Check plots in field breeding experiments

Stanisław Mejza, Iwona Mejza

Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Poland, e-mail: smejza@up.poznan.pl, imejza@up.poznan.pl

SUMMARY

This paper deals with the problems of selection in the early stages of a breeding program. During the improvement process, it is not possible to use an experimental design that satisfies the requirement of replicating all the treatments, because of the large number of genotypes involved, the small amount of seed and the low availability of resources. Hence unreplicated designs are used. To control the real or potential heterogeneity of experimental units, control (check) plots are arranged in the trial.

There are many methods of using the information resulting from check plots. All of the usually applied adjusting methods for unreplicated experiments are appropriate for some specific structure of soil fertility. Their disadvantage is the fact that, before and also after the experiment, we usually do not know what a kind of soil structure is present in the experiment. Hence we cannot say which of the existing methods is appropriate for a given experimental situation. The method of inference presented below avoids this disadvantage. It is always appropriate, because of the fact that a trend of soil variability is identified and estimated. In the paper the main tool used to explore this information will be based on a response surface methodology. To begin with we will try to identify a response surface characterizing the experimental environments. We assume that observed yield (or another trait) results directly from two components, one of them due to soil fertility and the other due to the genotype effect. This means that difference between observed yield and forecast can be treated as the estimate of a genotype effect. The obtained response surface will then be used to adjust the observations for genotypes. Finally, the data so adjusted are used for inferences concerning the next stage of the breeding program. The theoretical considerations are illustrated with an example involving yields of spring barley.

Key words: breeding field experiments, check plots, phenotypic selection, unreplicated experiments.

1. Introduction

This work deals with selection experiments in the early stages of breeding programs. In the early stages of plant breeding trials, it is often difficult to use

an experimental design that satisfies, for example, Fisher's principles. This is connected with the number of genotypes (experimental objects) evaluated, which usually ranges from several hundred to thousands. The breeder is mainly interested in the selection and identification of superior genotypes for further breeding. Additionally to the very large number of genotypes (lines) to be evaluated, these experiments often have a limitation resulting from the small amount of seeds and the low availability of resources. Thus replication may not always be possible, especially if the plots are to be large enough for proper yield assessment (Kempton 1984). Consequently, in this case unreplicated experiments are commonly adopted. Then, to control the real or potential heterogeneity of experimental units (field plots), replicated check variety plots are usually distributed over the field experiment area. Control plots are only effective if the yield (or another trait) of the checks shows the same general pattern of response across the trial as the test genotypes. Otherwise, the adjustment of test plot yields by the yields of the checks may lead to wrong estimation of genotype effects and finally to a wrong selection. This means that the arrangements and frequencies of check plots play a crucial role in selecting genotypes in such experiments.

Different random and systematic arrangements of check plots have been used (see for example Kempton 1984, Sebolai et al. 2005, Cullis et al. 1989, Baker and McKenzie 1967). Moreover, inference methods for unreplicated breeding trials are proposed, for example, by Cullis et al. 1989, Kempton and Fox 1997. The latter additionally conducted a large simulation study to compare adjustments by the methods of spatial analysis, moving averages and check plots. The variability of units with their geometrical structure in the experiment is used by the proposed methods to adjust the average values of observed characteristics on genotypes. More exactly, the yields of the check plot variety are used as the yardstick against which to assess the yield of each test (genotype) plot.

The simplest experimental designs are based on a systematic arrangement of equally spaced checks. Often the check plots are used to construct an

environmental index. The variability of units with their geometrical structure in the experiment is used by the proposed methods to adjust the average values of observed characteristics on genotypes. Usually, to construct the environmental index, we use, for example, the yield from the nearest check plots, the mean yield of the two nearest checks, one on either side of the test plot, the weighted mean of those two checks, where weights are inversely related to the distance from the genotype plots (in all directions), etc. Also, there are many other suggestions as to how to use check plots, boarding check plots, to estimate the spatial variability of environment. Recently the method called "moving average" has frequently been recommended for use (see for example Utz 1997).

All of the usually applied adjustment methods for unreplicated experiments are appropriate for some specific structure of soil (environment) fertility. Their disadvantage is the fact that, before and also after the experiment, we do not know what kind of soil (environmental) structure is present in the experiment. Hence we cannot say which of the existing methods is appropriate to a given experimental situation. The method of inference given in this paper avoids this disadvantage. It is always appropriate for any structure of soil (environment). This results from the fact that we estimate the environmental structure independently for each experiment. The problem which arises concerns only the quality of the response surface estimated in the experiment. To choose the proper response surface estimate, any selection criteria for linear or nonlinear models may be used.

2. Method

In this paper we propose a new approach to the design and analysis of unreplicated breeding experiments. The density and arrangement of check plots play the main role.

Let us assume that the experimental units have a row-column structure. We denote by *(i,j)* the coordinate of the check plot center. Let us note that the same genotype occurs on the check plots. The environment (soil variability) is the

main source of the variability of observations. Then the yield on the check plot can be expressed as:

$$Y_{ij} = F(i,j) + e_{ij}, \ E(Y_{ij}) = F(i,j),$$

where F(i,j) is some response surface function, while e$_{ij}$ denotes an experimental error.

The response surface function F(i,j) characterizes the true, real environmental variability (for example variation in soil fertility). In fact the function F(i,j) describes the yield response of the check plot genotype over the experimental plots. In genotype selection we do not need to consider the genotype that occurs on the check plot.

This results from the fact that the same genotype occurs on the check plots. In the selection problem we are not interested in making a ranking with respect to the check plot object, but with respect to the test genotypes. Hence we can treat the function F(i,j) as in fact describing the true, real environmental fertility and variability.

As usual we assume that random terms e$_{ij}$ are independently and identically normally distributed over all experimental units (not only over check plots) with expected value equal to 0 and common variance equal to σ^2. We can write this in short as e$_{ij} \sim$ N(0, σ^2). Additionally all covariances are equal to 0. The form of the function F(i,j) is unknown and it is necessary to estimate it.

Let $F(i,j)$ be the estimate of the function F(i,j). To estimate the response surface we can use any relevant available statistical tools (see for example: Box and Draper 2007, Bradley 2007, Khuri and Cornell 1987, Khuri 2006, Myers and Montgomery 2001).

Let us assume that the estimate $F(i,j)$ obtained is acceptable and sufficiently explains the environmental response over the experimental material (an experimental field). Additionally, based on the check plot observations, we estimate the common variance σ^2. We assume that this estimate is valid for all genotype plots.

In the next step we generate a forecast for all plots with genotypes. Let $\hat{F}(p,q)$ denote the forecast of the response on the plot *(p,q)* (for genotype occurring on this plot). Then the difference

$$\mathrm{Y}_{pq} - \hat{F}(p,q) = \hat{G}(p,q)$$

can be treated as the estimate of the effect of genotype G*(p,q)* occurring on the plot (*p,q*), where as previously Y_{pq} denotes the observation obtained for the genotype occurring on the plot (*p,q*). In the case of unreplicated experiments it is impossible to separate the environmental effect from the genotype effect.

Finally we have the estimate of the genotype effects and estimate of the error variance $\mathrm{MSE} = \hat{\sigma}^2$. These facts can be used to build a ranking (selection) of the genotypes. There are many ways to build the ranking of genotypes. Here we suggest the use of some test for grouping genotypes. We propose to apply a simultaneous test procedure, such as Tukey's test or the approximated (Bonferroni) t-test.

The hypotheses to be tested can be expressed as:

$$H_0 : G(p,q) - G(p',q') = 0 \quad \text{for all } (p,q), (p',q'),\ p \neq q,\ p' \neq q',\ p \neq p',\ q \neq q'$$
$$H_1 : G(p,q) - G(p',q') > 0$$

Two additional issues should be discussed. The first concerns check plot arrangements, and the second concerns check plot frequency (see for example Mejza and Marczyńska 2011).

Traditional arrangements include schemes in which the check plots are arranged, for example, in every third row (Holtsmark and Larsen 1905), in every fifth or sixth row in experimental units laid out in two ways (see example), and some others. Between these rows the analyzed genotypes are arranged. There are no clear arguments for such arrangements. Such supporting points of a design do not make it possible to control environmental heterogeneity over the experimental field. Therefore, we propose a systematic and even arrangement of check plots over the whole experimental area. Such arrangements allow one to estimate the environmental response function more exactly. The location scheme of the supporting points in experiments (check

S. Mejza, I. Mejza

plots) has a direct impact on the method of estimation of the response surface function and on its goodness of fit. Also, the density of check plots plays an important role in the selection of an appropriate response function. Using systematic designs we have the following potential and useful arrangements. The scheme depends on the frequency (density) of the check plots that the breeder intends to apply in the experiment. For example, if the breeder decides to use about 50% of units for check plots, he can use one of the schemes:

X			X
	X	X	

For a density of about 33% of check plots we can use one of the following potential schemes (from 3!= 6):

X		
	X	
		X

X		
		X
	X	

		X
X		
	X	

	X	
X		
		X

		X
	X	
X		

	X	
		X
X		

For a density of about 25% of check plots we can use one of the potential schemes from 4!= 24. Here we present only four such arrangements.:

X			
	X		
		X	
			X

X			
		X	
			X
		X	

X			
		X	
	X		
			X

X			
			X
		X	
		X	

For a frequency of about 20% of check plots we have 5! =120 potential arrangements of check plots. Here we present three of them.

X				
	X			
		X		
			X	
				X

X				
		X		
	X			
			X	
				X

	X			
X				
		X		
				X
			X	

3. Example

The above considerations are illustrated with the results obtained for grain yield in unreplicated breeding trials with standards, including 333 lines of spring barley, performed at Modzurów (Poland) plant breeding station. The observed trait in the experiment was the grain yield in kg per plot. The experiment was conducted in the 2005/2006 season. In the statistical analysis the yields of 333 experimental plots were taken into account. The plots were arranged in 37 rows and 9 columns. In the experiment 63 plots were designated as check plots, and the analyzed genotypes were assigned to 270 plots. The measured (observed) area of the plots was 1m x 10 m, i.e. $10m^2$. The geometrical structure was characterized by (*x,y*) coordinates with the following values: x = 5, 15, 25, 35, 45, 55, 75, 85[m]; y = 0.5, 6.5, 12.5, 18.5, 24.5 30.5, 36.5[m]. The experimental scheme is given in Table 1. Codes printed in bold and italics denote the check plots (the same genotype). Other codes denote the genotypes (test lines).

The statistical analysis was performed in two stages. At the start we estimate the two-dimensional surface function for check plot yield (marked in bold and italics in Table 1) to characterize the variability of the soil (environ-ment) in the experimental field. Using the methods of model selection, we decided to adopt the estimate of the response surface of the yield in the form: *F(x,y)* = 4.574 + 0,160x - $0.006x^2$ + $0.0001x^3$ + 0.227y - $0.016y^2$ + $0.0003y^3$ + 0.0001xy. We reject the hypothesis concerning the adequacy of the model at a significance level of alpha = 0.05. The coefficient of determination is equal to 74%. The shape of the response surface characterizing the environmental

S. Mejza, I. Mejza

Table 1. Scheme of the experiment

4813	*4948*	*5069*	*5204*	*5325*	*5460*	*5581*	*5654*	*5775*
4814	4947	5070	5203	5326	5459	5582	5653	5776
4815	4946	5071	5202	5327	5458	5583	5652	5777
4816	4945	5072	5201	5328	5457	5584	5651	5778
4817	4944	5073	5200	5329	5456	5585	5650	5779
4818	4943	5074	5199	5330	5455	5586	5649	5780
4819	*4942*	*5075*	*5198*	*5331*	*5454*	*5587*	*5648*	*5781*
4820	4941	5076	5197	5332	5453	5588	5647	5782
4821	4940	5077	5196	5333	5452	5589	5646	5783
4822	4939	5078	5195	5334	5451	5590	5645	5784
4823	4938	5079	5194	5335	5450	5591	5644	5785
4824	4937	5080	5193	5336	5449	5592	5643	5786
4825	*4936*	*5081*	*5192*	*5337*	*5448*	*5593*	*5642*	*5787*
4826	4935	5082	5191	5338	5447	5594	5641	5788
4827	4934	5083	5190	5339	5446	5595	5640	5789
4828	4933	5084	5189	5340	5445	5596	5639	5790
4829	4932	5085	5188	5341	5444	5597	5638	5791
4830	4931	5086	5187	5342	5443	5598	5637	5792
4831	*4930*	*5087*	*5186*	*5343*	*5442*	*5599*	*5636*	*5793*
4832	4929	5088	5185	5344	5441	5600	5635	5794
4833	4928	5089	5184	5345	5440	5601	5634	5795
4834	4927	5090	5183	5346	5439	5602	5633	5796
4835	4926	5091	5182	5347	5438	5603	5632	5797
4836	4925	5092	5181	5348	5437	5604	5631	5798
4837	*4924*	*5093*	*5180*	*5349*	*5436*	*5605*	*5630*	*5799*
4838	4923	5094	5179	5350	5435	5606	5629	5800
4839	4922	5095	5178	5351	5434	5607	5628	5801
4840	4921	5096	5177	5352	5433	5608	5627	5802
4841	4920	5097	5176	5353	5432	5609	5626	5803
4842	4919	5098	5175	5354	5431	5610	5625	5804
4843	*4918*	*5099*	*5174*	*5355*	*5430*	*5611*	*5624*	*5805*
4844	4917	5100	5173	5356	5429	5612	5623	5806
4845	4916	5101	5172	5357	5428	5613	5622	5807
4846	4915	5102	5171	5358	5427	5614	5621	5808
4847	4914	5103	5170	5359	5426	5615	5620	5809
4848	4913	5104	5169	5360	5425	5616	5619	5810
4849	*4912*	*5105*	*5168*	*5361*	*5424*	*5617*	*5618*	*5811*

response (soil fertility) of the experimental field on the basis of check plots is presented in Figure 1.
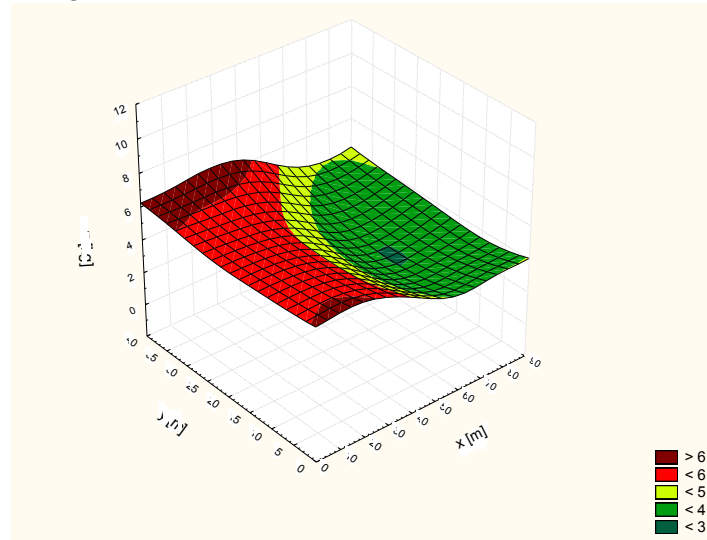

Figure 1. The estimate of the response function for check plots

In the next step we calculate forecasts for all genotypes (all plots with test genotypes). The coordinates of the genotype plot were the central points of the experimental fields. This means that the distances in one direction were 5, 10, 15, …, 85[m] while those in the second were 0.5, 1.5, 2.5, …, 36.5[m].

As a result of analysis of variance performed for check plot yield, we obtain $LSD_{0.05} = 3.82$ for Tukey's test. The ranking of the lines is presented in Table 2, ordered from the genotype with the smallest yield to the largest. This means that the genotype coded 4844 is the worst, while the genotype coded 5336 is the best. Using Tukey's test we observe that 62 lines (up to code 5334) are of small value. In the case where we skip the line coded 4844, the next 220 genotypes can be considered as being of small value. The best genotype, code 5336, is situated close to a check plot on the field. This means that the forecast can be expected to be very good.

In the sense of Tukey's test the last 44 genotypes (from code 5445 to the end) have almost identical yields. This means that for the next step of selection the last 44 genotypes can be recommended.

S. Mejza, I. Mejza

Tabela 2. Genotype ranking

4844	-4,194	4946	-0,548	5612	-0,105	5190	0,254	5608	0,662	5440	1,003
5653	-2,85	5589	-0,547	5457	-0,088	5801	0,268	5194	0,668	5445	1,034
5652	-2,635	5645	-0,537	5354	-0,077	4926	0,287	5451	0,668	4931	1,056
5356	-2,572	5179	-0,519	5796	-0,075	4834	0,289	5100	0,67	5450	1,069
5651	-2,448	4846	-0,489	4832	-0,068	5637	0,302	5347	0,676	4933	1,071
5650	-2,076	5628	-0,486	5073	-0,041	5426	0,323	5084	0,678	5176	1,099
5778	-1,822	5200	-0,484	5798	-0,036	5594	0,327	5351	0,682	5089	1,111
5776	-1,805	5585	-0,477	5427	-0,007	5789	0,335	5804	0,683	4938	1,113
4845	-1,803	4814	-0,472	4824	-0,006	5102	0,337	5441	0,699	4941	1,116
5582	-1,799	5622	-0,471	5429	-0,005	5358	0,346	5078	0,708	5443	1,132
5326	-1,505	5786	-0,47	5788	0,01	5091	0,346	5332	0,709	5074	1,134
5649	-1,489	4841	-0,451	5076	0,029	4945	0,347	5619	0,722	5444	1,165
5583	-1,485	5780	-0,443	5792	0,033	5095	0,378	5103	0,725	5341	1,183
5584	-1,368	4847	-0,422	5344	0,036	4927	0,388	5616	0,73	5182	1,19
5172	-1,359	5783	-0,408	5625	0,038	5345	0,42	5171	0,745	5435	1,233
5459	-1,329	5592	-0,401	5614	0,055	5610	0,439	5187	0,746	5434	1,247
5357	-1,31	5627	-0,389	5600	0,055	5330	0,452	5085	0,747	5339	1,257
5644	-1,286	5794	-0,368	5621	0,056	5452	0,471	5346	0,756	5177	1,269
4838	-1,285	4915	-0,346	5640	0,056	5425	0,478	5596	0,759	5079	1,278
5779	-1,17	5094	-0,345	4827	0,07	5352	0,497	5438	0,77	5188	1,29
5101	-1,158	5072	-0,341	5348	0,102	5803	0,506	5620	0,786	5088	1,318
5646	-1,155	5634	-0,334	4823	0,109	5169	0,509	5597	0,79	5098	1,357
5777	-1,13	5639	-0,317	5791	0,115	5184	0,515	4822	0,82	5439	1,359
4916	-1,07	5590	-0,309	5797	0,117	5338	0,523	5595	0,842	5808	1,382
5638	-1,015	5633	-0,307	4815	0,13	4937	0,529	5096	0,843	5433	1,413
5202	-0,97	5191	-0,3	5170	0,134	5071	0,533	5080	0,844	5077	1,423
5173	-0,962	5603	-0,293	5455	0,14	5432	0,543	5329	0,846	5810	1,429
5183	-0,96	5428	-0,272	5199	0,142	4842	0,544	5195	0,848	4840	1,451
5784	-0,94	5598	-0,263	4816	0,145	5615	0,545	5335	0,85	5809	1,513
5083	-0,938	4828	-0,235	4821	0,155	4830	0,548	5453	0,857	4929	1,52
5795	-0,933	5360	-0,23	5328	0,155	5606	0,561	5446	0,857	5447	1,553
4947	-0,921	5607	-0,224	5800	0,173	5609	0,563	4914	0,862	5086	1,563
5327	-0,901	5629	-0,221	4829	0,174	5193	0,564	4917	0,868	5175	1,614
5591	-0,888	4922	-0,212	4817	0,174	5802	0,565	4943	0,871	4944	1,626
5647	-0,86	5643	-0,208	5333	0,183	5353	0,587	4934	0,876	5092	1,631
5586	-0,809	5602	-0,204	5782	0,187	4932	0,591	5197	0,878	5097	1,652
5631	-0,789	5350	-0,182	4833	0,194	4836	0,602	5342	0,88	4940	1,669
5604	-0,776	5613	-0,161	5359	0,195	5456	0,603	4818	0,908	5082	1,769
5588	-0,691	5201	-0,144	5807	0,196	5203	0,607	5340	0,953	4923	1,995
4839	-0,633	5189	-0,14	4935	0,203	5090	0,616	5449	0,956	4913	2,236
5641	-0,619	5178	-0,136	5181	0,206	4826	0,617	5334	0,969	4848	2,282
5632	-0,596	5437	-0,124	5601	0,219	4939	0,624	5196	0,972	4921	2,492
5070	-0,59	5635	-0,119	5790	0,223	5623	0,636	4928	0,973	5785	3,362
5806	-0,579	4925	-0,118	4835	0,238	5185	0,641	5431	0,979	5336	4,846
5458	-0,555	4820	-0,118	5626	0,252	4920	0,651	5104	0,98		

4. Remarks

This paper has dealt with early generation selection experiments in which various random or systematic arrangements of genotypes as well as various statistical techniques have been developed to increase the effectiveness and efficiencies of inference in such experiments. Usually the techniques are connected with spatial analysis. Because of certain limitations, mentioned earlier, it is often impossible to replicate the examined genotypes. Then it is necessary to adopt some assumptions concerning the spatial structure of the experimental material or to introduce into the experiment some plots with the same lines (very often varieties) called check plots. To the assumed correlation structure of experimental plots, geostatistical methods are applied (see for example Briggs and Shebeski, 1967; Zimmerman and Harville, 1991; Brownie and Gumpertz, 1997; Martin, 1986; Gołaszewski, 1999, 2002). In another approach, to recognize the spatial variability of the environment (soil), check plots are distributed over all experimental units. The problem is the density of the check plots and the way of using them in statistical analysis of the breeding experiments and in further inferences.

The literature contains many methods of using the information resulting from check plots. All of these methods have at least one weak point. They are developed for some specific proper structure of the environment, usually soil fertility. Their main disadvantage is the fact that before and also after the experiment we do not know what kind of the soil (environmental) structure is present in the experiment. Hence, we cannot say which of the existing methods is appropriate to a given experimental situation.

We have proposed here a method of using information from check plots that practically has no limitations on its use. In the proposed method we explore this information using a response surface methodology. Initially we try to identify the response surface characterizing the structure of an experimental environment. The obtained response surface is then used to adjust the

observations for genotypes. Finally, the data so adjusted are used for statistical inference concerning selection for the next stages of the breeding program (ranking of genotypes).

Acknowledgement

REFERENCES

Baker R.J., McKenzie R.I.H. (1967): Use of control plots in yield trials. Crop. Sci. 7:335-337.

Box G.E.P., Draper N. (2007): Response Surfaces, Mixtures, and Ridge Analyses, Wiley, New York.

Bradley N. (2007): The response surface methodology. Indiana University South Bend.

Briggs K.G., Shebeski L.H. (1967): Implications concerning the frequency of control plots in wheat breeding nurseries. Can. J. Plant Sci. 48: 149 - 153.

Cullis B.R., Warwick J.A., Fisher B.J., Read B.J., Gleeson A.C. (1989): A new procedure for the analysis of early generation variety trials. Appl. Statist. 38: 361-375.

Brownie C., Gumpertz M.L. (1997): Validity of Spatial Analyses for Field Trials. Journal of Agricultural, Biological, and Environmental Statistics 2(1): 1-23.

Gołaszewski J. (1999): Application of geostatistical methods to analysis of the data from a pea breeding trial. Biometrical Letters 36(2): 145-157.

Gołaszewski J. (2002): Geostatistical approach to data from field experiments with check plots – Electronic Journal of Polish Agricultural Universities, EJPAU, Ser. Agronomy 5(2).

Holtsmark G., Larsen B.R. (1905): Om multigheder for at indskraenke de fejl, som ved marksforsog betinges af jordens uensartethed. Tidsskrift for Landbrugets Planteavl, 12: 330-351.

Kempton R.A. (1984): The design and analysis of unreplicated field trials. Vortr. Pflanzenzuchtg 7: 219-242.

Kempton R.A., Fox P.N. (1997): Statistical methods for plant variety evaluation. Chapman & Hall.

Khuri A.I., Cornell J.A. (1987): Response Surfaces. Designs and Analysis. Marcel Dekker, Inc. New York

Khuri A.I. (2006): Response Surface Methodology and Related Topics. World Scientific, Singapore.

Martin R.J. (1986): On the design of experiments under spatial correlation. Biometrika 73: 247-277.

Mejza S., Marczyńska K. (2011): Check plot density in estimation of soil fertility. Proceedings: Biometric Methods and Models in Current Science and Research, D.

Hampel, J. Hartmann, J. Michalek , (eds) Central Institute of Supervising and Testing in Agriculture, Brno: 167-172.

Myers an R.H., Montgomery D.C. (2001): Response Surface Methodology, John Wiley and Sons, 2nd edition.

Sebolai B., Pedersen J.F., Marx D.B., Boykin D.L. (2005): Effect of control plot density, control plot arrangements, and assumption of random or fixed effects on nonreplicated experiments for germplasm screening using spatial models. Crop Sciences: 1978-198.

Utz H.F. (1997): PLABSTAT. A computer program for statistical analysis of plant breeding experiments. Version 2N. Institute of Plant Breeding, Seed Science and Population Genetics. University of Hohenheim, Germany.

Zimmerman D.L., Harville D.A. (1991): A Random Field Approach to the Analysis of Field – Plot Experiments and Other Spatial Experiments. Biometrics 47: 223-239.