher to predict the values for points of the field in which the measurements have not been made. Unlike interpolation methods, kriging makes use of the information on spatial variability of the property or trait analysed. Semivariance fixed for different distances (*lag h*) between the sampling points serves as a measure of spatial variability. Detailed statistical assumptions and description of the methods one can find in the books by Journel and Huijbregts (1978) and Cressie (1991), application of the spatial analyses in pedology in the book by Webster and Oliver (1990), and practical aspects of the utilisation of the methods in the book by Clark (1979), respectively. Besides, in polish journals the papers of Kristensen and Ersboll (1992) and Gołaszewski (1997) deal with the methods from the perspective of field experimentation methodology.

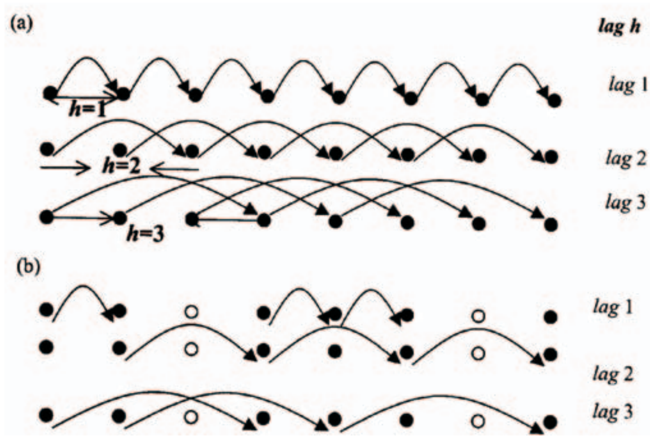

Fig.1. The scheme of semivariance calculation for complete observations – a, and missing observations - b (acc. to Gołaszewski 1997)

One-dimensional semivariance is calculated according to the formula:
for *i = 1, 2, 3, ..., N*(*h*); where *N*(*h*) - number of pairs observations [*z*(*i*), *z*(*i*

$$\hat{y}(h) = \frac{1}{2 \times N(h)} \sum_{i=1}^{N(h)} \left[z(i) - z(i+h) \right]^2$$

+ *h*)] spaced by lag *h*

The above procedure deals with pairs of observations recorded in one direction, but the idea applies to all possible directions according to the net of measurements. *Lag h* is an integer, and a multiple of subsequent distances between the points of measurements.

Graphically, semivariance distribution related to distance *h*, called a variogram, may have different shapes, depending on the character of spatial variability of the variable analysed. A model of such a variogram is described with the function for which the dispersion of the semivariance values is the smallest. The model, which allows the researcher to estimate relationship between semivariances and distances can be linear, logarithmic, exponential, spherical, etc., but the question which form to chose is difficult and requires some experience on behalf of the researcher. The key to the problem is that any model is no more than an approximation of the actual variogram; if defined incorrectly, it may become the main source of a bias of the results interpolated with kriging. Only two models: linear and spherical, seem to be of practical importance in field experimentation (Gołaszewski 1997, Trangmar *et al.* 1985, Stroup *et al.* 1994).

A linear model of semivariogram with a threshold is as follows:

$$\left\{ \begin{array}{l} \gamma(h) = C_0 + C \times \dfrac{h}{a} \quad dla \quad 0 < h \le a \\ \gamma(h) = C_0 + C \quad dla \quad h > a \end{array} \right\}$$

and a spherical model of semivariogram has a form:

$$\left\{ \begin{array}{l} \gamma(h) = C_0 + C \times \left(\dfrac{3}{2} \times \dfrac{h}{a} - \dfrac{1}{2} \dfrac{h^3}{a^3} \right) \quad dla \quad 0 < h \le a \\ \gamma(h) = C0 + C \quad dla \quad h > a \end{array} \right\}$$

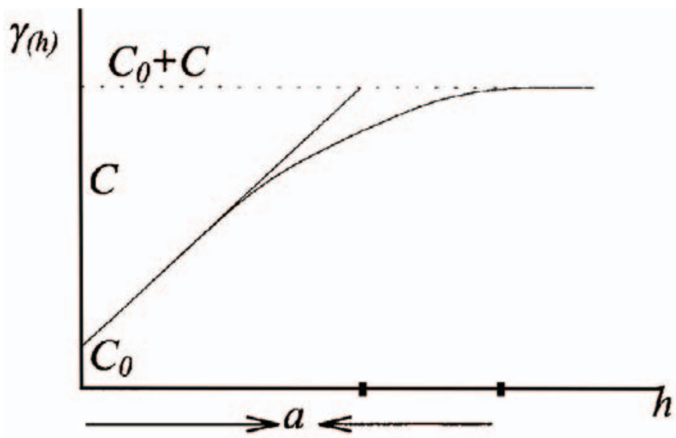

Fig.2. Theoretical semivariogram

Typically, a semivariogram is defined with the following parameters (Fig.2):

- range (a)

  defines the spatial boundary depending on the observation (it is assumed that semivariances exceeding range a equal σ^2 and are accounted for by random variability)

  defines the maximum radius, within which adjacent observations are collected for kriging.

- nugget variance (nugget effect) (C_0)

  defines non-continuity of the variogram in relation to the central point of the coordinate system,

  results from measurement errors and microvariability at distances smaller than sampling.

- structural variance (C)

  a true spatial component of a sample variance; defines this part of variance which results from the spatial aspect of the observation.

- threshold ($C_0 + C$)

  defines the value at which the variance becomes stabilised; corresponds to lack of spatial correlation of variables $Z(x)$ and $Z(x+h)$, the fact which can be interpreted as a spatial independence of the observation, with the best estimator - mean value of variability for the whole field, pure nugget effect and an increasing variance of sample s^2 suggest the occurrence of microvariability, which becomes evident at larger distances,

  the threshold is defined by the value of C when spatial variability of the observation is present and C_0 does not occur.

RESULTS AND DISCUSSION

Kriging of chemical properties of soil

For the study presented in this paper, no spatial relation was determined for pH, P and K (Fig.3). Estimates of semivariances set for subsequent distances between points of soil sampling were grouped around the variance of a sample. Magnesium semivariance distribution was described by the linear model with a parameter which determined the nugget variance $C_0 = 0.1053$. Contour maps of the soil properties analysed in the study, presented in Fig.4, were produced with kriging, therefore they contain information on spatial variability. The parameters included in the kriging of pH, P and K referred to the maximum scope of sampling and sample variance, but the kriging of Mg additionally included the variance of nugget. In order to compare the effect of the introduction of additional nugget variability to kriging, a map of the Mg content was produced using the assumption of absence of spatial variability in the observation.

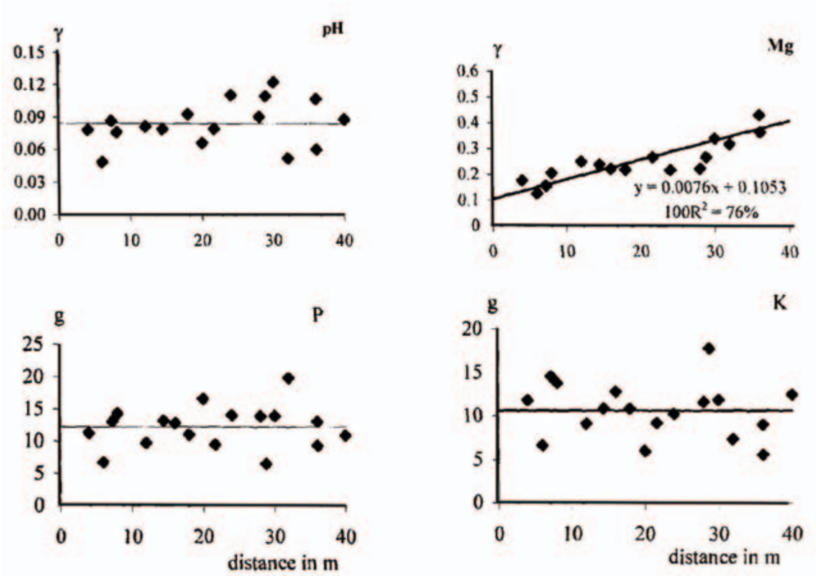

Fig.3. Semivariances of pH and available mineral compounds in the soil (mg/100 g soil)

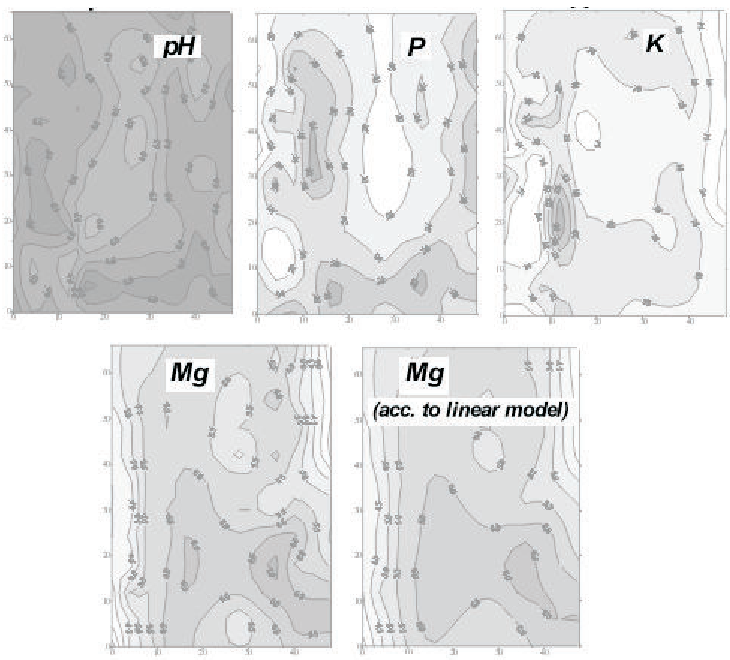

Fig.4. Contour maps of pH and available macronutrients produced by kriging in the experimental field (16 m of the field breadth corresponds to the pea experimental stripes).

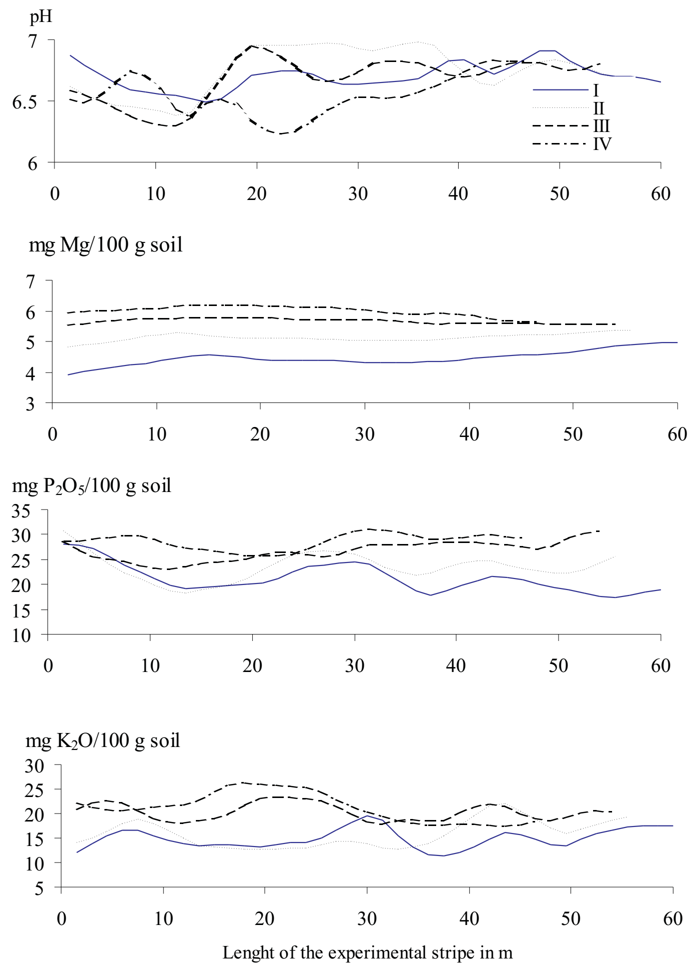

Fig.5. pH and available mineral components in plots at succesive replications (I, II, III, IV) of the experiment with pea (data after kriging).

Figure 5 shows the values of pH predicted by kriging and the content of mineral components for each pea experimental plot in successive replications. A possibility of producing a semivariance model and defining model parameters was reflected in the distributions obtained. The Mg content showed a high dimensional uniformity, with any large local variations ruled out. As regards the other soil properties, the variability of the values predicted between the plots was much higher.

Mean squares of error (MSE) from covariance analysis

Table 1 contains mean squares of error from the analysis variance and analysis of covariance with the concomitant variable according to the NNA for the first ten iterations for the classical designs: the CRD and RBD. The mean square error for the plant height became stabilised from iteration II, and for the seed yield - from iteration IV.

Table 1
Mean square error in ANOVA and ANCOVA in the following iterations according to NNA for CRD and RBD.

Specification	Plant height		Seed yield	
	CRD	RBD	CRD	RBD
Analysis of variance (ANOVA):	498	485	136335	121572
Analysis of covariance (ANCOVA): Iterations:				
I	479	487	132001	123282
II	467	478	120751	118466
III	467	478	121086	118744
IV	466	477	120276	118172
V	466	477	120318	118214
VI	466	477	120228	118145
VII	466	477	120235	118153
VIII	466	477	120222	118142
IX	466	477	120223	118143

Table 2
Mean square error in ANOVA and ANCOVA with concomitant variables obtained by kriging for CRD and RBD.

Specification	Plant height		Seed yield	
	CRD	RBD	CRD	RBD
Analysis of variance (ANOVA)	498.0	485.0	136335	121572
Analysis of covariance (ANCOVA): Concomitant variables:				
pH	498.0	494.2	136335	123283
Mg	499.7	460.5	120726	123047
K2O	495.2	485.8	133380	120730
P_2O_5	504.5	493.1	131313	120443
pH, Mg, P_2O_5, K_2O	500.2	468.6	121519	119284
NNA[1]	467.0	477.7	120751	118466

[1]- concomitant variables from iteration II for plant height and iteration IV for seed yield.

Table 2 shows the mean square errors for the plant height and seed yield in the analyses of variance and analysis of covariance with concomitant variables obtained by kriging for the completely randomised deign (CRD) and randomised block design (RBD). For both traits, the highest reduction of error relative to the mean square error from the analysis of variance in the completely randomised design was observed for the analysis of covariance with the concomitant variable determined according to the NNA method and kriging with the content of Mg as a concomitant variable.

Relative efficiency of different methods of data analysis

Table 3 presents an evaluation of the relative efficiency (RE) of different methods of data analysis. The results of variance analysis for the completely randomised design (CRD) and randomised block design (RBD) served as a reference point for the analysis methods.

Table 3

Relative efficiency (in %) of different methods of data analysis to CRD and RBD.

Specification	Plant height		Seed yield	
	CRD	RBD	CRD	RBD
	Analysis of variance (ANOVA)			
Randomized Block Design (RBD)	101.3	-	109.2	-
Incomplete Block Design (IBD)	-	101.5	-	100.2
Concomitant variables	Covariance analysis (ANCOVA)			
pH	98.2	98.5	99.5	98.1
Mg	99.6	106.2€	112.9	98.7
K2O	98.3	98.9	103.4	100.5
P2O5	100.2	100.4	101.8	100.3
pH, Mg, P_2O_5, K_2O	103.5	104.4	111.9	101.9
NNA[1]	110.7	102.0	111.2	100.2

[1]- concomitant variables from iteration II for plant height and iteration IV for seed yield.

Results obtained in the pea experiment discussed in this paper should be analysed with the analysis of variance according to incomplete block design. Real variability of the experimental field made the efficiency of incomplete blocks minimal relative to the RBD method. In a case of the plant height its efficiency was only 101.5%, and for the seed yield it was equal to 100.2%. In a similar experiment with pea performed by Gołaszewski (1999) established by the incomplete block method in a balanced design, the efficiency of incomplete blocks relative to the RBD was equal to 120% for the plant height and 100% for the seed yield.

Analysis of covariance with concomitant variables set according to the NNA and kriging for seed yield with the completely randomised design was more efficient as compared to ANOVA with the randomised block design (RBD). This is in accord with the methodological suggestion of the superiority of the randomised block design over the completely randomised design, as it rules out effects of soil variability between replications from mean square experimental error.

Regarding the plant height, a morphological trait, the analysis of covariance with such concomitant variables as the Mg content, and the four parameters of the chemical status of the soil - pH, Mg, P and K, together with the values of the trait on the adjacent plots (NNA), turned out to be efficient.

As far as the seed yield is concerned, the mean square error in the covariance analysis with the concomitant variables set according to the NNA and kriging was comparable to ANOVA for the randomised block design (RBD). It was only when all the analysed soil properties were introduced to the analysis as a concomitant variable, that the efficiency was 2% higher relative to the RBD method. According to the publication by Gołaszewski (1999) cited earlier, the respective efficiency was 10% higher.

In the literature on spatial methods there are many papers in which authors consider various methodological aspects of field trials data elaboration with spatial statistics, but only a few examples of use the methods in experimentation practice (Binns 1986, Brownie *et al.* 1993, Stroup *et al.* 1994, Gołaszewski 1999).

In conclusion, in the pea experiment presented hereby the analysis of covariance with the concomitant variable set according to the nearest neighbour analysis produced more accurate comparisons between entries in terms of the plant height than the analysis of variance with the CRD and the RBD. On the other hand, the seed yield can be efficiently analysed with the standard analysis of randomized block design.

The results from the pea experiment presented above are an example that the application of much sophisticated methods dealing with spatial variability can not be done routinely, even if the soil is heterogeneous. In our case the efficiency of the spatial methods were relatively low in comparison with standard data elaboration methods. It is clear that field trials conducted on spatially variable soil should be carefully designed by selection of experimental design, appropriate block orientation, proper selection of plot shape and size but it is the first step to the efficient treatment comparison. These obvious rules of field experimentation do not secure before possible soil trends or interplot interference. It suggests that quick methods for evaluation of purposefulness of correction the data on spatial variability should be incorporated. It could be done on the data of our interest or on the additional data. Of the two possibilities, the former could be based on the analysis of residuals by the autocorrelation technique or in the case of block designs by the calculation of intrablock correlation and Smith's index of soil heterogeneity (Gołaszewski 2000). In this case, the confirmed effect of autocorrelation or low value of Smith's index point to low efficiency of blocking and should lead to neighbour analysis. It can be said that these kinds of information are also the good prerequisites for application of spatial methods and use of additional data to improve efficiency of treatment comparisons.

The best additional information on spatial variability of the experimental field in macroscale (trend) is to measure soil properties that determine soil fertility. Including the information on a single or a complex soil proprieties predicted by kriging for each plot as concomitant variable (s) in ANCOVA seems to be the natural way to further increment of experiment precision. However, mainly due to costs the measurements are not made commonly before establishing of every experiment and when they are done the sampling net is usually not appropriate to valid estimation of spatial variation of the soil property. One can assume that in the future the measure of soil properties will be made directly in the field and their cost will be relatively low. On the other hand, the soil fertility trend can be assessed not only by direct measure of soil properties but also by the use of some accessory easy measurable plant traits noted from check plots in the block or in the vicinity of the experiment. In further methodical research the usefulness of these traits for spatial analysis should be evaluated in relation to the real factors of soil fertility.

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