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REGULAR ARTICLE

Evaluation of nearest neighbor methods in wheat genotype experiments

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ABSTRACT
Spatial variability among experimental plots may be a relevant problem in field genotype experiments, especially when a large number of entries are involved. Whenever variability cannot be controlled by blocking, nearest neighbor methods can be helpful. The purpose of this study is to evaluate nearest neighbor methods and to present other control methods for intense soil heterogeneity. Three experimental trials with wheat varieties were analyzed by using the following spatial techniques: Papadakis method, modified Papadakis methods, moving average, first-order autoregressive and first differences. The results of the above analyses were compared with those of a traditional Randomized Complete Block (RCB) ANOVA. It can be concluded that, in the presence of soil heterogeneity, nearest neighbor analysis (NNA) improves the experimental accuracy, by significantly decreasing the residual (unexplained) variability. For these three experiments, the first-difference model was the most effective among all the tested NNA methods.

Key Words: *Papadakis method; spatial variability control; block design; agricultural experiments.*

INTRODUCTION

The accurate interpretation of the results of agricultural experiments requires the correct estimation of experimental error. One of the main sources of variation in field experiments is soil heterogeneity, which can create severe problems in genotype evaluation experiments, especially when these experiments involve a great number of varieties. The traditional methods of local control, such as blocks, rows and columns or lattice designs, may be unable to account for variation due to intense soil heterogeneity, especially when its pattern is complex. Thus, spatial or nearest neighbor analysis (NNA) methods can be used. These methods exploit the fact that neighboring experimental plots in the field should be more similar to one another than distant plots. Therefore, nearest neighbor methods use information from the neighboring plots in order to reduce or remove the undesirable effect of heterogeneity and hence improve the estimation of model parameters (Dixon 2013). These methods are characterized as *aposteriori* techniques, in contrast to the classical techniques of local control, which are incorporated at the onset of the experiment (designstage) (Gezan et al. 2010).

The first nearest neighbor method was presented by the agronomist John Papadakis in 1937 with the title 'Méthode statistique pour des expériences sur champ' and published in the proceedings of the Institute of Plant Breeding in Salonika (Papadakis 1937). Papadakis was not satisfied with the traditional use of blocks according to Fisher, which ignored the inter-block heterogeneity of soil fertility. Instead, he proposed an analysis of covariance to reduce the effects of spatial heterogeneity in yields. The value of the covariate for each plot was obtained by averaging the residuals for the neighboring plots. The residual for one plot is given by both the difference between the observed yield for the variety in that plot and the average yield for the same variety in the whole experiment. According to Papadakis (1937), the advantages of this method are (i) that it does not require any *ad hoc* layout of plots, and (ii) that it can be used in large scale fields with a high number of varieties and different number of replicates. The Papadakis method was examined and initially improved by Bartlett (1938), who proposed the traditional use of blocks together with a simultaneous adjustment based on nearest neighboring plots. Two years later, Papadakis (1940) proposed the use of two regression coefficients, one for the inner and one for the outer plots of the experimental field. However, his method remained neglected for a long time, due to the beginning of 2nd World War, the lack of the necessary theoretical background, and the need for difficult calculations (Papadakis 1991). Bartlett (1978) extended the method of Papadakis in two dimensions (rows and columns). He also suggested that the method could be improved by using an iterative process in which means for treatments from the previous iteration could be used to recalculate the residuals in the following iteration. Wilkinson et al. (1983) mentioned that the Papadakis methods tended to be conservatively biased, so they proposed both a modification based on the use of the mean of the four neighboring plot residuals and the moving block nearest-neighbor method.

Furthermore, Townley-Smith and Hurd (1973) proposed the moving average method (MA), based on the use of the arithmetic mean for the two neighboring plots. The least-squares smoothing method (LSS) proposed by Green et al. (1985) estimates a smooth curve for the trend effect operating in blocks of three adjacent plots. They assume that the trend effect should be locally linear and choose a smoothing constant to ensure a given degree of smoothness. The first difference with errors-in-variables model discussed by Besag and Kempton (1986) provides a method for explicitly estimating the above trend effect.

Gleeson and Cullis (1987) suggested that spatial variability in field trials can be modeled by the general class of autoregressive integrated moving average (ARIMA) (p, d, q) random processes, where p and q refer to the order of the autoregressive (AR) and moving average (MA) processes, and d refers to the degree of differencing required to achieve stationarity.

Zimmerman and Harville (1991) introduced a random field linear model (RFLM) by directly using a geostatistical approach. This model is developed by viewing heterogeneity as the result of two sources of spatial dependence: 'large-scale' (or global trend), which is modeled through the mean structure, and 'small-scale' (or local trend), which is modeled through the correlation structure. Their model is based on the theory of certain stochastic processes known as 'random fields'.

The purpose of this study is to evaluate the nearest neighbor methods in comparison to a randomized complete block ANOVA model, which is the most traditional method for controlling intense soil heterogeneity in field experiments. Moreover, the role of nearest neighbor methods in the design and analysis of agricultural experiments in breeding programs is discussed.

MATERIALS AND METHODS

Yield data of three variety-evaluation experiments in wheat were used. The first experiment comprised nine bread wheat varieties and was laid down according to a randomized complete block design with four replicates (Figure 1). The experiment took place in the experimental station of the Agricultural University of Athens, in the area of Copais in the growing season 2008/2009. The experimental plots consisted of five six-meter-long rows, spaced 20 cm apart.

The second experiment took also place in the area of Copais in the growing season 2013/2014. This experiment comprised 24 durum wheat varieties and was laid down according to a randomized complete block design with three replicates (Figure 2). The experimental plots consisted of four two-meter-long rows, spaced 20 cm apart.

The third experiment comprised ten durum wheat varieties and was laid down according to a randomized complete block design with two replicates. This experiment took place in the area of Votanikos in the growing season 2000/2001. The experimental plots consisted of five individual plants (experimental units) at a distance of 30 cm and plot size was 150 x 30 cm (length x width).

Yield data for each trial were analyzed with a spatial model as proposed by Brownie et al. (1993):

$$Y_{ij} = \mu + \tau_{k(ij)} + T_{ij} + \varepsilon_{ij} \quad (1)$$

where Y_{ij} is the observed yield (kg ha^{-1}) in the jth plot within the ith block or plot ij, the term $\mu + \tau_{k(ij)}$ represents the mean performance of variety k grown in plot ij, T_{ij} is the trend effect representing systematic spatial variation in this plot, and ε_{ij} is the random error. For the baseline RCB analysis, the trend effects T_{ij} were assumed to be constant for all plots within the same block, that is, $T_{ij} = \beta_i$ (the effect of the ith block).

The seven nearest neighbor methods considered in this study were:

a) Papadakis method (PAP^1) where the trend effect T_{ij} in Eq. [1] became bX_{ij}, where $X_{ij} = (e_{i,j-1} + e_{i,j+1})/2$, b = the regression coefficient associated with the covariate X_{ij}, and $e_{ij} = Y_{ij} - \overline{Y}_{k(ij)}$ with $\overline{Y}_{k(ij)}$ being the mean for the variety k grown in the plot ij.

$$Y_{ij} = \mu + \tau_{k(ij)} + b\,(e_{i,j-1} + e_{i,j+1})/2 + \varepsilon_{ij} \quad (2)$$

For the edge plot the covariate is the residual from the adjusted interior plot; the same applies to the modified Papadakis methods.

b) Papadakis method plus block (PAP$^{1+\beta}$) (Bartlett 1938), where the trend effect T_{ij} is defined as $\beta_i + bX_{ij}$ (β_i = block effect).

c) Papadakis method with four residuals e_{ij} (PAP^{4res}) (Wilkinson et al. 1983) where $X_{ij} = (e_{ij-2} + e_{ij-1} + e_{ij+1} + e_{ij+2})/4$.

d) The iteration Papadakis method (PAP$^{iter.}$), in which Eq. [2] represents Step 1, and adjusted means from Eq. [2] are used to calculate Step 2 residuals $e^{(2)}_{ij}$ and $X^{(2)}_{ij}$. The process is repeated until adjusted means from consecutive steps are approximately the same (Bartlett 1978).

e) Moving average method (MA) (Townley-Smith and Hurd 1973), where X_{ij} is the arithmetic mean of yields of the neighbor experimental plots, $X_{ij} = (Y_{ij-1} + Y_{ij+1})/2$; for the edge plot the covariate is the value from the adjusted interior plot.

f) Autoregressive method (AR1). The used spatial correlation model for AR(1) is: $Cov(\varepsilon_{ij}, \varepsilon_{ij'}) = \sigma^2 Corr(\varepsilon_{ij}, \varepsilon_{ij'}) = \sigma^2 \rho^{|i-j'|}$, where σ^2 is the residual variance, and ρ is the autocorrelation parameter (Zimmerman and Harville 1991, Brownie et al. 1993, Yang et al. 2004).

g) First-difference model (FD). The used model is: $Y_{ij} - Y_{ij-1} = \overline{Y}_{ij} - \overline{Y}_{ij-1} + T_{ij} - T_{ij-1} + \varepsilon_{ij}$, where T_{ij} and T_{ij-1} represent the trend effects for plots i and i-1, and ε_{ij} is the random error (Besag and Kempton 1986, Loo-Dinkins 1992). The first observation was ignored.

For the third experiment, spatial heterogeneity was tested in two dimensions, among experimental plots (in columns) and among experimental units (plants in rows) (see later). Accordingly, two covariates were used in the Papadakis method.

The models were compared by the coefficient of determination (R^2), the adjusted coefficient of determination (R^2_{adj}), the residual standard deviation (SD), the coefficient of variation (CV%), and the Akaike Information Criterion (AIC) (Akaike 1974). The R software (R Core Team 2014) was used for the above estimations.

RESULTS

Table 1 shows wheat yields from the first experiment along with the procedure for calculating the residuals with the Papadakis method. Figure 1 presents the heterogeneity of the experimental field according to the residuals of the Papadakis method.

Table 1. Yield (kg ha^{-1}) of the varieties and the residuals of Papadakis method for the first experiment.

	Variety	Block	Y_{ij}	$\overline{Y}_{k(ij)}$	e_{ij}	$e_{i,j-1}$	$e_{i,j+1}$	X_{ij}
1	1	1	4030	4475	-445		-885	-885
2	2	1	3070	3955	-885	-445	-1038	-741
3	3	1	2830	3868	-1038	-885	-310	-598
4	4	1	1910	2220	-310	-1038	-985	-1011
5	5	1	2530	3515	-985	-310	-10	-160
6	6	1	4090	4100	-10	-985	100	-443
7	7	1	4550	4450	100	-10	-128	-69
8	8	1	3610	3738	-128	100	443	271
9	9	1	5040	4598	443	-128		-128
10	6	2	4050	4100	-50		-275	-275
11	1	2	4200	4475	-275	-50	103	267
12	8	2	3840	3738	103	-275	133	-71
13	3	2	4000	3868	133	103	-548	-223
14	9	2	4050	4598	-548	133	-255	-61
15	2	2	3700	3955	-255	-548	-150	-349
16	7	2	4300	4450	-150	-255	-200	-228
17	4	2	2020	2220	-200	-150	275	63
18	5	2	3790	3515	275	-200		-200
19	4	3	1840	2220	-380		10	10
20	6	3	4110	4100	10	-380	-155	-268
21	2	3	3800	3955	-155	10	-188	-89
22	8	3	3550	3738	-188	-155	135	-10
23	1	3	4610	4475	135	-188	623	218
24	3	3	4490	3868	623	135	-318	-91
25	9	3	4280	4598	-318	623	235	429
26	5	3	3750	3515	235	-318	-100	-209
27	7	3	4350	4450	-100	235		235
28	7	4	4600	4450	150		213	213
29	8	4	3950	3738	213	150	423	286
30	9	4	5020	4598	423	213	50	131
31	6	4	4150	4100	50	423	890	656
32	4	4	3110	2220	890	50	283	166
33	3	4	4150	3868	283	890	585	738
34	1	4	5060	4475	585	283	1295	789
35	2	4	5250	3955	1295	585	475	530
36	5	4	3990	3515	475	1295		1295

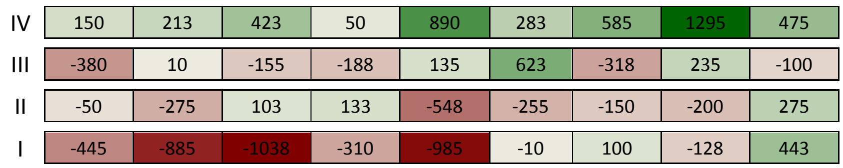

Figure 1. Heterogeneity of the experimental plots for the first experiment, according to the Papadakis residuals. The shade of the colour represents the value of Papadakis residuals in the plots. Dark green represents the highest values while dark red the lowest ones.

The analysis of variance showed that both effects of variety ($p<0.001$) and blocks ($p<0.001$) were statistically significant. The Papadakis method (PAP^1) did not improve the randomized complete block design (Table 2). The experimental accuracy improved when the effect of the blocks ($PAP^{1+\beta}$) was added to the model. Considering the coefficient of determination-R^2, the best results came from the method of first differences (0.82). The modified Papadakis method with the average of the four nearest neighbor residuals (PAP^{4res}) showed better results than all the other methods used, based on the adjusted coefficient of determination-R^2_{Adj} (0.74), the standard deviation of the experiment (43.3), the coefficient of variation CV% (11.7), and the AIC criterion (394.8).

In the second experiment, both effects of variety ($p<0.001$) and blocks ($p<0.001$) were significant. Figure 2 shows the heterogeneity of the experimental field according to the residuals *e* of the Papadakis method.

Table 2. The summary of the models fitted for the first experiment.

	RCBD	PAP^1	PAP^{4res}	$PAP^{iter.}$	$PAP^1+\beta$	MA	AR(1)	FD
R^2	0.81	0.77	0.80	0.78	0.81	0.82	0.81	0.82
R^2 Adj	0.72	0.70	0.74	0.71	0.72	0.73	0.72	0.72
Residual SD	442.3	464.1	433.6	453.9	449.6	439.9	447.4	439.5
CV %	11.40	11.96	11.18	11.70	11.59	11.52	11.53	11.32
AICc	402.9	399.7	394.8	398.1	408.1	406.5	407.7	406.4
Mean (kg ha^{-1})					3879			
N					36			

RCBD = Randomized Complete Block Design, PAP^1 = Papadakis method, PAP^{4res} = Papadakis method with four residuals, PAP^{iter} = The iteration Papadakis method, $PAP^1+\beta$ =Papadakis method + blocks, MA = Moving average method, AR1 = Autoregressive method and FD = First-difference model.

Figure 2. Heterogeneity of the experimental plots for the second experiment, according to the Papadakis residuals. The shade of the colour represents the value of Papadakis residuals in the plots. Dark green represents the highest values while dark red the lowest ones.

In the second experiment, the classical method of Papadakis and its modifications had similar results to the RCB design, while the iteration method (PAP$^{iter.}$) showed slightly better results. After comparing all methods, it can be concluded that the best results in terms of experimental accuracy came from the method of first differences (Table 3).

In the analysis of variance of the third experiment, we observed a statistically significant effect of variety ($p<0.001$) and blocks ($p<0.001$).

Table 3. The summary of the models fitted for the second experiment.

	RCBD	PAP^1	PAP^{4res}	PAP$^{iter.}$	PAP^1+β	MA	AR(1)	FD
R^2	0.79	0.78	0.80	0.82	0.80	0.80	0.81	0.84
R^2 Adj	0.68	0.67	0.70	0.72	0.69	0.69	0.69	0.74
Residual SD	393.7	400.7	385.2	369.1	391.2	389.2	390.7	350.8
CV %	16.00	16.29	15.66	15.00	15.90	15.80	15.88	14.26
AICc	789.3	788.3	782.6	776.4	792.2	791.5	794.6	780.4
Mean (kg ha^{-1})					2460			
N					72			

RCBD = Randomized Complete Block Design, PAP^1 = Papadakis method, PAP^{4res} = Papadakis method with four residuals, PAP^{iter} = The iteration Papadakis method, PAP^1+β =Papadakis method + blocks, MA = Moving average method, AR1 = Autoregressive method and FD = First-difference model.

	169	128	189	76	236	209	173	40	539	-171
	-43	181	-119	188	-36	-44	216	179	426	690
I	21	105	9	243	-52	479	254	246	478	57
	40	147	259	214	563	539	445	765	267	647
	103	285	77	254	240	228	282	504	218	699

	-434	-438	-248	-584	-136	-576	-208	-153	-221	-31
	-111	-80	-284	-253	-207	-247	-37	-69	-7	-82
II	-644	-373	-394	-477	-250	-370	-228	-193	-275	-170
	-193	-161	-275	-299	-256	-33	-57	-95	-163	-336
	-538	-358	-448	-401	-344	-284	-119	-98	-160	-60

Figure 3. Heterogeneity of the experimental field for the third experiment, according to the Papadakis residuals. Each column consist an experimental plot which contains five plants. The shade of the colour represents the value of Papadakis residuals. Dark green represents the highest values while dark red the lowest ones.

The Papadakis method with using either rows or columns did not show satisfactory results (Table 4). The use of Papadakis method with two covariates (PAP^{C+R}) clearly improved experimental accuracy, reaching almost the results of RCBD analysis. However, the use of one covariate with blocks showed better results than the RCBD analysis. Yet, when evaluating the overall results, the method of first differences overrode all other methods.

Although the nearest neighbor methods improved experimental accuracy in all three experiments, the final ranking of wheat varieties did not change at all.

Table 4. The summary of the models fitted for the third experiment.

	RCBD	PAP^{Col}	PAP^{Row}	PAP^{C+R}	$PAP^{iter.}$	PAP^{4res}	$PAP^{Col}+\beta$	MA	AR(1)	FD
R^2	0.66	0.59	0.55	0.63	0.58	0.58	0.67	0.67	0.67	0.79
R^2Adj	0.62	0.54	0.50	0.58	0.53	0.54	0.63	0.63	0.63	0.77
Residual SD	254.8	279.6	293.5	269.0	283.3	281.2	252.7	251.2	252.0	196.5
CV %	14.02	15.38	16.14	14.79	15.58	15.47	13.90	13.81	13.86	10.80
AICc	947.3	965.9	975.6	959.6	968.6	967.1	947.17	945.9	937.3	888.1
Mean (kg ha^{-1})						1818				
N						100				

RCBD = Randomized Complete Block Design, PAP^{Col} = Papadakis method for columns, PAP^{Row} = Papadakis method for rows or columns, PAP^{4res} = Papadakis method with four residuals, PAP^{iter} = The iteration Papadakis method, $PAP^{Col}+\beta$ = Papadakis method + blocks, MA = Moving average method, AR1 = Autoregressive method and FD = First-difference model.

DISCUSSION AND CONCLUSIONS

The findings of this study showed that significant improvements can be achieved in controlling spatial variability by nearest neighbor methods. From the statistical analysis of the yield data of all the experiments, it appears that using the classical method of Papadakis does not improve the experimental accuracy of the experiments since it was less effective than the traditional ANOVA with randomized complete blocks. The results of this work can be summarized as follows:

- Although Papadakis introduced the method of replacing blocks, we observed that in all three experiments, the experimental accuracy was further enhanced when the effect of blocks was added to the original model surpassing that of the randomized complete block design (Brownie et al. 1993, Vollmann et al. 1996, Pearce 1998). Furthermore, the use of blocks in many agricultural experiments is considered necessary because of the large number of treatments or a lack of staff and adequate infrastructure. The significance of block effects does not necessarily mean the presence or absence of spatial variability. Stroup et al. (1994) reported that, although there was a significant effect of blocks, the application of nearest neighbor methods further improved the experimental accuracy. However, Yang et al. (2004) stated that although in some of the experiments there was no statistically significant effect of blocks, there was a major spatial variability, and application of nearest neighbor methods was more effective.
- The use of several covariates in the original Papadakis model or the use of more neighboring residuals displayed significantly better results compared to the original model and similar or slightly better results than the RCB design in all three experiments. The results agree with previous studies of Kempton and Howes (1981), Vollmann et al. (1996) and Genaz et al. (2010).
- The iteration Papadakis method, proposed by Bartlett (1978), showed better results than regular ANOVA for the RCB design in one of the three experiments. Although Wilkinson et al. (1983) reported an increase of bias, Kempton and Howes (1981), Pearce (1998) and Seena et al. (1998) reported that the iteration method was effective in some cases.
- The method of first differences was better than the other nearest neighbor methods in two out of three experiments. Wu et al. (1998), Wu and Dutilleul (1999) and Piepho et al. (2008) also reported that the method of first differences was more effective than the Papadakis method.

In conclusion, although using nearest neighbor techniques significantly improved experimental accuracy, this should not invalidate the classic design of experiments. As

Sarker et al. (2001) suggest, it is important to design an experiment according to some preferred design and perform the analyses accordingly. The nearest neighbor methods should be applied whenever there is suspicion or some relevant information of intense spatial heterogeneity.

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