

USING OF COVARIANCE ANALYSIS TO CONTROL FERTILITY GRADIENT IN FIELD EXPERIMENTS

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Abstract

Adjustment for fertility trends within a trial may increase precision. Two methods of controlling fertility trends were used in this study. The first was moving mean covariance analysis (MMCA) and the second was check-row mean covariance analysis (CRMCA).

The effectiveness of using moving mean covariance analysis (MMCA) for experimental error control was compared in soybean yield trial. The MMCA was superior to the RCBD, since it significantly reduced the experimental error and the coefficient of variation (CV.) Inclusion of five neighboring plots in moving mean computation provided better error control. However, the estimation of optimum number of neighboring plots to be used and moving mean were not easily calculated.

The feasibility of using check-row measurements such as mean seed yield/m of row as a covariate in an analysis of covariance (CRMCA) was examined in a separate wheat yield trial in which check rows were planted with check cultivar. Check-row measurements were effective in reducing the experimental error. Check-row measurements could be easily used as a covariate in analysis of covariance without need for moving mean computation from the response variable.

INTRODUCTION

Various experimental designs are considered for controlling soil heterogeneity in experimental sites. The most widely used design is RCBD or Latin square design for error control. The RCBD is effective in experimental sites with a single fertility gradient. Latin square is recommended in case of double fertility gradient. Incomplete block designs are used for trials consisting of large number of treatments; however, the analysis is relatively complicated and is restricted to certain fixed numbers of treatment. (Snedecor and Cochran, 1980; Stroup and Miltze, Clarke et al. 1994).

Clarke and Baker, (1996) reported that, randomization of treatments provides unbiased treatment estimates but does not deal with the high soil variability that results from large systematic variation. Blocking assumes homogeneity between plots within blocks. This method of accounting for soil heterogeneity is usually effective when blocks are small but becomes less effective as the number of treatments within a block increases. Experimental errors are expected to be high under these conditions and high C.V. values are usually associated with the results.

Check plot designs in which a systematic arrangement of check plots is superimposed on a RCBD have been proposed as a method of error control in experiments with several treatments (Baker and McKenzie, 1968; LeClerge, 1966). The performance of a cultivar is expressed as the differences between it and the adjacent check plot and test plot exists. If the check plot misrepresents the soil variation, improper adjustment might result (Mak et al., 1978; Smith and Hurd, 1973; Rosielle, 1980).

To prevent such an error in check plot using moving mean covariance analysis (MMCA) has been recommended (Mak et al., 1978; Smith and Hurd, 1973; Rosielle, 1980). The mean of the neighboring plots, excluding the test plot in question, is assumed to be a measure of fertility of the test plot and is used as a covariate to adjust the treatment effect in an analysis of covariance. If there is no linear association between the test plot yield and the covariate, the regression coefficient of the covariate would be about zero and any adjustment would be avoided. Although MMCA has been used successfully to adjust treatment effects (Mak et al., 1978; Smith and Hurd, 1973; Rosielle, 1980), the computation of moving means is difficult and the optimum number of neighboring plots included in moving mean calculation varies, depending on the variability of the experiment.

In yield trials of many row crops, plot yield is usually measured by excluding the two border rows and harvesting from the middle rows. If uniform plant types are to be evaluated in yield trials and a check cultivar is planted in the border rows of every experimental unit, then suitable measurements from the border rows could be substituted for the moving mean in the analysis of covariance to adjust the treatment effect for local soil variation.

The objectives of this study were to investigate the effectiveness of the moving mean covariance analysis (MMCA) in error control and to test the feasibility of using check row mean covariance analysis (CRMCA) when each test plot is bordered by a single check cultivar.

MATERIALS AND METHODS

1. The moving mean covariance analysis:

Data used in the moving mean covariance analysis (MMCA) were obtained from soybean yield trials during 1995 and 1996 growing seasons at Sakha Experimental Station. The experimental design was a randomized complete block design with four replications and fourteen genotypes of soybean namely; (Clark, Crawford, Catler 71, Elgin 87, H2L13, H2L21, H2L35, H2L24, H3L6, H15L4, H15L2, H15L17 and Columbus). The experimental plot consisted of six ridges 60 cm apart and 2.5 meters long (3.6 x 2.5 m. = 9m²). Seed yield (ton/fed.) was determined from a central area of 6 m² from each plot and used for this analysis.

Statistical analysis for (MMCA):

Data were subjected to two statistical analysis procedures, the first was randomized complete block design analysis and the second was the moving mean covariance analysis. MMCA has been suggested (Mak et al., 1978; Smith and Hurd, 1973; Rosielle, 1980). The statistical model of the MMCA in RCBD is as follows:

$$Y_{ij} = \mu + \beta (X_{ij}) + B_i + G_j + \epsilon_{ij}$$

Where : Y_{ij} = yield of j th genotypes in its block; μ = grand mean; β = the regression coefficient, measure of linear relationship between the test plot yield and the moving mean covariate; X_{ij} = moving mean covariate, the mean yield of the neighboring plots of j th genotypes in its block; B_i = the effect of its block; G_j = the effect of j th genotypes; ϵ_{ij} = random error.

For each test plot, three types of moving means were computed from the adjacent plot within a column. The number of adjacent plot included in the computation of the moving mean covariate varied from 3 (Cov.1) to 5 (Cov. 3) plots. In each case, the moving mean was computed using an equal number of plots on each side of the plot in question, except at the end of the blocks, where the appropriate number of plots adjacent to the test plot within a column was used (Table 1). For example, the moving mean in cov.1 of plot No. 1 (as the end of block in the experimental design) was calculated from values of plots No. (2, 3, 4), and moving mean of plot No.33 (as the middle in the block) calculated from mean values of plots No. ((1+2+4) + (2+4+5))/6, and so on for another moving mean (Table 1). Separate MMCA were performed using Cov1 to Cov.3.

Table 1. The plot layout and the moving mean computations.*

Test plot	Cov. λ	Cov. τ	Cov. ρ
Plot no. 1 (as the end of the block)	$(\#2 + \#3 + \#4)/3$	$(\#2 + \#3 + \#4 + \#5)/4$	$(\#2 + \#3 + \#4 + \#5 + \#6)/5$
Plot no. 3 (as the middle in the block)	$((\#1 + \#2 + \#4) + (\#2 + \#4 + \#5))/6$	$(\#1 + \#2 + \#4 + \#5)/4$	$(\#1 + \#2 + \#4 + \#5 + \#6)/5$

* Numerals following # refer to yield of plot indicated.

8	16	24	32	40	48	56*	*plot No.
7	15	23	31	39	47	55	Block 4
6	14	22	30	38	46	54	
5	13	21	29	37	45	53	Block 3
4	12	20	28	36	44	52	
3	11	19	27	35	43	51	Block 2
2	10	18	26	34	42	50	
1	9	17	25	33	41	49	Block 1

c1 c2 c3 c4 * c5 c6 c7

Columns

Genotypic means adjusted for the covariate were estimated by the least square method. To draw the trend of variability between residuals for RCBD analysis and plot location represented by serially numbered plot, we must calculate residual values. The residuals consisted from the difference between each actual plot yield and the corresponding treatment mean ($Y_i - y_i$). To confirm that the error term was positively autocorrelated we used Durbin Watson D statistic (Neter et al., 1983). Testing for significance of the genotypes differences were tested using Duncan's multiple range test at the 5% significance level in both RCBD and MMCA analyses.

2. Check row mean covariance (CRMCA):

To evaluate the effectiveness of CRMCA, two wheat yield trials were conducted at Sakha Experimental Station during 1995/96 and 1996/97 seasons. The experimental design was laid out as a randomized complete block design with three replications and nine cultivars of wheat namely; (Giza 155, Giza 156, Giza 157, Giza 162, Giza 163, Giza 164, Sakha 8, Sakha 61, Sakha 69).

The experimental units consisted of 12 rows (3m.), with 20 cm between

rows. In each plot, the two check rows, (first and seventh rows) were planted by Giza 155 cultivar (as the standard check cultivar, Figure 1). The fertility levels of each plot were estimated by seed yield of the check rows. Mean seed yield was recorded per plot at harvest (10 rows), and the yield for two check row means were used in an analysis of covariance to control the experimental error associated with the total seed yield.

The relative efficiency (RE) of CRMCA over RCBD was computed as follows:

$$RE = \frac{EMS1}{EMS2} \times \frac{(F1+1)(F2+3)}{(F2+2)(F1+3)}$$

Where : EMS1 = error mean square of RCBD; EMS2 = error mean square of CRMCA; F1= error df. of CRMCA; F2= error df. of RCBD.

RESULTS AND DISCUSSION

Moving mean covariance analysis:

The analysis of variance with the randomized complete block design (RCBD) and the results of moving mean covariance analysis (MMCA) are shown in Tables 2 & 3. Non-significant differences between genotypes were found in both seasons. The high CV. values in the two seasons of experimentation indicated the presence of an inflated error terms caused by local soil variation within the experimental site.

Table 2. Mean squares (M.S.) for randomized complete block design (RCBD) and moving mean covariance analysis (MMCA) in soybean yield trial in 1995 season.

Source of variance	RCBD		Type of analysis							
	D.F	M.S.	S.S.%	D.F	Cov.1 M.S.	S.S. %	Cov.2 M.S.	S.S.%	Cov.3 M.S.	S.S. %
Covariate	—			1	0.143***	18.33	0.113***	0.53	0.038***	7.58
Block	3	0.046	3.30	3	0.006	2.32	0.001	47.37	0.002	1.39
Genotypes	13	0.023**	7.16	13	0.021*	35.00	0.023***	17.90	0.038***	54.29
Error	39	0.096	89.54	38	0.009	44.35	0.006	34.20	0.005	36.5
C.V.%			19.39			5.98		4.71		4.34

Cov.1- Cov3 = moving means computed from three, four and five adjacent plots, respectively.
 *, **, *** Significant at p = 0.05, 0.01 or 0.001, respectively.
 n.s. non significant

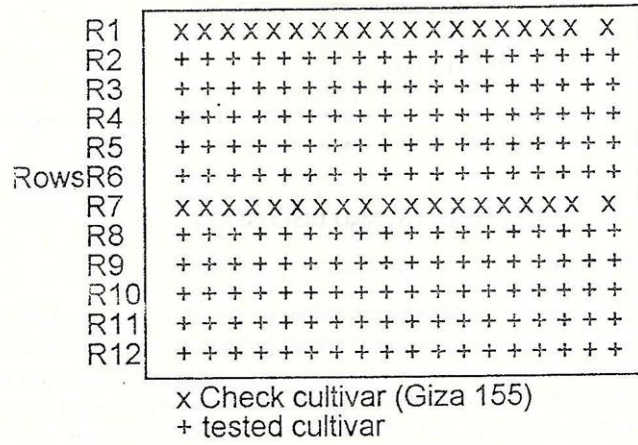


Fig. 1. The plot layout for check row mean covariance.

A significant trend was observed between residuals from the RCBD analysis and plot location represented by serially numbered plot (Fig. 2 {A,B}). The error terms were, therefore, serially correlated or autocorrelated with the adjacent observations (Li and Keller, 1951; Neter et al., 1983). Testing for significance by Durbin-Watson "D" statistic (Neter et al., 1983) confirmed that the error terms were positively autocorrelated (Fig. 2 {A,B}). For such date, the assumption of an uncorrelated or independent error terms are not appropriate because the error terms were positively correlated with the location of the plots.

To overcome the effects of autocorrelation on the error terms, MMCA has been applied. The covariance adjustments (Cov. 1 to Cov. 3) reduced the errors and CV. in two seasons of experimentation (Tables 2 & 3). The covariate and genotype effects were significant, suggesting that the precision of the analysis increased significantly. The sequential mean squares attributed to each individual effect and their percentages of the total sum of squares are presented in Tables 2 & 3. The CV. and the percentage variability attributed to the random error decreased as the number of plots included in the moving mean analysis increased. In similar studies, a maximum reduction in CV. was obtained with 8 to 18 neighboring plots in moving mean estimation (Mak et al., 1978; Rosielle, 1980). In the current study, five neighboring plots gave the lowest CV. Between five and four neighboring plots, the differences in mean comparisons were not different. The optimum number of adjacent plots to be included in the MMCA would logically vary, depending on the variability of the experimental area (Mak et al., 1978). However, too large numbers should be discouraged, because the size of the block would be increased accordingly, making the estimates less sensitive to changes in the micro soil variation.

Table 3. Mean squares (M.S.) for randomized complete block design (RCBD) and moving mean covariance analysis (MMCA) in soybean yield trial in 1996 season.

Source of variance	Type of analysis									
	RCBD			MMCA						
	DF	M.S.	S.S. %	DF	Cov 1 M.S.	S.S. %	Cov 2 M.S.	S.S. %	Cov 3 M.S.	S.S. %
Covariate	---			1	0.124***	14.05	0.050***	15.5	0.067***	17.35
Block	3	0.033	2.31	3	0.011	3.74	0.004	3.4	0.003	2.59
Genotypes	13	0.045**	12.80	13	0.027*	39.45	0.011**	43.16	0.018***	63.37
Error	39	0.098	81.89	38	0.010	42.74	0.003	38.19	0.002	16.32
C.V. %			19.85			6.26		3.51		2.76

Cov. 1- Cov.3 = moving means computed from three, four and five adjacent plots, respectively.
 *, **, *** Significant at $p = 0.05$, 0.01 or 0.001 , respectively.
 n.s. non significant

With the covariance adjustment, the mean yield adjusted to the local fertility trend, also differed significantly from the RCBD analysis in two seasons of experimentation (Table 4). These results suggest that covariance adjustment using moving means of neighboring plots gives superior control of experimental error in situations where many genotypes are evaluated in sites with large micro soil variation.

A partially balanced lattice design and MMCA have been compared in error control (Mak et al., 1978). Both methods gave a similar level of error control. In cases where the number of genotypes is large to accommodate in an incomplete block design, MMCA was found to be advantageous (Mak et al., 1978). Furthermore, use of MMCA has advantages over lattice designs and to lay out treatment and calculation of the moving mean and estimation of the optimum number of neighboring plots in MMCA (Rosielle, 1980).

Table 4. Mean seed yield in randomized complete block design (RCBD) vs. the adjusted seed yield (Ton/fad) in the moving mean covariance analysis (MMCA) in soybean yield trial.

Genotypes	1996			1997		
	RCBD	MMCA		RCBD	MMCA	
		Cov.2	Cov.3		Cov.2	Cov.3
Clark	1.520 a	1.569 ab	1.531 b	1.564 a	1.689 b	1.614 ab
Crawford	1.770 a	1.758 c	1.748 c	1.704 a	1.606 a	1.662 bc
Cattler 71	1.662 a	1.629 b	1.621 c	1.650 a	1.679 b	1.708 c
Elgin 87	1.555 a	1.563 ab	1.582 bc	1.585 a	1.597 a	1.610 ab
H2L13	1.565 a	1.606 ab	1.594 bc	1.590 a	1.584 a	1.584 ab
H2L21	1.860 a	1.837 c	1.839 e	1.593 a	1.676 b	1.684 bc
H2L35	1.747 a	1.695 bc	1.660 cd	1.479 a	1.579 a	1.612 ab
H2L24	1.743 a	1.695 bc	1.706 cd	1.588 a	1.592 a	1.549 a
H3L6	1.614 a	1.578 ab	1.594 bc	1.498 a	1.567 a	1.640 bc
H3L12	1.709 a	1.624 b	1.629 bc	1.613 a	1.649 ab	1.683 bc
H15L4	1.556 a	1.718 bc	1.717 cd	1.648 a	1.625 ab	1.630 bc
H15L2	1.681 a	1.556 ab	1.560 bc	1.586 a	1.587 a	1.617 ab
H15L17	1.553 a	1.629 b	1.635 bc	1.719 a	1.652 b	1.666 bc
Columbus	1.487 a	1.422 a	1.441 a	1.293 a	1.638 ab	1.557 a

Not : Any 2 means take the same letter are not significantly different.
Cov.2-Cov.3 =moving means computed from four and five adjacent plots, respectively.

Check-row mean covariance analysis:

In the second yield trial, blocking was not effective in reducing the experi-

mental error for yield trial in 1995/96 and 1996/97 seasons (Table 5). About 49.83% to 52.03% of the total variability was attributed to experimental error in RCBD. Check-row mean covariates, reduced CV, from 23.67% to 13.36% in the first season and from 18.25% to 9.86% in the second season and the experimental errors about 14.46% to 17.24% from the total variability in two seasons of experimentation. The relative efficiency of CRMCA over RCBD in the error control varied from 299.77% to 323.00%. These results indicated that CRMCA could be effectively used for error control in field experiments.

Table 5. Mean squares for randomized complete-block design (RCBD) and checkrow mean covariance analysis (CRMCA) in wheat yield trial in 1995/96 and 1996/97 seasons.

Source of variance	D.F.	Mean square of RCBD				Mean square of CRMCA				
		1995/96		1996/97		1995/96		1996/97		
		M.S.	S.S.%	M.S.	S.S.%	M.S.	S.S.%	M.S.	S.S.%	
Block	3	0.125	5.48	0.099	6.31	3	0.170*	7.13	0.016	0.75
Varieties	8	0.382*	44.68	0.245*	41.65	8	0.236**	26.38	0.110**	21.99
Covariat	---	---	---	---	---	1	3.723***	52.03	2.401**	60.01
Error	24	0.142	49.83	0.102	52.03	23	0.045	14.46	0.030	17.24
C.V. %		23.67		18.25		13.36		9.86		
R.E. %						299.77%		323.00%		

*, **, *** Significant at $p = 0.05$, 0.01 or 0.001 , respectively

Table 6 gives the cultivar means, before and after adjusting for CRMCA analysis in RCBD experiment. Adjusting the means using CRMCA improved treatment estimates. The adjusted means gave significant differences between the cultivars, whereas cultivars differences were not significant in RCBD. These results are in harmony with those reported by Baker and McKenzie, 1967; LeClerge, 1966 Mak et al., 1978; Smith and Hurd, 1973; Rosielle, 1980).

Mak, et al. (1978) used control plots and moving mean of adjacent plots as methods of controlling error in single-row plots. They reported that control plots and moving mean gave similar control of experimental error, but control plots had less practical application than moving means. In this study, check-row measurements could be used as covariates without any further computations in the covariance analysis than moving means. Computation of moving means or finding the optimum number of neighboring plots in moving means calculations is not necessary. Unlike MMCA, where moving means are computed from the response variable, check-row measurements are not dependent on the response variable.

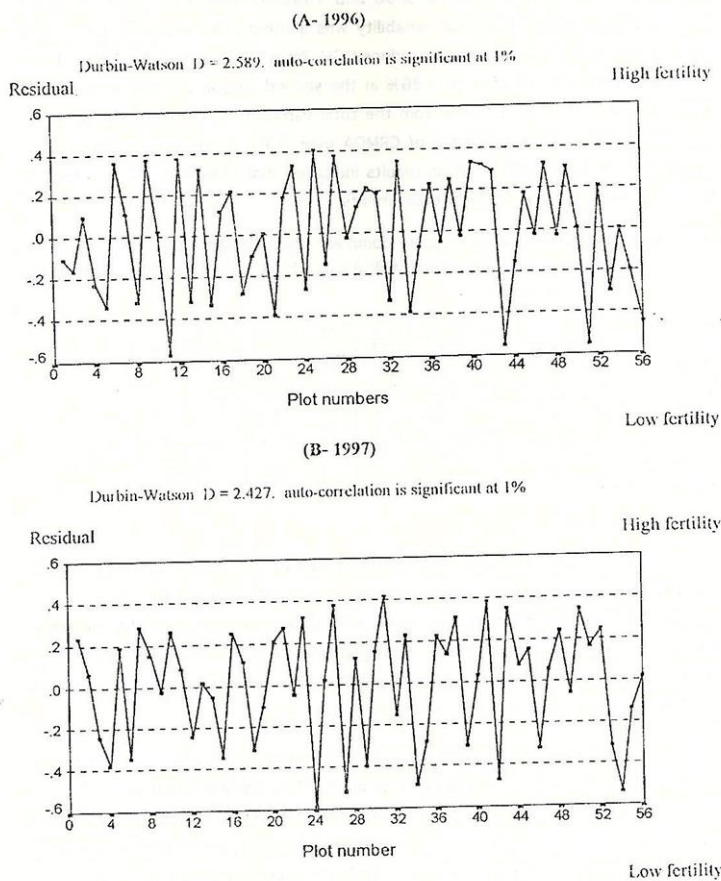


Fig. 2 A, B. Autocorrelation between the residuals from the RCBD and the plot arrangement (plot number) in both seasons (1996 & 1997) of experimentation for soybean.

Table 6. Mean seed yield (kg/2.8m²) in randomized complete-block design (RCBD) vs.the adjusted seed yield in check-row mean covariance analysis (CRMCA).

Varieties	1995/96		1996/97	
	RCBD	CRMCA	RCBD	CRMCA
1- Giza 155	1.450 b	1.678 a	1.645 ab	1.681 bc
2- Giza 156	1.465 ab	1.576 ab	1.635 ab	1.582 c
3- Giza 157	1.623 ab	1.509 ab	2.023 ab	1.862 ab
4- Giza 162	1.843 a	1.644 ab	1.920 ab	1.791 abc
5- Giza 163	1.442 b	1.457 b	1.585 b	1.678 bc
6- Giza 164	1.778 ab	1.636 ab	1.670 ab	1.667 bc
7- Sakha 8	1.540 ab	1.696 a	1.585 b	1.769 bc
8- Sakha 61	1.647 ab	1.681 a	2.050 a	2.029 a
9- Sakha 69	1.545ab	1.457 b	1.638 ab	1.690 bc

Not : Any 2 means take the same letter are not significantly different.

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إستخدام تحليل التغيرات للتحكم فى اختلاف درجات الخصوبة فى التجارب الحقلية

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قسم الحساب العلمى - العمل المركزى لبحوث التصميم والتحليل الأحصائى مركز البحوث الزراعية.

يعتبر عدم تجانس خصوبة التربة من العوامل الهامة التى تؤدى الى انخفاض دقة نتائج التجارب الحقلية. ولتحسين نتائج هذه التجارب يجب على الباحث استخلاص هذا التأثير من قيمة الخطأ التجريبي عند تحليل البيانات مما يؤدى الى زيادة دقة النتائج المتحصل عليها. لذلك يهدف هذا البحث الى ايضاح امكانية أستخدام طريقتين من طرق تحليل التغيرات لأظهار أهميتهما فى تحسين دقة النتائج التجريبية هما المتوسط المتحرك ومتوسط خط الأختبار.

ولقياس كفاءة أسلوب تحليل التغيرات للمتوسط المتحرك أقيمت تجربة على مجموعة من التراكيب الوراثية لمحصول فول الصويا فى قطاعات كاملة العشوائية وتم تحليل النتائج بالطريقتين، حيث أظهرت النتائج تفوق أسلوب تحليل التغيرات للمتوسط المتحرك فى اظهار معنوية العوامل المستخدمة تحت الدراسة عن طريق خفض قيمة الخطأ التجريبي بعد استخلاص نسبة التباين الراجع الى عدم تجانس خصوبة القطع التجريبية حيث أنخفضت قيمة معامل الاختلاف. وقد أظهرت النتائج أن استخدام خمس قطع تجريبية متجاوزة فى حساب المتوسط المتحرك كافياً للحصول على دقة عالية من المعنوية. أما بالنسبة لقياس كفاءة أسلوب تحليل التغيرات عن طريق أسلوب متوسط خط الأختبار، فقد أقيمت تجربة على تسع أصناف من القمح فى تصميم قطاعات كاملة العشوائية، ثم حلت النتائج بالطريقتين حيث تفوق أسلوب تحليل التغيرات فى خفض قيمة الخطأ التجريبي بشكل ملحوظ مما أدى الى ظهور الفروق المعنوية بدقة اعلى بين الأصناف عن الطريقة التقليدية. كما ارتفعت الكفاءة النسبية لأسلوب تحليل التغيرات لمتوسط خط الأختبار الى ثلاث أضعاف الأسلوب التقليدى، ومما سبق يتبين كفاءة كل من الطريقتين بالنسبة لتحليل القطاعات الكاملة العشوائية ويمكن مقارنتهما ببعض عند تطبيقهما فى تجربة واحدة لبيان أيهما أكفأ.