

Stability analysis for single-site long term experiments planned in split plot design

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Abstract : Long term fertility experiments with replications are often statistically analyzed as split plots in time. Due to advancement in agrotechnology, scientists are sometimes changing the package of practices (input factor) but not the basic treatments. The statistical procedure for analyzing the long term experiment with change in input is lacking. The objective of this study was to evaluate long term fertility experiments by stability analysis. Stability analysis was performed on bidi tobacco single-site long term fertility experiment. Stability analysis can be inappropriate without prior evaluation of the relationship between treatment yield and year in single-site long term experiment.

Key words: Long term experiments, stability analysis, regression analysis, split plot design

Introduction

Long term field experiments provide valuable information that cannot be obtained from short term experiments. They are essential to understanding the many slow changes that occur in soil under constant fertility, tillage, irrigation or cropping management treatments.

If a long term experiment is considered (by strict definition) to be one in which the original treatments are repeated for many years, it probably would be impossible to manage; and it would be impractical even if could be done. For valid results from long term experiments primary treatments must be maintained continually for some long time, however, flexibility is necessary in secondary treatments and management aspects of the study to make it practical.

Technical changes such as varietal improvement, nutrient management, pest control, complex agronomic practices etc. are in ways that cannot be foreseen. The incorporation of technical advances during the course of a long term study can greatly alter the results; however, the adoption of new technology is necessary. Crop variety is one of the most important technological advances. Failure to use new variety would result in unrealistic data. The method of adjustment of the effect of new technology in long term experiment is lacking (Frey and Thomas, 1991).

The technique of stability analysis (Eberhart and Russel, 1966) rarely been used applied beyond the realm of plant breeding. However, Raun *et al.* (1993) and Guertal *et al.* (1994) used stability analysis to evaluate fertility treatments from multilocal and single-site experiments. Stability analysis was assumed to be a means of visually partitioning the treatment $\times$ environment interaction, illustrating the effect of fertilizer treatments on grain yield within changing environments.

The treatment yields may reveal a significance relationship with year (time) in a single-site long term experiment. The relationship could confound stability analysis, as an external factor (e.g. soil acidity) has a significant increasing or decreasing effect on the environmental mean with time. This problem could be important in long term fertility studies where treatment effects are determined in sequential years (Guertal *et al.* 1994).

The technique of stability analysis was, however, applied to evaluate long term experiment laid down in split plot design.

Material and methods

The data for the analysis was taken from the single-site long term experiment on *bidi* tobacco running at Bidi Tobacco Research Station (BTRS), Gujarat Agricultural University, Anand, since 1960-61. The experiment is running with four bulky manure treatments as main plot treatments and five fertilizers treatments as sub-plot treatments. These basic 20 treatments were continued as such throughout experimentation. Year effects coupled with variety effect (being confounded with year effect) were considered as environments. Treatments are likely to show stability over environments. This was tested by stability analysis (Eberhart and Russel, 1966). The data were subjected to analysis of variance (ANOVA) technique. The ANOVA table for the same is given in Table 1.

Where, $m = 1, 2, 3, 4$ (Main plot) $s = 1, 2, 3, 4, 5$ (Sub plot)
 $r = 1, 2, 3$ (Replication)
 $y = 1, 2, \dots, 34$ (Year)

Different sum of squares were computed as follows

Subscript i= 1, 2, 3, 4 for main plot j=1, 2, . . ., 5 for sub plot
k = 1, 2, . . ., 34 for year

Variable X, Y and I represent individual year data, Pooled year data and Interaction data with respect to the treatment with which it is appearing, respectively.

$$\text{Main plot SS [SS(M)]} = \left[\sum_{ik} X_{ik}^2 - \frac{(\sum_{ik} X_{ik})^2}{my} \right] - \left[\sum_i^m X_{i.}^2 - \frac{(\sum_i X_{i.})^2}{y} \right]$$

$$\text{Env(linear)M SS [ELMSS]} = \frac{1}{m} \left[\frac{(\sum Y_{(m)k} I_k)^2}{I_k} \right]$$

$$\text{Env} + (\text{M} \times \text{Env}) \text{SS [EMSS]} = \sum \left[\sum_i X_{i.}^2 - \frac{(\sum_i X_{i.})^2}{m} \right]$$

$$\text{M} \times \text{Env(linear)} \text{SS} = \text{EMSS} - \text{ELMSS}$$

$$\text{Sub plot SS [SS(S)]} = \left[\sum_{jk} X_{jk}^2 - \frac{(\sum_{jk} X_{jk})^2}{my} \right] - \left[\sum_j^s X_{j.}^2 - \frac{(\sum_j X_{j.})^2}{y} \right]$$

$$\text{Env(linear)S SS [ELSSS]} = \frac{1}{s} \left[\frac{(\sum Y_{(s)k} I_k)^2}{I_k} \right]$$

$$\text{Env} + (\text{S} \times \text{Env}) \text{SS [ESSS]} = \sum \left[\sum_j X_{j.}^2 - \frac{(\sum_j X_{j.})^2}{s} \right]$$

$$\text{S} \times \text{Env(linear)} \text{SS} = \text{ESSS} - \text{ELSSS}$$

$$\text{M} \times \text{S Interaction SS}$$

$$[\text{SS(MS)}] = \left[\sum_{ik} X_{ik}^2 - \frac{(\sum_{ik} X_{ik})^2}{my} \right] - \left[\sum_j X_{j.}^2 - \frac{(\sum_j X_{j.})^2}{y} \right] - \text{SS(M)} - \text{SS(S)}$$

$$\text{Env(linear)MS SS [ELSS]} = \frac{1}{ms} \left[\frac{(\sum Y_{(ms)k} I_k)^2}{I_k} \right] - \text{ELMSS} - \text{ELSSS}$$

$$\text{Env} + (\text{MS} \times \text{Env}) \text{SS [ESSS]} = \sum \left[\sum_{ij} X_{ij}^2 - \frac{(\sum_{ij} X_{ij})^2}{ms} \right] - \text{EMSS} - \text{ESSS}$$

$$\text{MS} \times \text{Env(linear)} \text{SS} = \text{ESSS} - \text{ELSS}$$

In order to test the significance of the difference among the treatment means i.e. $H_0: \mu_1 = \mu_2 = \dots = \mu_t$ the appropriate F test is defined as

$$\text{Main plot treatment } F = \frac{MS_1}{MS_3} \quad \text{Sub plot treatment } F = \frac{MS_5}{MS_7}$$

$$\text{MS interaction } F = \frac{MS_8}{MS_{11}}$$

To test that the treatment do not differ for their regression on the environmental index i.e. $H_0: \beta_1 = \beta_2 = \dots = \beta_t$ the appropriate F test is defined as

$$\begin{aligned} \text{Main plot} \times \text{Env(linear)} \quad F &= \frac{MS_2}{MS_3} & \text{Sub} \\ \text{plot} \times \text{Env(linear)} \quad F &= \frac{MS_6}{MS_7} & \text{MS} \times \text{Env(linear)} \\ F &= \frac{MS_{10}}{MS_{11}} \end{aligned}$$

The regressions were found treatment-wise for each variety and overall (ignoring variety). Eberhart and Russel (1966) model of stability parameters was used for calculating the regressions.

The calculated regression coefficients were then tested by t-test for their stability ($H_0: \beta = 1$) and also for difference between regression coefficients obtained from overall and variety-wise regression coefficients.

$$t = \frac{|1 - \beta_i|}{SE(b_i)} \quad \text{and} \quad t = \frac{|b_{kj} - b_{ij}|}{\sqrt{S^2 \left(\frac{1}{TSS_1} + \frac{1}{TSS_k} \right)}}$$

where

b_{ij} = regression coefficient of jth treatment from overall basis

b_{kj} = regression coefficient of jth treatment of kth variety

TSS_1 = Treatment Sum of square for overall basis

TSS_k = Treatment Sum of square for kth variety

$$S^2 = \frac{[(n_1 - 2)S_{Y,X(1)}^2 + (n_2 - 2)S_{Y,X(2)}^2]}{n_1 + n_2 - 4}$$

n_1 & $S_{Y,X(1)}^2$ = no. of observations and error mean square for overall basis, respectively.

n_2 & $S_{Y,X(2)}^2$ = no. of observations and error mean square for respective variety, respectively.

Since five varieties were changed during the experimentation, the analysis was carried out overall (ignoring variety) and variety-wise. The variety 1 (K20) was run for 8 years from 1961-62 to 1968-69, variety 2 (Anand 2) for 8 years from 1969-70 to 1976-77, variety 3 (GT 4) for 4 years from 1977-78 to 1980-81, variety 4 (Anand 119) for 4 years from 1981-82 to 1984-85 and variety 5 (GT 5) for 10 years from 1985-86 to 1994-95, respectively.

Results and discussion

Pooled analysis through ANOVA showed presence of interaction between main plot and sub plot treatments also with year effect. Sub plot analysis further revealed disturbance in error distribution. The ANOVA pooled as well as variety-wise in presented in Table 2.

The results presented in Table 2 showed significant variation among main plots, sub plots and interaction between main $\times$ sub plot treatments. Environment (linear) component was also significant in majority of the cases. As evinced from non significant $\text{M} \times \text{Env(linear)}$ in pooled analysis, there was no difference among main plot treatments for their regression on the environmental index. In spite of

having differences in yield of main plots $H_0: \beta_1 = \beta_2 = \beta_3 = \beta_4$ for main plot treatments could not be rejected. The regression coefficients are presented in Table 3. Further analysis related to sub-sets (variety-wise) revealed significant deviation among main plots for their regression on environmental index for all the varieties barring V5.

The regression on the environmental index for sub plot [$S \times Env(\text{linear})$] treatments showed significant differences on pooled analysis basis and variety-wise. This suggested that the sub plot treatments were influenced more by varietal variation being confounded with year effect. The estimates presented in Table 3 indicate large variation among regression coefficient (variety-wise) within and between treatments in comparison to those obtained on pooled basis.

The estimates (b_i) were tested for stability ($H_0: \beta = 1$). The significant estimates are marked with star (*) in Table 3. From this criterion it could be generalized that varietal variation had influenced the stability of treatment effects. Therefore, inference based on pooled data may not prove valid. This is supported by the t-values presented in Table 4. Guertal *et al.* (1994) suggested that in a continuous single-site experiment stability analysis should not be performed on data sets that contain significant slope components from regression of treatment mean on year. The present investigation leads to the same conclusion.

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Table 1. Analysis of variance for testing significance of treatment means and deviation from their regression

Source of variation	Degrees of freedom	Sum of Squares	Mean Sum of Square
Replication/Environment	$y(r - 1)$		
Main plot (M)	$(m - 1)$	SS(M)	MS ₁
$Env + (M \times Env)$	$m(y - 1)$	EMSS	
Env(linear)M	1	ELMSS	
$M \times Env(\text{linear})$	$(m - 1)$	MELSS	MS ₂
Pooled deviation (PM)	$m(y - 2)$		MS ₃
Pooled Error (a)	$y(m - 1)(r - 1)$		MS ₄
Sub plot (S)	$(s - 1)$		MS ₅
$Env + (S \times Env)$	$s(y - 1)$		
Env(linear)S	1		
$S \times Env(\text{linear})$	$(s - 1)$		MS ₆
Pooled deviation (PS)	$s(y - 2)$		MS ₇
$M \times S$ interaction	$(m - 1)(s - 1)$		MS ₈
$Env + (MS \times Env)$	$(ms - m - s)(y - 1)$		MS ₉
Env(linear)	1		
$S \times Env(\text{linear})$	$(m - 1)(s - 1)$		MS ₁₀
Pooled deviation (PS)	$(ms - m - s)(y - 2) - 2$		MS ₁₁
Pooled Error (b)	$my(s - 1)(r - 1)$		MS ₁₂
Total	$msyr - 1$		

Table 2. Overall and variety-wise ANOVA (Regression analysis)

Source of variation	Pooled		Variety 1		Variety 2		Variety 3		Variety 4		Variety 5	
	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Replication/Environment	68		16		16		8		8		20	
Main plot (M)	3	5.61*	3	0.85*	3	1.93*	3	10.63*	3	3.79*	3	10.24*
Env(linear)M	1	223.14	1	0.58	1	0.54	1	0.11	1	0.13	1	2.37
M × Env(linear)	3	0.01	3	0.06*	3	0.09*	3	0.12*	3	0.07*	3	0.02
Pooled deviation (PM)	128	0.07	24	0.01	24	0.03	8	0.04	8	0.01	32	0.02
Pooled Error (a)	204	0.59	48	0.37	48	0.84	24	0.64	24	0.70	60	0.51
Sub plot (S)	4	2.68*	4	1.74*	4	1.30*	4	0.28*	4	0.85*	4	3.50*
Env(linear)S	1	278.92	1	0.72	1	0.68	1	0.14	1	0.17	1	2.96
S × Env(linear)	4	0.08*	4	0.50*	4	0.37*	4	0.28*	4	0.29*	4	0.13*
Pooled deviation (PS)	160	0.01	30	0.03	30	0.04	10	0.02	10	0.04	40	0.01
M × S interaction	12	8.50*	12	4.23	12	10.79*	12	11.51*	12	5.68*	12	30.33*
Env(linear)	1	613.62	1	1.59	1	1.50	1	0.30	1	0.37	1	6.52
S × Env(linear)	12	0.30	12	0.90*	12	0.90	12	0.78	12	0.59	12	3.22*
Pooled deviation (PS)	350	0.43*	64	0.38*	64	0.83*	20	0.62*	20	1.10*	86	0.49*
Pooled Error (b)	1088	0.15	256	0.07	256	0.16	128	0.17	128	0.16	320	0.20
Total	2039		479		479		239		239		599	

Table 3. Variety –wise and overall calculated regression coefficients for raw data

Treatment		V1	V2	V3	V4	V5	Overall
Main plot (M)	1	1.07	2.22	-0.12	-1.13	1.20	0.99
	2	1.14	0.47	1.79	1.67*	0.97	1.01
	3	1.64	0.70	3.54	1.80	1.01	1.00
	4	0.15	0.61	-1.21	1.66	0.82	1.01
Sub plot (S)	1	-1.70*	-1.30	-2.34*	-3.53	1.48*	0.94
	2	0.24	0.49	5.33	3.32	0.76	1.04
	3	3.08	2.88	-0.46	-0.09	0.57	1.00
	4	2.30	2.33	-0.81	1.59	1.54*	1.00
	5	1.09	0.61	3.28*	3.71	0.64	1.02
	1	-2.43*	-1.06	2.99	-3.34	4.26*	0.90
	2	0.12	1.22	0.64	2.22	-0.30	1.04
	3	5.07*	6.61	0.42	-5.77	1.25	0.92
M × S interaction	4	1.05	3.04	-3.16	0.87	-0.81*	1.07
	5	1.52	1.26	-1.51	0.37	1.61	1.00
	6	0.06	-1.97	0.49	-3.67	0.61	0.91
	7	-0.56*	1.37	5.06	6.76*	2.35*	0.97
	8	2.65	0.81	-0.25	0.87	-1.46*	1.02
	9	2.74	0.50	-1.15	0.05*	3.04*	1.09
	10	0.83	1.64	4.82*	4.33*	0.31	1.05
	11	-1.59*	-2.11	-4.16*	-2.91	1.37	0.95
	12	0.60	0.63	7.80*	1.48	-1.40*	1.08
	13	5.49*	4.07	3.87	5.39	3.35*	1.03
	14	2.93	2.92	4.25*	2.00	-0.28*	0.93
	15	0.76	-2.02	5.93*	3.06	2.03	0.98
	16	-2.86*	-0.07	-8.70*	-4.21	-0.32*	0.98
	17	0.79	-1.27*	7.81	2.82	2.40	1.08
	18	-0.88	0.01	-5.86*	-0.85	-0.84*	1.04
	19	2.46	2.84	-3.18	3.46	4.23*	0.93
	20	1.24	1.55	3.89	7.08	-1.40	1.03

Table 4. t-test statistic of different varieties with respect to overall

Treatment		V1	V2	V3	V4	V5
Main plot (M)	1	-0.51	-10.40*	14.57*	22.20*	-3.05*
	2	-0.86	4.52*	-10.32*	-6.95*	0.52
	3	-4.03	2.51*	-33.35*	-8.48*	-0.23
	4	5.39*	3.38*	29.13*	-6.78*	2.76*
Sub plot (S)	1	45.91*	33.64*	30.96*	62.13*	-13.73*
	2	13.98*	8.35*	-40.45*	-31.65*	7.06
	3	-36.16*	-28.21*	13.76*	15.17*	10.79*
	4	-22.53*	-19.92*	17.11*	-8.25*	-13.74*
	5	-1.22	6.17	-21.35*	-37.43*	9.62*
M × S interaction	1	29.93*	20.81*	-23.95*	38.45*	-45.19*
	2	8.25*	-1.96	4.59*	-10.66*	17.94*
	3	-37.33*	-60.55*	5.72*	60.64*	-4.48*
	4	0.13	-20.96*	48.44*	1.77	25.18*
	5	-4.65*	-2.74*	28.80*	5.73*	-8.10*
	6	7.66*	30.61*	4.83*	41.54*	4.06*
	7	13.73*	-4.31*	-46.91*	-52.56*	-18.59*
	8	-14.65*	2.16*	14.53*	1.35	33.30*
	9	-14.90*	6.22*	25.64*	9.39*	-26.24*
	10	1.96	-6.28*	-43.12*	-29.76*	9.95*
	11	22.83*	32.55*	58.56*	34.98*	-5.60*
	12	4.35*	4.82*	-76.99*	-3.60*	33.40*
	13	-40.15*	-32.28*	-32.48*	-39.52*	-31.10*
	14	-18.04*	-21.22*	-38.03*	-9.73*	16.27*
	15	1.99*	31.92*	-56.61*	-18.79*	-14.05*
	16	34.63*	11.17*	110.85*	47.09*	17.55*
	17	2.61*	25.07*	-77.07*	-15.74*	-17.76*
	18	17.28*	10.89*	79.02*	17.10*	25.21*
	19	-13.82*	-20.38*	47.03*	-22.95*	-44.40*
	20	-1.83	-5.47*	-32.69*	-54.82*	32.65*

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