LATIN SQUARE DESIGN (LS)

Facts about the LS Design

-With the Latin Square design you are able to control variation in two directions.

-Treatments are arranged in rows and columns

-Each row contains every treatment.

-Each column contains every treatment.

-The most common sizes of LS are 5x5 to 8x8

Advantages of the LS Design

1. You can control variation in two directions.

2. Hopefully you increase efficiency as compared to the RCBD.

Disadvantages of the LS Design

1. The number of treatments must equal the number of replicates.

2. The experimental error is likely to increase with the size of the square.

3. Small squares have very few degrees of freedom for experimental error.

4. You can't evaluate interactions between:
   a. Rows and columns
   b. Rows and treatments
   c. Columns and treatments.

Effect of the Size of the Square on Error Degrees of Freedom

SOV	Df	2x2	3x3	4x4	5x5	8x8
Rows	r-1	1	2	3	4	7
Columns	r-1	1	2	3	4	7
Treatments	r-1	1	2	3	4	7
Error	(r-1)(r-2)	0	2	6	12	42
Total	$r^2 - 1$	3	8	15	24	63

Where r = number of rows, columns, and treatments.

-One way to increase the Error df for small squares is to use more than one square in the experiment (i.e. repeated squares).

Example
Two 4x4 Latin squares.

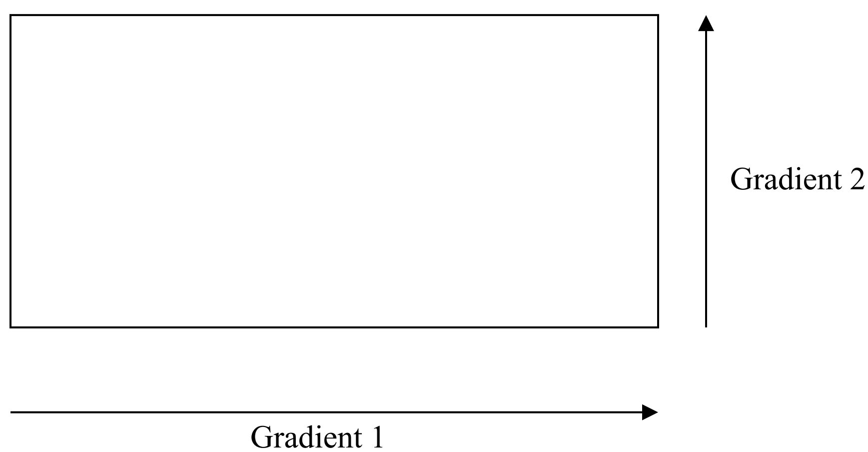
	SOV	Df
	Squares	sq – 1 = 1
*	**Row(square)**	sq(r-1) = 6
*	**Column(square)**	sq(r-1) = 6
	Treatment	r-1 = 3
	Square x Treatment	(sq-1)(r-1) = 3
*	**Error**	sq(r-1)(r-2) = 12
	Total	$sqr^2 – 1 = 31$

*Additive across squares.

Where sq = number of squares.

Examples of Uses of the Latin Square Design

1. Field trials in which the experimental error has two fertility gradients running perpendicular each other or has a unidirectional fertility gradient but also has residual effects from previous trials.



2. Animal science feed trials.

3. Insecticide field trial where the insect migration has a predictable direction that is perpendicular to the dominant fertility gradient of the experimental field.

4. Greenhouse trials in which the experimental pots are arranged in a straight line perpendicular to the glass walls, such that the difference among rows of pots and distace from the glass wall are expected to be the major sources of variability.

A	D	C	B	B	C	A	D	D	A	B	C	C	B	D	A

Randomization Procedure

-Depends on the type of Latin Square you use.

3x3 Latin Square

-Start with the standard square and randomize all columns and all but the first row.

	1	2	3
1	A	B	C
2	B	C	A
3	C	A	B

Standard square

Randomize columns $\longrightarrow$

3	1	2
C	A	B
A	B	C
B	C	A

Randomize all but the first row $\downarrow$

C	A	B
B	C	A
A	B	C

4x4 Latin Square

-Randomly choose a standard square.
-Randomize all columns and all but the first row.

5x5 Latin Square

-Randomly choose a standard square.
-Randomize all columns and rows.

Analysis of a Single Latin Square

Example

Grain yield of three maize hybrids (A, B, and D) and a check (C).

Row	Column 1	Column 2	Column 3	Column 4	Row $\left(\sum R\right)$
1	1.640 (B)	1.210 (D)	1.425 (C)	1.345 (A)	5.620
2	1.475 (C)	1.185 (A)	1.400 (D)	1.290 (B)	5.350
3	1.670 (A)	0.710 (C)	1.665 (B)	1.180 (D)	5.225
4	1.565 (D)	1.290 (B)	1.655 (A)	0.660 (C)	5.170
Column total $\left(\sum C\right)$	6.350	4.395	6.145	4.475	21.365

Step 1. Calculate treatment totals.

Treatment	Total
A	5.855
B	5.885
C	4.270
D	5.355

Step 2. Compute the Correction Factor (CF).

$$CF = \frac{Y_{..}^2}{r^2}$$

$$= \frac{21.365^2}{4^2}$$

$$= 28.53$$

Step 3. Calculate the Total SS

$$TotalSS = \sum Y_{ij}^2 - CF$$

$$= (1.64^2 + 1.210^2 + 1.425^2 + ... + 0.66^2) - CF$$

$$= 1.4139$$

Step 4. Calculate the Row SS

$$RowSS = \frac{\sum Row^2}{r} - CF$$

$$= \frac{(5.62^2 + 5.35^2 + 5.225^2 + 5.17^2)}{4} - CF$$

$$= 0.0302$$

Step 5. Calculate the Column SS.

$$Col.SS = \frac{\sum Col^2}{r} - CF$$

$$= \frac{(6.35^2 + 4.395^2 + 6.145^2 + 4.475^2)}{4} - CF$$

$$= 0.8273$$

Step 6. Calculate the Treatment SS

$$TrtSS = \frac{\sum Y_{i.}^2}{r} - CF$$

$$= \frac{(5.855^2 + 5.885^2 + 4.270^2 + 5.355^2)}{4} - CF$$

$$= 0.4268$$

Step 7. Calculate the Error SS

Error SS = Total SS – Row SS – Column SS – Trt SS

= 0.1296

Step 8. Complete the ANOVA table

SOV	Df	SS	MS	F
Row	r-1 = 3	0.030		
Column	r-1 = 3	0.827		
Trt	r-1 = 3	0.427	0.142	Trt MS/Error MS = 6.60*
Error	(r-1)(r-2) = 6	0.129	0.0215	
Total	r^2-1 = 15	1.414		

Step 9. Calculate the LSD.

$$LSD = t_{\alpha/2}\sqrt{\frac{2ErrorMS}{r}}$$

$$= 2.447\sqrt{\frac{2(.0215)}{4}}$$

$$= 0.254$$

Linear Model

$$Y_{ij(t)} = \mu + \beta_i + \kappa_j + \tau_t + \varepsilon_{ij(t)}$$

where: μ = the experiment mean.
β_i = the row effect,
κ_j = the column effect,
τ_t = the treatment effect, and
$\varepsilon_{ij(t)}$ = the random error.

Latin Square - Combined Analysis Across Squares

-The squares can be at the same location, or three different locations, or three different years, etc.

Example

Three 3x3 Latin squares

Square 1

				$\sum R$	
	41 (B)	25 (C)	15 (A)	81	SS Row_1 = 126.89
	20 (A)	32 (B)	24 (C)	76	SS $Column_1$ = 89.55
	22 (C)	12 (A)	21 (B)	55	SS $Treatment_1$ = 368.22
$\sum C$	83	69	60	212	SS $Error_1$ = 21.56

Square 2

				$\sum R$	
	27 (C)	28 (B)	3 (A)	58	SS Row_2 = 130.89
	4 (A)	17 (C)	9 (B)	30	SS $Column_2$ = 110.22
	22 (B)	4 (A)	17 (C)	43	SS $Treatment_2$ = 534.22
$\sum C$	53	49	29	131	SS $Error_2$ = 14.89

Square 3

				$\sum R$	
	43 (B)	27 (C)	17 (A)	87	SS Row_3 = 126.89
	22 (A)	34 (B)	26 (C)	82	SS $Column_3$ = 89.55
	24 (C)	14 (A)	23 (B)	61	SS $Treatment_3$ = 368.22
$\sum C$	89	75	66	230	SS $Error_3$ = 21.56

Step 1. Test the homogeneity of the Error MS from each square using Bartlett's Chi-square test.

Step 1.1 Calculate the Error SS for each square.

Step 1.2 Calculate the Error MS for each square.

Step 1.3 Calculate the Log of each Error MS

Square	Error SS	Error df	Error MS	Log Error MS
1	21.56	2	10.78	1.0326
2	14.89	2	7.45	0.8722
3	21.56	2	10.78	1.0326
			$\sum s_i^2 = 29.01$	$\sum \log s_i^2 = 2.9374$

Step 1.4 Calculate the Pooled Error MS (s_p^2)

$$s_p^2 = \frac{\sum s_i^2}{\#sq} = \frac{29.01}{3} = 9.67$$

Step 1.5 Calculate Bartlett's χ^2

$$\chi^2 = \frac{2.3026(Errordf)\left[\left(sq \log s_p^2\right) - \sum \log s_i^2\right]}{1 + \left[\frac{(sq+1)}{3 * sq * Errordf}\right]}$$

Where Error df = df for one square.

$$\chi^2 = \frac{2.3026(2)\left[\left(3 \log 9.67\right) - 2.9374\right]}{1 + \left[\frac{(3+1)}{3 * 3 * 2}\right]}$$

$$= \frac{0.0869}{1.2222}$$

$$= 0.0711$$

Step 1.6 Look up the Table χ^2-value at the 99.5% level of confidence and df = #sq-1.

$$\chi^2_{0.005;2df} = 10.6$$

Step 1.7 Make conclusions

Since $\chi^2{}_{calc} < \chi^2_{table}$ we fail to reject $H_o: \sigma_1^2 = \sigma_2^2 = \sigma_3^2$ at the 99.5% level of confidence; thus, we can do the combined analysis across squares

Step 2. Calculate Treatment Totals for each square.

Treatment	Square 1	Square 2	Square 3	$\sum TRT$
A	47	11	53	111
B	94	59	100	253
C	71	61	77	209
$\sum Square$	212	131	230	573

Step 3. Calculate the Correction Factor (CF).

$$CF = \frac{Y_{...}^2}{sq * r^2}$$

$$= \frac{573^2}{3 * 3^2}$$

$$= 12{,}160.333$$

Step 4. Calculate the Total SS

$$TotalSS = \left(41^2 + 25^2 + 15^2 + ... + 23^2\right) - CF$$
$$= 2{,}620.67$$

Step 5. Calculate the Square SS

$$SquareSS = \frac{\sum Sq^2}{r^2} - CF$$

$$= \frac{\left(212^2 + 131^2 + 230^2\right)}{3^2} - CF$$

$$= 618.0$$

Step 6. Calculate the Row(Square) SS (Additive across squares)

Row(Square) SS = Row_1 SS + Row_2 SS + Row_3 SS

= 384.67

Step 7. Calculate the Column(Square) SS (Additive across squares)

Column(Square) SS = Column_1 SS + Column_2 SS + Column_3 SS

= 289.32

Step 8. Calculate the Treatment SS

$$TrtSS = \frac{\sum TRT_i^2}{sq * r} - CF$$

$$= \frac{(111^2 + 253^2 + 209^2)}{3*3} - CF$$

$$= 1{,}174.22$$

Step 9. Calculate the Square X Treatment SS.

$$Sq * TrtSS = \frac{\sum (SqXTrt)^2}{r} - CF - SquareSS - TrtSS$$

$$= \frac{(47^2 + 94^2 + 71^2 + ... + 77^2)}{3} - CF - SquareSS - TrtSS$$

$$= 96.45$$

Step 10. Calculate Error SS (Additive across squares)

Error SS = Error_1 SS + Error_2 SS + Error_3 SS

Error SS = 58.01

Step 11. Complete the ANOVA Table.

SOV	Df	SS	MS	F (Squares and Trt are Fixed effects)
Square	Sq-1 = 2	618.0		Non-valid F-test
Row(Sq)	Sq(r-1) = 6	384.67		Non-valid F-test
Column(Sq)	Sq(r-1) = 6	289.32		Non-valid F-test
Trt	r-1 = 2	1174.22	587.11	Trt MS/Error MS = 60.73^{**}
Sq X Trt	(sq-1)(r-1) = 4	96.45	24.11	Sq X Trt MS/Error MS = 2.49^{ns}
Error	Sq(r-1)(r-2) = 6	58.01	9.67	
Total	Sqr^2-1 = 26	2620.67		

Conclusions:

1. The non-significant Square X Treatment interaction indicates that treatments responded similarly in all squares.

Table 1. Mean for the square x treatment interaction.

	Treatment		
Square	A	B	C
1	15.7	31.3	23.7
2	3.7	19.7	20.3
3	17.7	33.3	25.7
LSD(0.05)	-------------------ns--------------------		

2. The significant F-test for Treatment indicates that averaged across all squares, there were differences between treatments.
3.

Table 2. Mean for the treatment main effect averaged Across squares.

Treatment	Mean
A	12.3
B	28.1
C	23.2
LSD(0.05)	3.6

Step 12. Calculate LSD's

Square X Trt: Normally, you would not calculate this LSD because the F-test for the interaction was non-significant. However, if it would have been significant, you would have calculated the LSD using the following method:

$$LSD_{SqXTrt} = t_{a/2;errordf}\sqrt{\frac{2ErrorMS}{r}}$$

$$= 2.447\sqrt{\frac{2(9.67)}{3}}$$

$$= 6.2$$

This LSD would be used for comparisons only in Table 1.

Treatment:

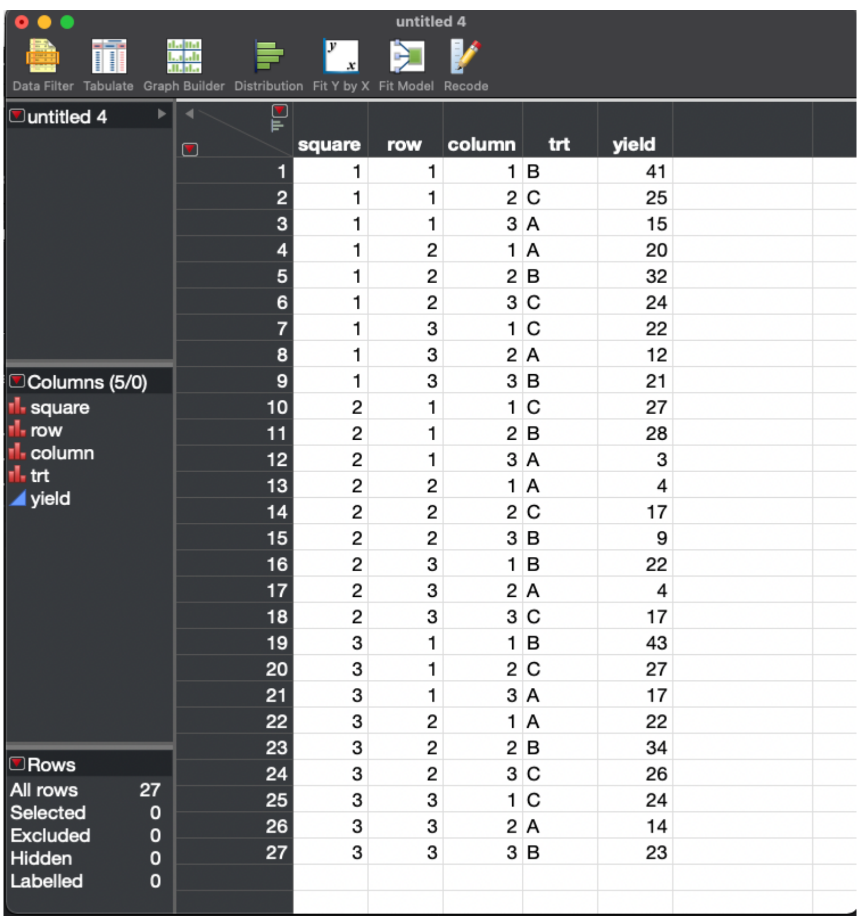
$$LSD_{Trt} = t_{a/2;errordf}\sqrt{\frac{2ErrorMS}{sq*r}}$$

$$= 2.447\sqrt{\frac{2(9.67)}{3*3}}$$
$$= 3.6$$

This LSD would only be used for comparisons in Table 2.

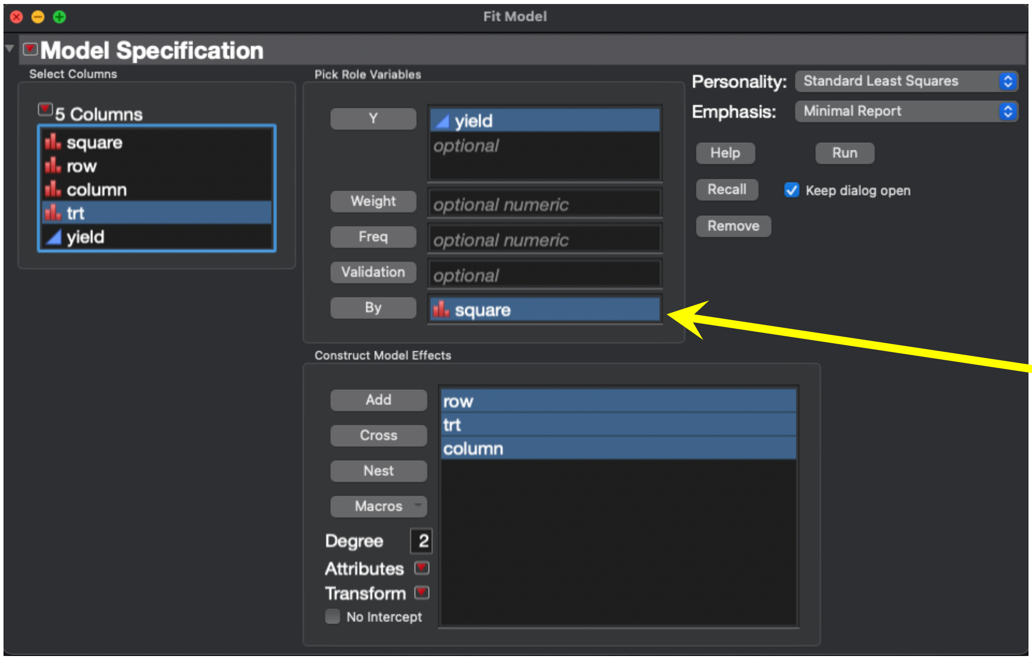
JMP Analysis for the Latin Square (individual squares and combined across squares).

Data



	square	row	column	trt	yield
1	1	1	1	B	41
2	1	1	2	C	25
3	1	1	3	A	15
4	1	2	1	A	20
5	1	2	2	B	32
6	1	2	3	C	24
7	1	3	1	C	22
8	1	3	2	A	12
9	1	3	3	B	21
10	2	1	1	C	27
11	2	1	2	B	28
12	2	1	3	A	3
13	2	2	1	A	4
14	2	2	2	C	17
15	2	2	3	B	9
16	2	3	1	B	22
17	2	3	2	A	4
18	2	3	3	C	17
19	3	1	1	B	43
20	3	1	2	C	27
21	3	1	3	A	17
22	3	2	1	A	22
23	3	2	2	B	34
24	3	2	3	C	26
25	3	3	1	C	24
26	3	3	2	A	14
27	3	3	3	B	23

JMP Model Specification for the analysis of individual squares



The *By* command is allowing us to analyze each of the squares separately

Output from the Analysis of Three Individual Latin Square Experiments

If our interest is doing the combined analysis across squares, then the only items of interest to us in these single experiment analyses are the *Error MS* values from each square (highlighted in yellow)

Output from the Analyses of Individual Squares

Response yield square=1
Summary of Fit

RSquare	0.964443
RSquare Adj	0.857771
Root Mean Square Error	3.282953
Mean of Response	23.55556
Observations (or Sum Wgts)	9

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	584.66667	97.4444	9.0412
Error	2	21.55556	10.7778	**Prob > F**
C. Total	8	606.22222		0.1029

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
row	2	2	126.88889	5.8866	0.1452
trt	2	2	368.22222	17.0825	0.0553
column	2	2	89.55556	4.1546	0.1940

Response yield square=2
Summary of Fit

RSquare	0.981159
RSquare Adj	0.924634
Root Mean Square Error	2.728451
Mean of Response	14.55556
Observations (or Sum Wgts)	9

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	775.33333	129.222	17.3582
Error	2	14.88889	7.444	**Prob > F**
C. Total	8	790.22222		0.0555

Output from the Analysis of Three Individual Latin Square Experiments

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
row	2	2	130.88889	8.7910	0.1021
trt	2	2	534.22222	35.8806	0.0271*
column	2	2	110.22222	7.4030	0.1190

Response yield square=3
Summary of Fit

RSquare	0.964443
RSquare Adj	0.857771
Root Mean Square Error	3.282953
Mean of Response	25.55556
Observations (or Sum Wgts)	9

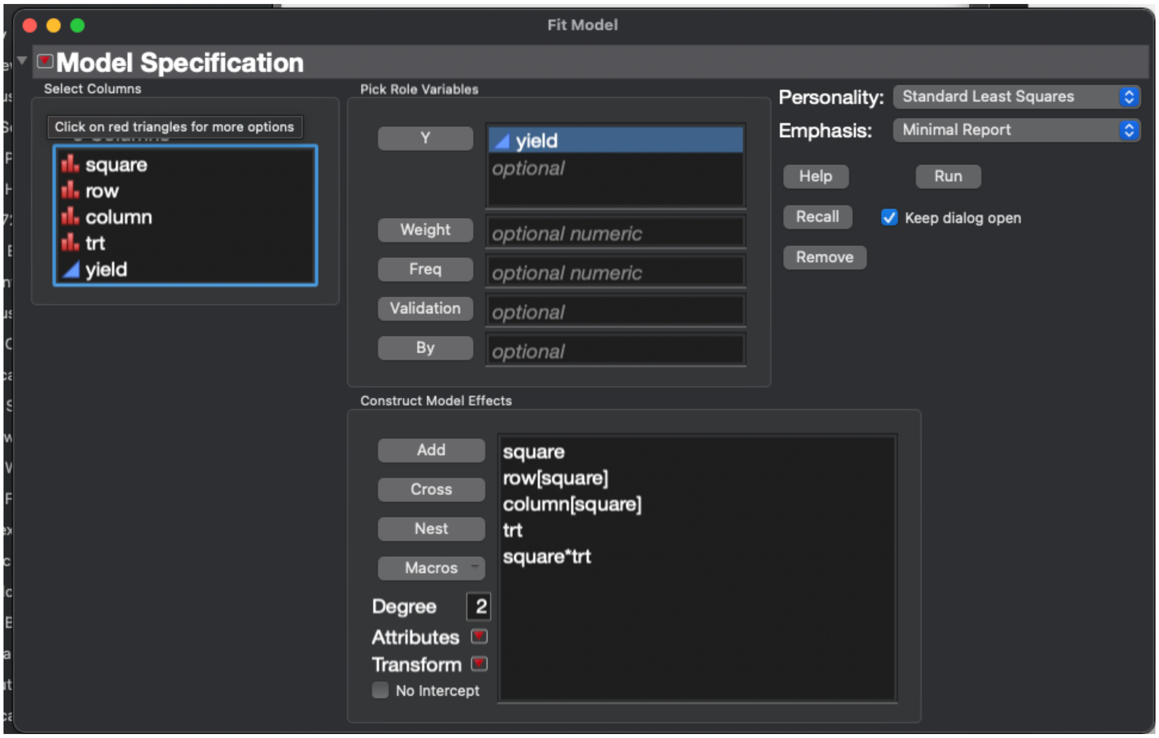
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	584.66667	97.4444	9.0412
Error	2	21.55556	10.7778	**Prob > F**
C. Total	8	606.22222		0.1029

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
row	2	2	126.88889	5.8866	0.1452
trt	2	2	368.22222	17.0825	0.0553
column	2	2	89.55556	4.1546	0.1940

JMP Model Specification for the combined analysis across squares (assuming square and treatments are both fixed effects



Output from the Combined Analysis Across Three Latin Square Experiments

Output from the Combined Analysis Across Squares

Response yield
Summary of Fit

RSquare	0.977868
RSquare Adj	0.904096
Root Mean Square Error	3.109126
Mean of Response	21.22222
Observations (or Sum Wgts)	27

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	20	2562.6667	128.133	13.2552
Error	6	58.0000	9.667	**Prob > F**
C. Total	26	2620.6667		0.0021*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
square	2	2	618.0000	31.9655	0.0006*
row[square]	6	6	384.6667	6.6322	0.0183*
column[square]	6	6	289.3333	4.9885	0.0357*
trt	2	2	1174.2222	60.7356	0.0001*
square*trt	4	4	96.4444	2.4943	0.1522

Effect Details
trt
Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
A	12.333333	1.0363755	12.3333
B	28.111111	1.0363755	28.1111
C	23.222222	1.0363755	23.2222

The *F*-test for trt is significant while the one for the square X trt interaction is not. Thus, mean separation will only be done on the means for trt.

Output from the Combined Analysis Across Three Latin Square Experiments

LSMeans Differences Student's t
α=0.050 t=2.44691
LSMean[i] By LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	A	B	C
A	0 0 0 0	-15.778 1.46566 -19.364 -12.191	-10.889 1.46566 -14.475 -7.3026
B	15.7778 1.46566 12.1914 19.3641	0 0 0 0	4.88889 1.46566 1.30256 8.47522
C	10.8889 1.46566 7.30256 14.4752	-4.8889 1.46566 -8.4752 -1.3026	0 0 0 0

Level				Least Sq Mean
B	A			28.111111
C		B		23.222222
A			C	12.333333

Levels not connected by same letter are significantly different.

square*trt
Least Squares Means Table

Level	Least Sq Mean	Std Error
1,A	15.666667	1.7950549
1,B	31.333333	1.7950549
1,C	23.666667	1.7950549
2,A	3.666667	1.7950549
2,B	19.666667	1.7950549
2,C	20.333333	1.7950549
3,A	17.666667	1.7950549
3,B	33.333333	1.7950549
3,C	25.666667	1.7950549