AGR5201
ADVANCED STATISTICAL METHODS

LATIN SQUARE DESIGN
GRAECO LATIN SQUARE DESIGN

Topic outline

1.0 Latin square design

- Introduction, advantages & disadvantages, usage
- Experimental layout and plot randomization
- ANOVA (manual calculation and table)
- Examples (1, 2 and 3)
- Layout randomization and analysis using R

2.0 Graeco Latin square design

- Introduction
- Example
- Layout randomization using R

1.0 Latin square design

Introduction

- An experimental design when you have two perpendicular sources of variation
- If you can block these two sources of variation (rows x columns) → you can reduce experimental error when compared to the RCBD
- More restrictive than the RCBD
- The total number of plots is the square of the number of treatments
- Each treatment appears once and only once in each row and column

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

1.0 Latin square design

Advantages and disadvantages

- ***Advantage:***
  - Allows the experimenter to control two sources of variation

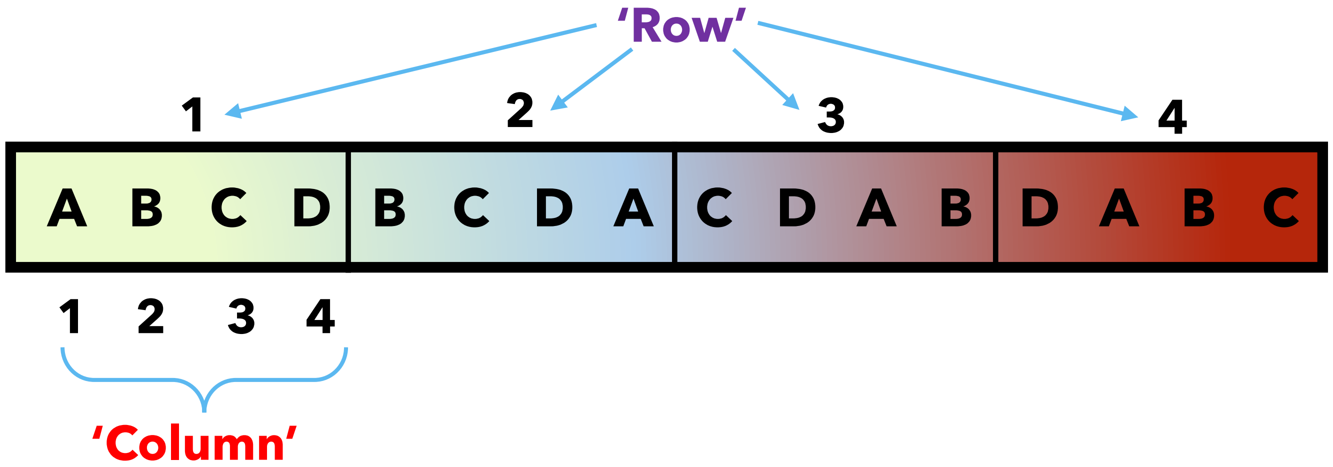
- ***Disadvantages:***
  - The experiment becomes very large if the number of treatments is large
  - The statistical analysis is complicated by missing plots and misassigned treatments
  - Error df is small if there are only a few treatments
    - This limitation can be overcome by repeating a small
    - Latin Square and then combining the experiments:
      - a 3x3 Latin Square repeated 4 times
      - a 4x4 Latin Square repeated 2 times

1.0 Latin square design

The usage of Latin square design

- When two sources of variation must be controlled:
  - Slope and fertility
  - Furrow irrigation and shading

✓ Practically speaking, use only when you have more than four but fewer than ten treatments
  - a minimum of 12 df for error



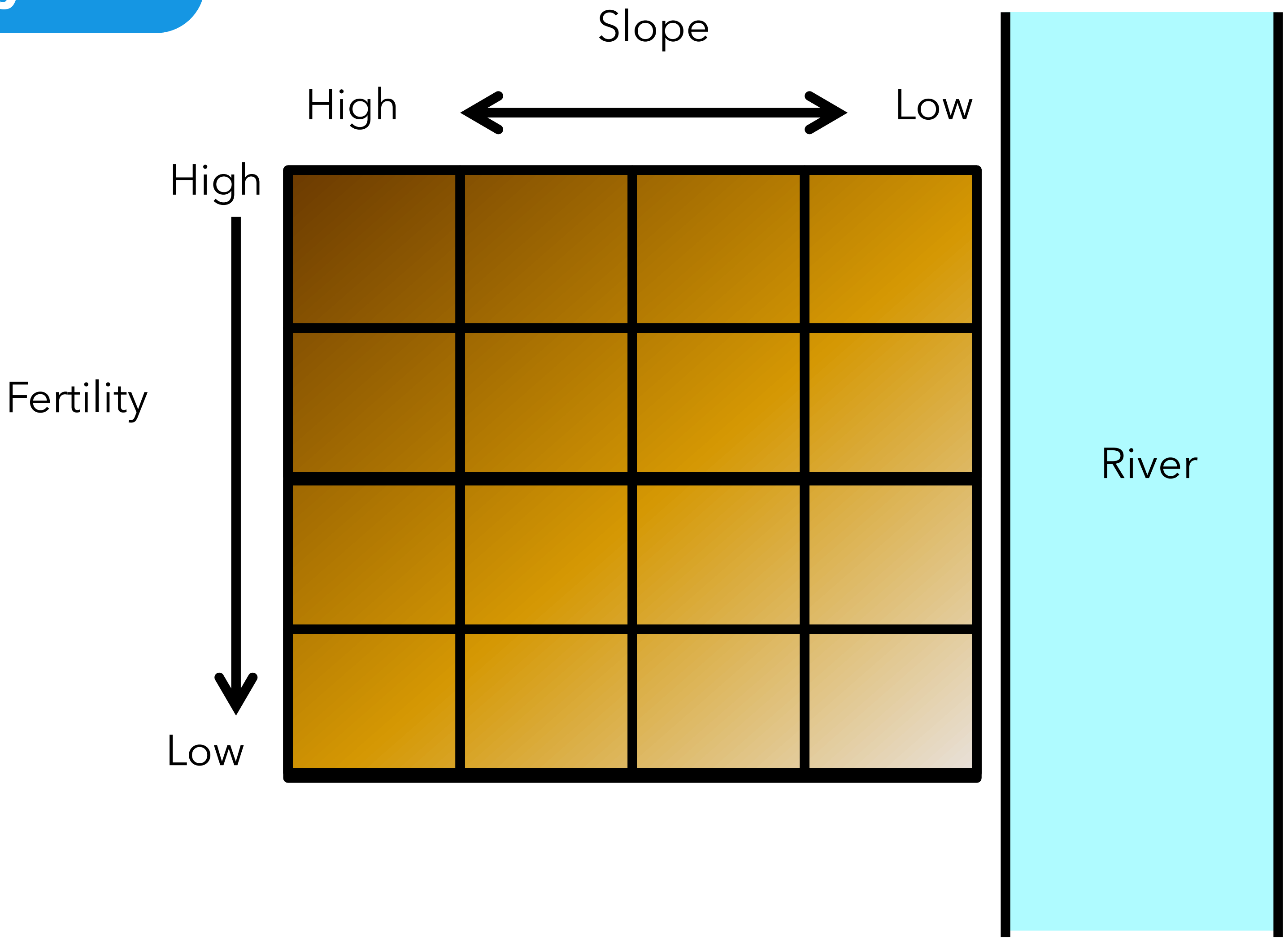
	Col 1	Col 2	Col 3	Col 4
Row 1	A	B	C	D
Row 2	B	C	D	A
Row 3	C	D	A	B
Row 4	D	A	B	C

1.0 Latin square design

Experimental design layout

- Two sources of variation in the field

1. Slope (horizontal)
2. Fertility (vertical)



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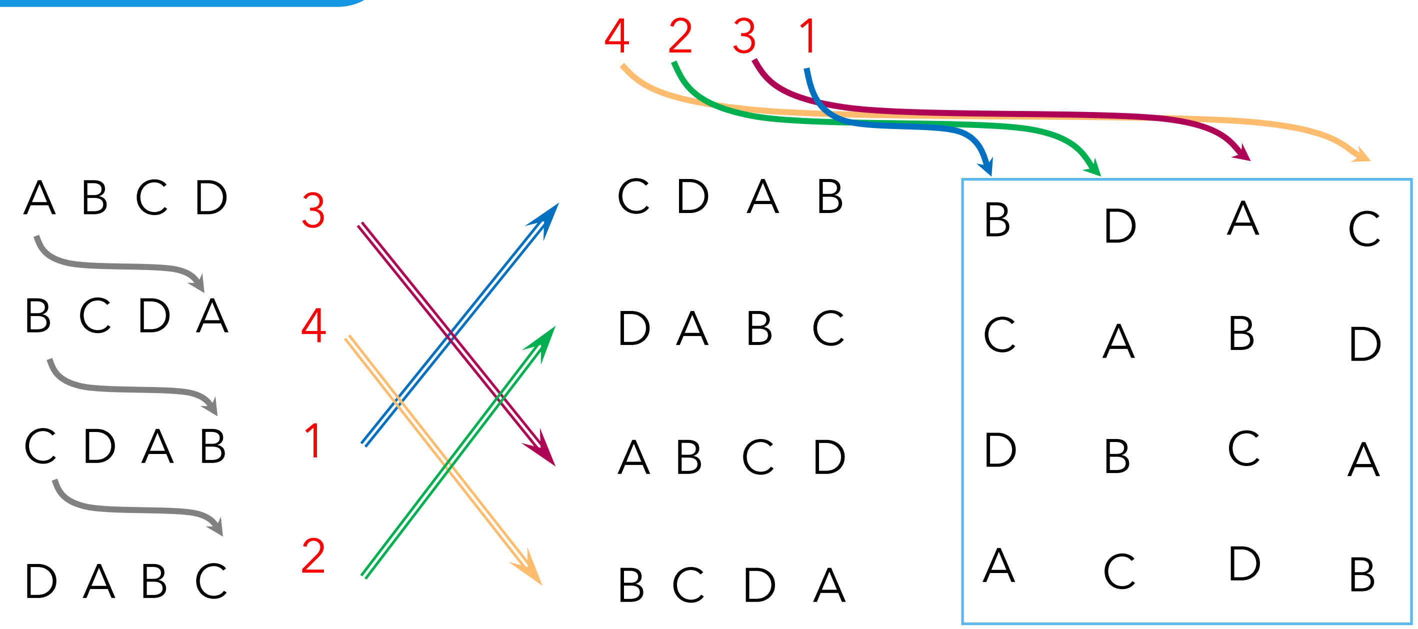
Plot randomization



1. On the first row, write the treatment name
2. Subsequent rows - shift letters one position
3. Assign random numbers to the rows
4. Sort the row in ascending order
5. Assign random numbers to the column
6. Sort the column in ascending order

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Linear additive model

$$Y_{ij} = \mu + \beta_i + \gamma_j + \tau k + \varepsilon_{ij}$$

$\mu =$	mean effect
$\beta_i =$	i^{th} block effect
$\gamma_j =$	j^{th} column effect
$\tau_k =$	k^{th} treatment effect
$\varepsilon_{ij} =$	random error

- Each treatment occurs once in each block and once in each column
  - $r = c = t$
  - $N = t^2$

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Analysis of variance (Manual calculation)

- Set up a two-way table and compute the row and column means and deviations
- Compute a table of treatment means and deviations
- Set up an ANOVA table divided into sources of variation
    - Rows
    - Columns
    - Treatments
    - Error
- Significance tests
    - F_T tests difference among treatment means
    - F_R and F_C test if row and column groupings are effective

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ANOVA table

Sources of variation	Degree of freedom	Sum of square	Mean square	F value (F ratio)	P-value (Pr (F > f))
Row	t-1	SSR = $t\sum_i \left(\bar{Y}_i - \bar{\bar{Y}}\right)^2$	$MS\ Row = \dfrac{SS\ Row}{df\ Row}$	$F = \dfrac{MS\ Row}{MS\ Error}$	The Pr (F) for Row
Column	t-1	SSC = $t\sum_j \left(\bar{Y}_j - \bar{\bar{Y}}\right)^2$	$MS\ Col = \dfrac{SS\ Col}{df\ Col}$	$F = \dfrac{MS\ Col}{MS\ Error}$	The Pr (F) for Col
Treatment	t-1	SST = $t\sum_k \left(\bar{Y}_k - \bar{\bar{Y}}\right)^2$	$MS\ Trt = \dfrac{SS\ Trt}{df\ Trt}$	$F = \dfrac{MS\ Trt}{MS\ Error}$	The Pr (F) for Trt
Error	(t-1)*(t-2)	SSE = SSTotal – SSE – SSC -SST	$MSError = \dfrac{SS\ Error}{df\ Error}$		
Total	t^2-1	SSTotal = $\sum_i \sum_j \left(Y_{ij} - \bar{\bar{Y}}\right)^2$			

Notes:

r = c = t, thus df for r, c and t = t-1

Total observation, N = t^2

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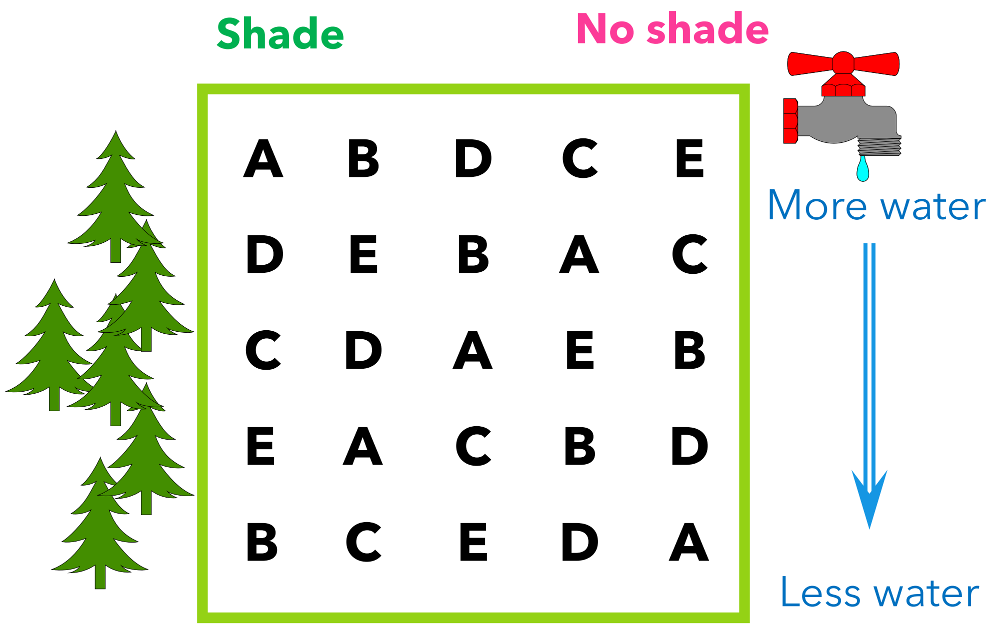
Example 1 | Shade and water

To determine the effect of four different sources of seed inoculum, A, B, C, and D, and a control, E, on the dry matter yield of irrigated alfalfa. The plots were furrow irrigated and there was a line of trees that might form a shading gradient.

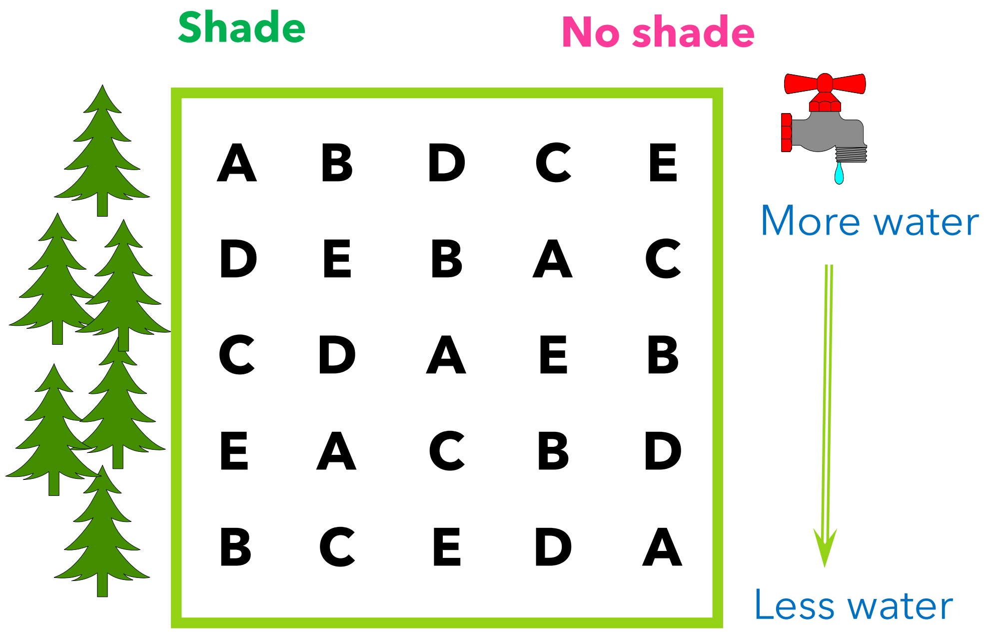


Questions:

1. Are the blocks for shading represented by the row or columns?
2. Is the gradient due to irrigation accounted for by rows or columns?

1.0 Latin square design

Example 1| Data collection



A	B	D	C	E
34	34	30	33	24
D	E	B	A	C
37	29	34	35	33
C	D	A	E	B
36	36	37	27	35
E	A	C	B	D
33	37	37	38	34
B	C	E	D	A
35	39	33	37	36

1.0 Latin square design

Example 1 | ANOVA table

Sources of variation	df	SS	MS	F value	F_{table} (5%)
Rows	4	87	22	7.13^{**}	3.23
Columns	4	17	4.1	1.35^{ns}	3.23
Treatments	4	156	39	12.71^{**}	3.23
Error	12	37	3.1		
Total	24	297			

Row = irrigation
Column = shading

** Significant at p< 0.01
ns Not significant at p>0.05

$F_{(0.05, 4,12)} = 3.23$

1.0 Latin square design

Report of statistical analysis

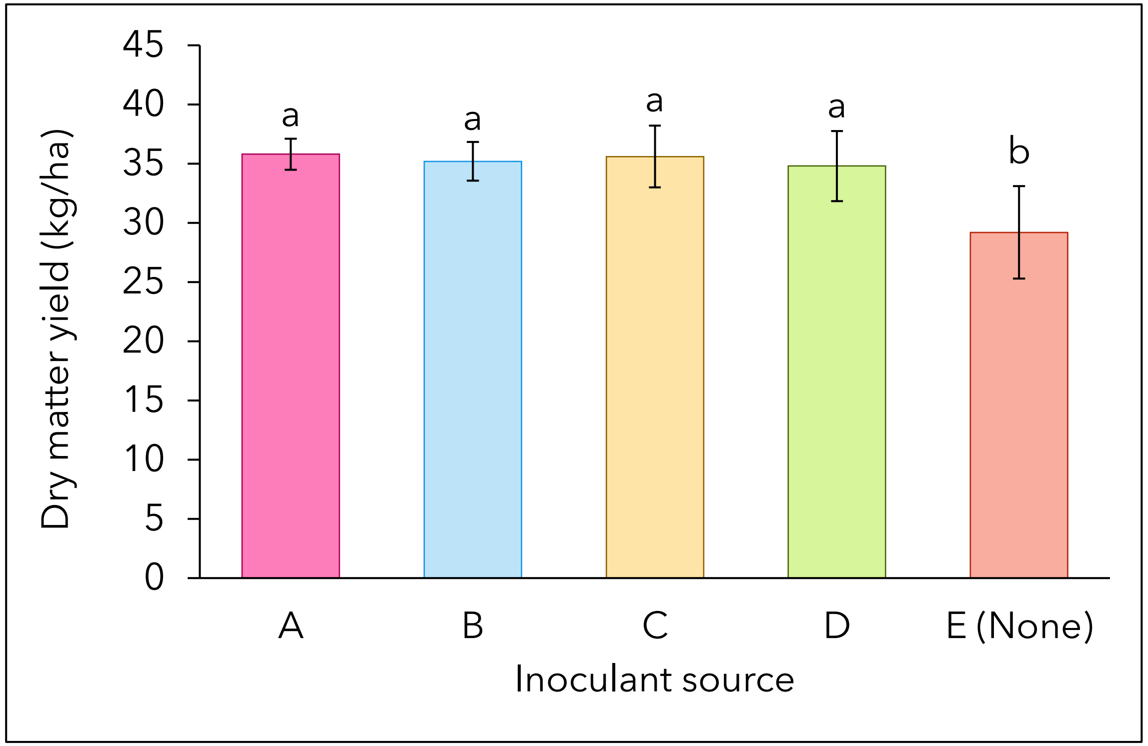


Figure 1. The effects of different source of seed inoculum on different dry matter yield of irrigated alfalfa. Means with the same letter are not significantly different at P>0.05 using LSD.

Interpretation

1. Differences among treatment means were highly significant
2. No difference among inocula. However, inoculation, regardless of source produced more dry matter than did no inoculation
3. Blocking by irrigation effect was useful in reducing experimental error
4. Distance from shade did not appear to have a significant effect

1.0 Latin square design

Example 2 | Driver and car

- A courier company is interested in deciding between five brands (D, P, F, C and R) of car for its next purchase of fleet cars.
- The brands are all comparable in purchase price.
- The company wants to carry out a study that will enable them to compare the brands with respect to operating costs.
- For this purpose, they select five drivers (Rows).
- In addition, the study will be carried out over a five-week period (Columns = weeks).

1.0 Latin square design

Example 2 | Data

- Each week a driver is assigned to a car using randomization and a Latin Square Design.
- The average cost per mile is recorded at the end of each week and is tabulated below:

		Week				
		1	2	3	4	5
Drivers	1	5.83 D	6.22 P	7.67 F	9.43 C	6.57 R
	2	4.80 P	7.56 D	10.34 C	5.82 R	9.86 F
	3	7.43 F	11.29 C	7.01 R	10.48 D	9.27 P
	4	6.60 R	9.54 F	11.11 D	10.84 P	15.05 C
	5	11.24 C	6.34 R	11.30 P	12.58 F	16.04 D

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Example 2 | ANOVA table

Source	df	S.S.	M.S.	F value	F table
Week	4	51.18	12.80	16.06	3.26
Driver	4	69.45	17.36	21.79	3.26
Car	4	70.90	17.73	22.25	3.26
Error	12	9.56	0.80		
Total	24	201.09			

1.0 Latin square design

Example 3 | Cow and diet

- A researcher wants to determine the digestibility of four feeds: D1, D2, D3, D4
- However, he only has 4 cattles. How can he do a replicated trial?
- Replicate in time: 4 periods – P1, P2, P3, P4 – will there be an error due to variations in time?
- Also, will there be an error due to variations among animals?
- He only wants to know effects of the diet.

1.1 Latin square design using R

Layout randomization of Latin square:

R codes:

```
trt <- c("A", "B", "C", "D")
outdesign <- design.lsd(trt, seed=543, serie=2)
print(outdesign$sketch)
```

- Output:

```
     [,1] [,2] [,3] [,4]
[1,] "B"  "C"  "A"  "D" 
[2,] "D"  "A"  "C"  "B" 
[3,] "C"  "D"  "B"  "A" 
[4,] "A"  "B"  "D"  "C" 
```

1.1 Latin square design using R

ANOVA of Latin square R codes

- Read the data into R:

```
ls<- read.csv ("alfalfa.csv", sep = "," , header = T)
```

- To fit a model for latin square:

```
myfit <- lm(yield ~ row + col + inoc, data=ls)
```

- ANOVA output:

```
anova(myfit)
```

ANOVA R output

```
Analysis of Variance Table

Response: yield
          Df Sum Sq Mean Sq F value    Pr(>F)    
row        4  81.84  20.460  6.8352 0.0041640 ** 
col        4  22.64   5.660  1.8909 0.1768100    
inoc       4 154.24  38.560 12.8820 0.0002665 ***
Residuals 12  35.92   2.993                      
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

2.0 Graeco Latin square design

Introduction

Graeco-Latin Square Design

A variation on the Latin square theme that uses a triple-grouping, or control of variability due to three factors.

E1	C4	B3	D5	A2
D4	B2	A1	C3	E5
B5	E3	D2	A4	C1
C2	A5	E4	B1	D3
A3	D1	C5	E2	B4

every isolates
and varieties
assigned to every
row and column

Letters refer to the five treatments.
Numbers refer to the five levels of the third blocking factor.

Note that the design is balanced and symmetrical for all four factors, just as for the Latin square.

2.0 Graeco Latin square design

Example:

<u>Hypothetical Example</u>:
Consider an experiment designed to test disease incidence on five varieties of a crop species. You want to control any spatial variability that might exist on the greenhouse bench as well as use five isolates of the pathogen to broaden your inferences. Using the typical factorial treatment design, you might conclude that you need 25 treatments (5 varieties x 5 isolates) and then still need to replicate each treatment. Not so. Here's the ANOVA, using the GLS design.

Source of variation	df	F-test
Rows (North-South)	r-1	MS_R/MS_e
Columns (East-West)	r-1	MS_C/MS_e
Isolates (numbers)	r-1	MS_I/MS_e
Varieties (letters)	r-1	MS_V/MS_e
Error	(r-1)(r-3)	
Total	r^2-1	

What is conspicuously missing from this ANOVA table/model?

We cannot test any interaction, similar with the latin sq

The interaction between two factors cannot be tested

2.1 Graeco Latin square using R

Layout randomization of Graeco Latin square

- R codes:

```
trt1 <- c("A", "B", "C", "D")
trt2 <- 1:4
outdesign <- design.graeco(trt1,trt2, seed=543, serie=2)
print(outdesign$sketch)
```

- Output:

```
     [,1]  [,2]  [,3]  [,4]
[1,] "D 2" "B 4" "A 3" "C 1"
[2,] "B 3" "D 1" "C 2" "A 4"
[3,] "A 1" "C 3" "D 4" "B 2"
[4,] "C 4" "A 2" "B 1" "D 3"
```