Common Experimental Designs in Agronomic Research and their Analaysis

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Conservation Agriculture Based Innovation Systems Course

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Introduction

- Statistics starts with a problem, continues with the collection of data, proceeds with the data analysis and finishes with conclusions
- It is a common mistake of inexperienced statisticians to plunge into a complex analysis without paying attention to what the objectives are or even whether the data are appropriate for the proposed analysis
- The formulation of a problem is often more essential than its solution itself, which may be merely a matter of mathematical or experimental skills, Albert Einstein.

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Formulation of the problem

- Understand the physical background
- Understand the Objective
- Make sure what the client wants
- Put the problem in statistical terms
- This is a challenge step and where irreparable errors are something made. Once that the prblem is translated into the statistics languaje, the solution is often routine.

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Data collection

- It is important to understand how the data was collected
- 1. Are the data observational or experimental
- 2. Are there missing values
- 3. How the data was coded
- 4. What are the units of measurement
- 5. Beware of data entry errors.
- The last problem is all too common, almost a certainly in any real dataset of at least moderate size. Perform some data sanity check

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Initial data analysis

- Numerical Summaries
  - means
  - Standard deviations
  - Standard deviationsfive-number summaries
  - Correlations
- Graphical summaries
  - One variable - Boxplot, histograms, etc.
  - Two variables -scatterplots
  - Many variables - interactive graphics

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- Many things, unfortunately
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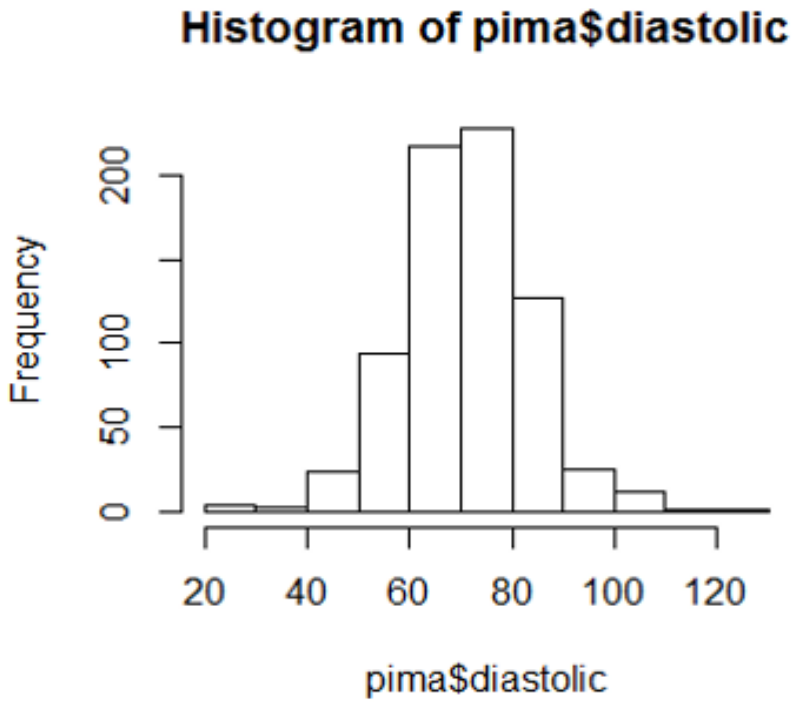
Histogram of pima$diastolic
Frequency
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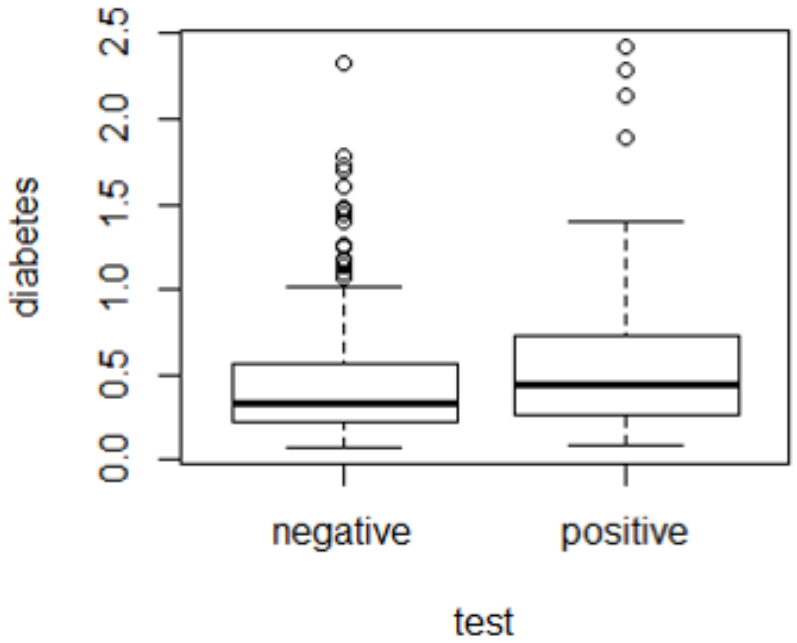
pima$diastolic


diabetes
0.0
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1.0
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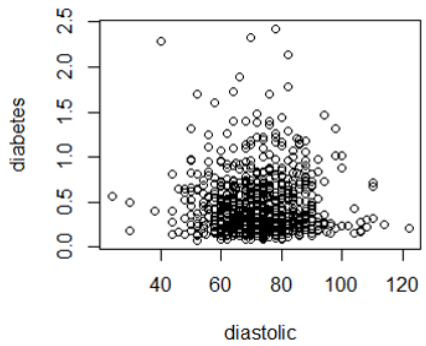
2.0
2.5
negative
positive
test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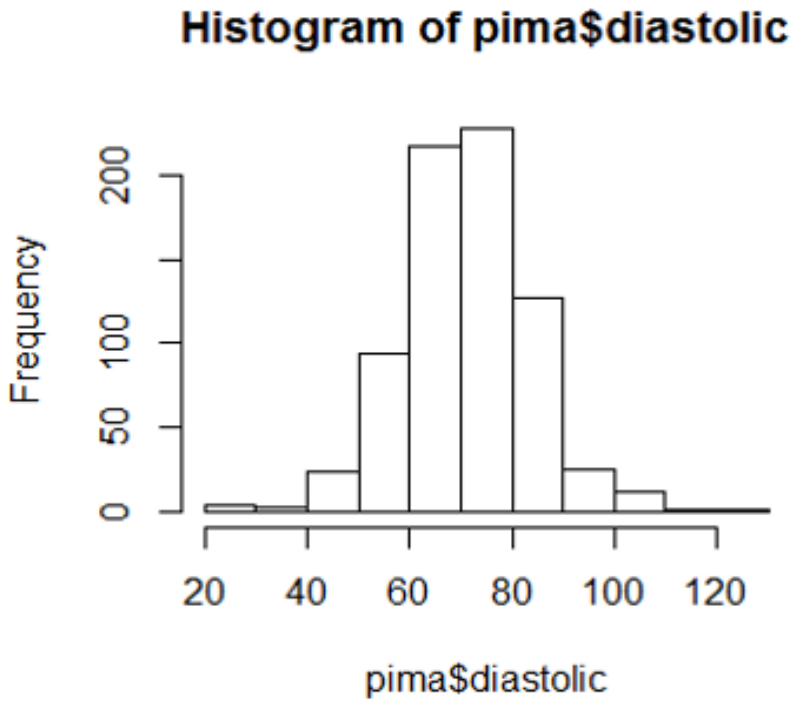
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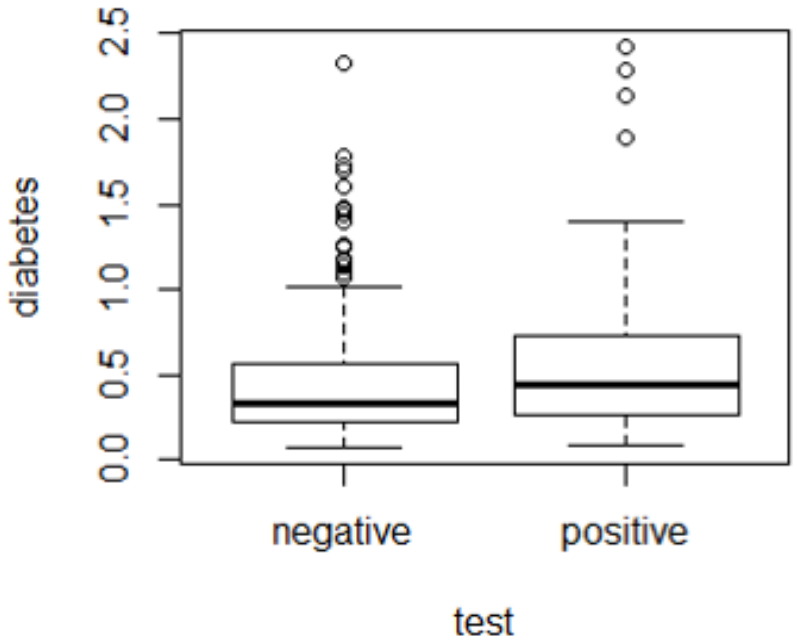
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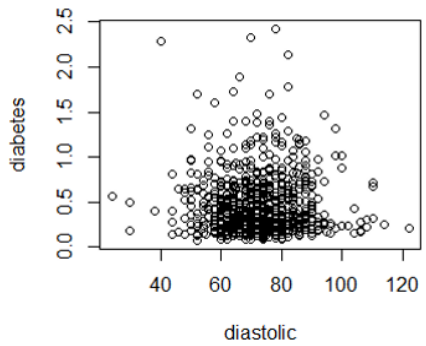
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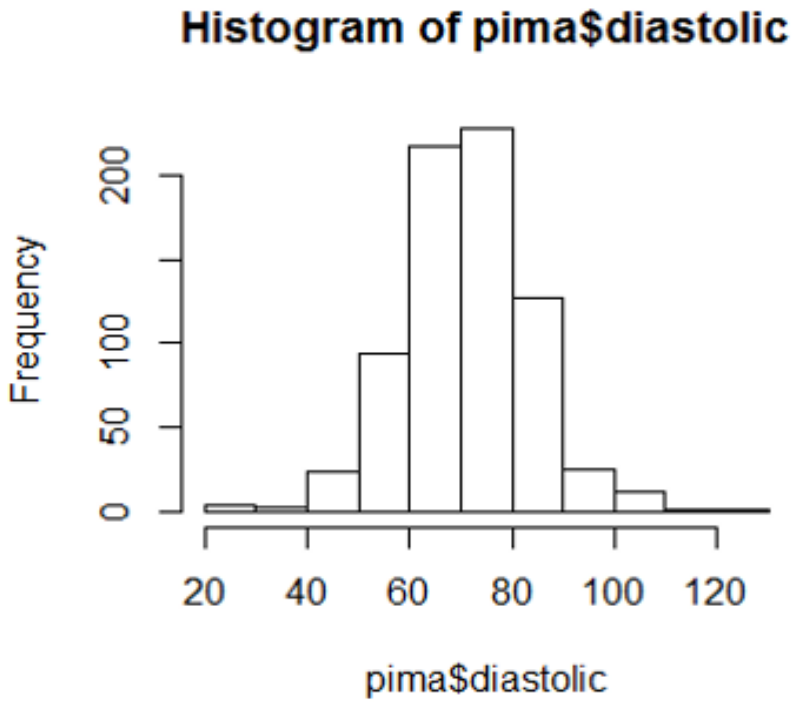
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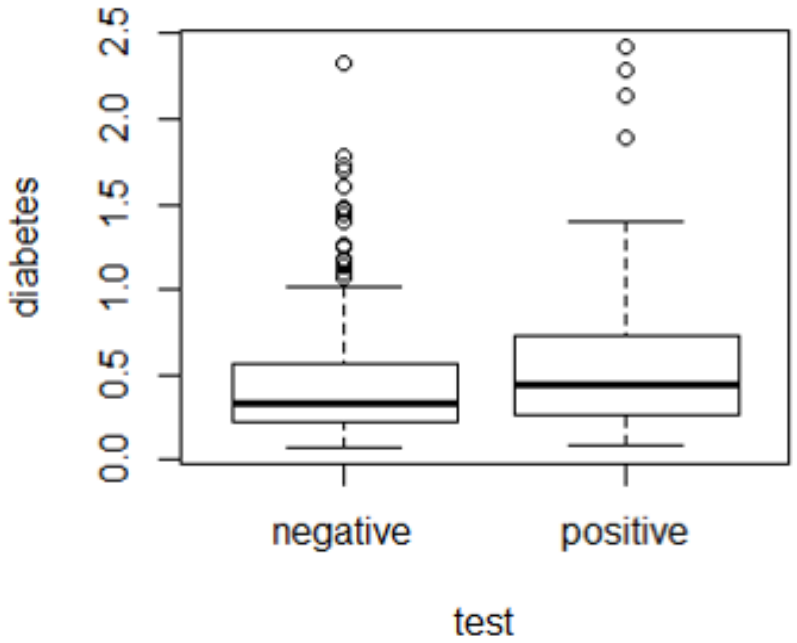
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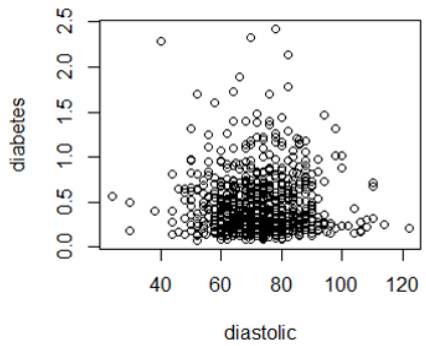
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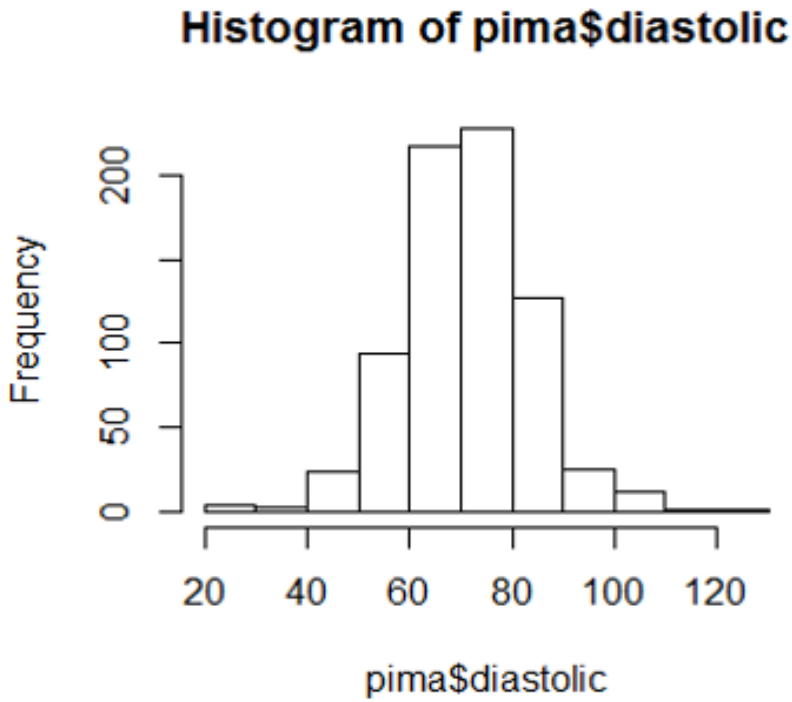
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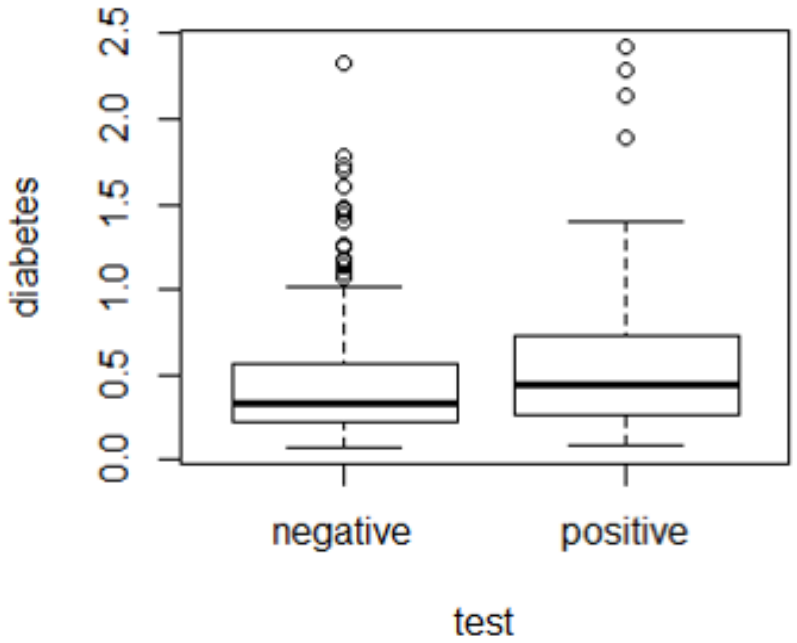
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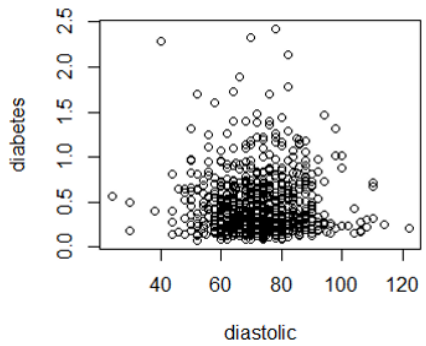
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- In the planning and conduction of field research, we can use different strategies to control the variability as:
  - 1. Selection of homogeneous material and/or environments
  - 2. Grouping (blocking, stratifying) material into homogeneous subgroups (blocks, strata), and
  - 3. Measurement of related variables and use of covariance analysis

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Basic concepts on experimental designs: The three Fisher basic principles

- Replication
- Why?
- It is the only way in which we are able to get an estimate of the experimental error
- How many replications?
- At least two. As higher number is better precision
- Unfortunately, there are a compromise between precision and cost
- Also, the number of replications to use depends of the response variable to be assessed
  - Continuous variables do not need too much replications
  - However for discrete variables (diseases, counts of insects), it is advisable to make more replications

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- Arrangement of experimental units (or experimental material) into similar groups reduces the sources of variation and allows greater precision
- The size, shape and orientation of the blocks affects the precision in the control of environmental noise sources

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- Randomization

➢ **Replication 1**

B10										
B2	20	19	18	17	16	15	14	13	12	11
B1	1	2	3	4	5	6	7	8	9	10

Replication 2

	16					19				B20
			6					14		
	1					15				
					2			9		
		5								
11						10				
		13		3				8		
					20					
		4				7			17	
12					18					B11

Basic concepts on experimental designs: The three Fisher basic principles

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The three Fisher principles: Randomization

- Reduces the bias, avoiding to favor some treatments.
- The main assumption in all statistical linear models related to the experimental error is

$$\epsilon_{ij} \sim NI(0, 1\sigma_{ij}^2) \tag{1}$$

- Therefore, the randomization guarantee that experimental units (e.u.) are independent to each other, in a such way that is possible to use the classical parametric statistical methodologies.
- Randomization is one of the most important components of a well-designed experiments.

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Basic terms and concepts

- Factor: Are the explanatory variable (independent) variables that the researcher are interested in evaluate their effect.
- Levels: Are the different categories in which a factor can be divided.
- Treatments: Are the different procedures we want to compare. Sometimes correspond to the combination of factors and their levels.
- Experimental Units (E.U.): Are the smallest physical area in which we apply one and only one treatment.
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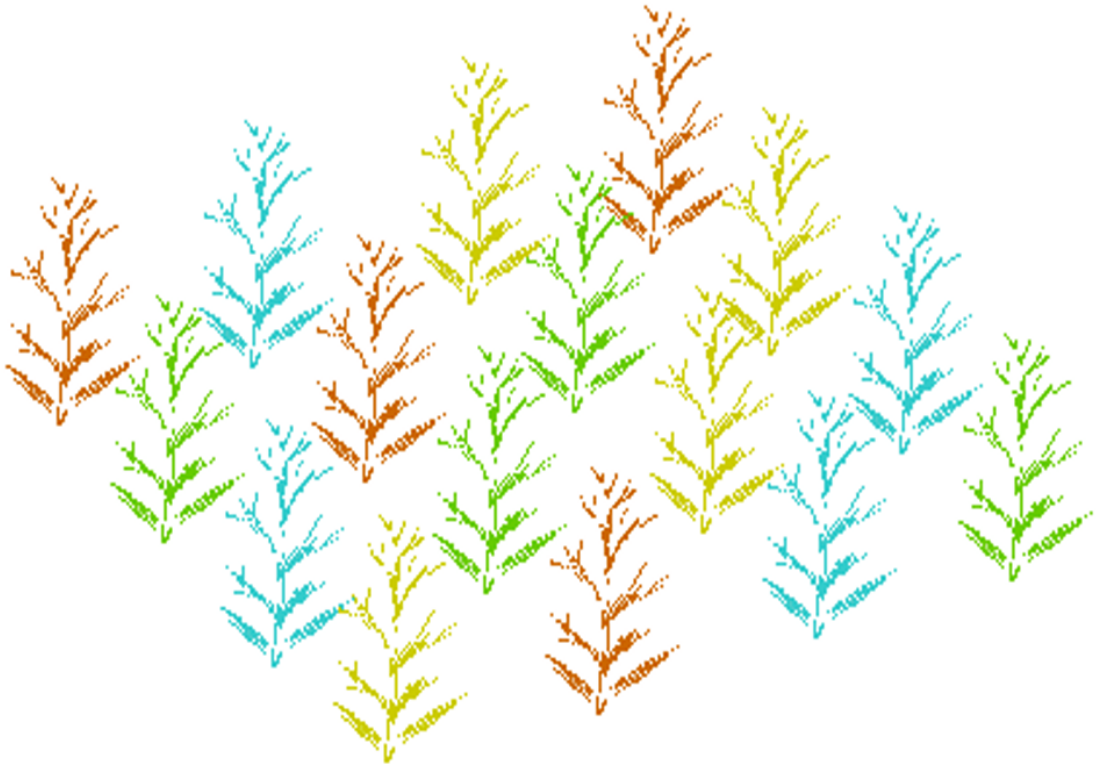
Experimental Designs: Completely Randomized Design (CRD)

A1	B1	C1	A2
D1	A3	D2	C2
B2	D3	C3	B3
C4	A4	B4	D4



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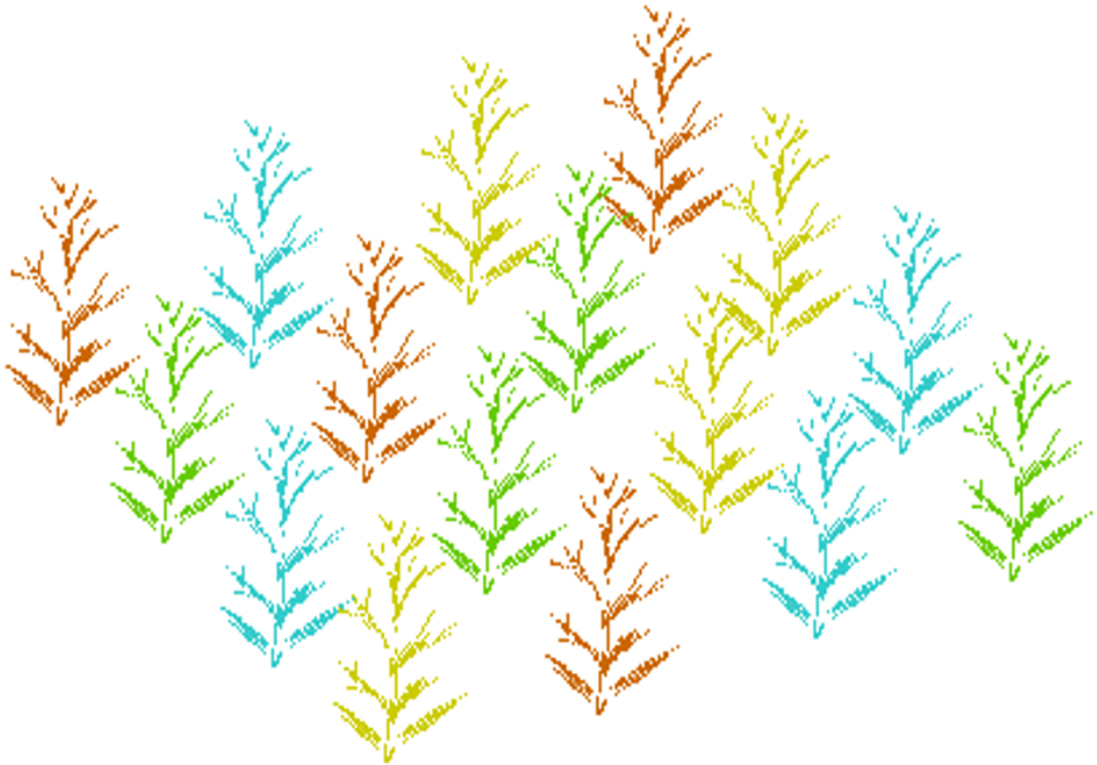

CRD

- statistical Linear model

$$y_{ij} = \mu + \tau_i + \epsilon_{ij} \tag{2}$$

- y_{ij}: Is the response associated with the effect of the i^{th} treatment in the j^{th} replication
- μ: Is the general grand mean common to all experimental units before applying the treatments
- τ_i: Is the effect of the i^{th} treatment
- ϵ_{ij}: Are the errors associated to the effect of the i^{th} treatment in the j^{th} replication
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CRD: Test of hypotheses and analysis of variance (ANOVA)

- We have the null hypotheses
- H_0: $\tau_i = \tau_j$, for all i $\neq$ j
- H_a: $\tau_i \neq \tau_j$; for at least one i $\neq$ j

Source of variation	df	Sum of squares	mean squares	F value	Pr > F
Treatments	(t-1)	$\sum_{i=1}^{t} \frac{(y_{i.})^2}{r} - \frac{(y_{..})^2}{t\,r}$	$\frac{ss\ treat}{(t-1)}$	$\frac{ms\ treat}{ms\ error}$	
Error	t(r-1)	$\sum_{i=1}^{t}\sum_{j=1}^{r}(y_{ij})^2 - \sum_{i=1}^{t}\frac{(y_{i.})^2}{r}$	$\frac{ss\ error}{t(r-1)}$		
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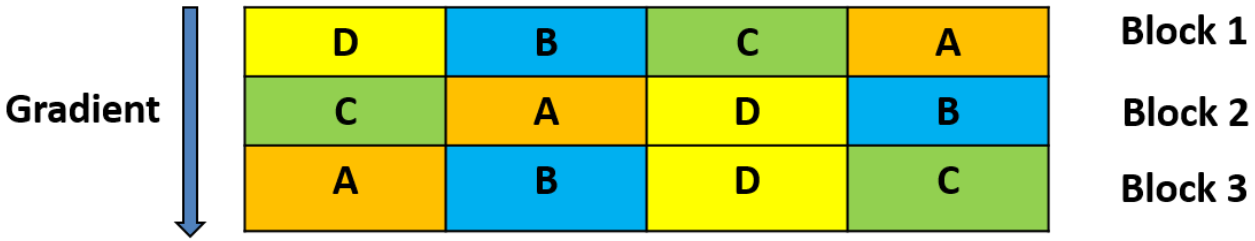
- The RCBD has as restriction that all the treatments are replicated once and only once in each block, using an unrestricted randomization independently in each block



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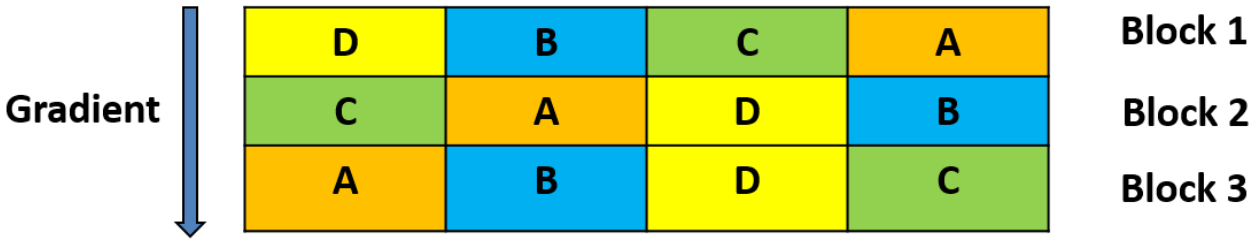

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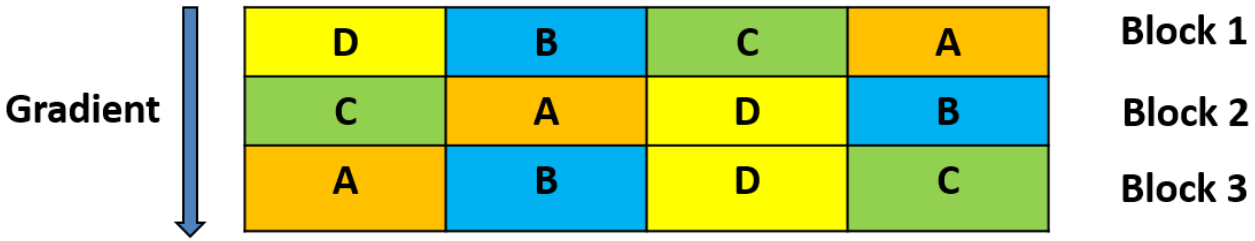

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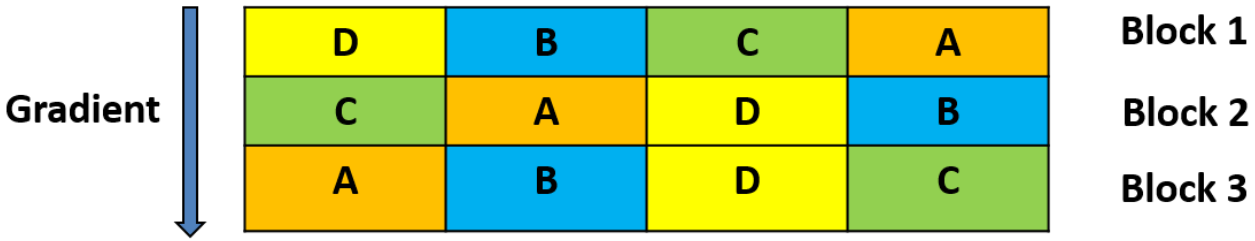

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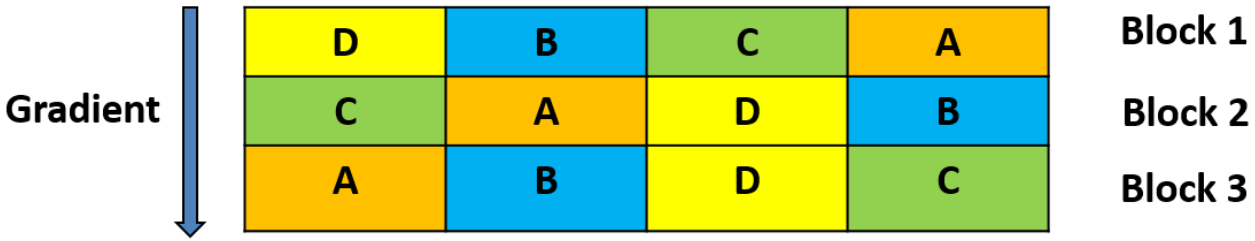

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- statistical Linear model

$$y_{ij} = \mu + \tau_i + \beta_j + \epsilon_{ij} \tag{3}$$

- y_{ij}: Is the response associated with the effect of the i^{th} treatment in the j^{th} replication
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RCBD: Test of hypotheses and analysis of variance

- We have the null hypotheses
- H_0: $\tau_i = \tau_j$, for all i $\neq$ j
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Source of variation	Df	Sum of squares	mean squares	F value	Pr > F
Treatments	(t-1)	$\sum_{i=1}^{t} \frac{(y_{i.})^2}{r} - \frac{(y_{..})^2}{t\,r}$	$\frac{ss\ treat}{(t-1)}$	$\frac{ms\ treat}{ms\ error}$	
Blocks	(t-1)(b-1)	$\sum_{j=1}^{b} \frac{(y_{.j})^2}{t} - \frac{(y_{..})^2}{t\,r}$			
Error	t(r-1)		$\frac{ss\ error}{t(r-1)}$		
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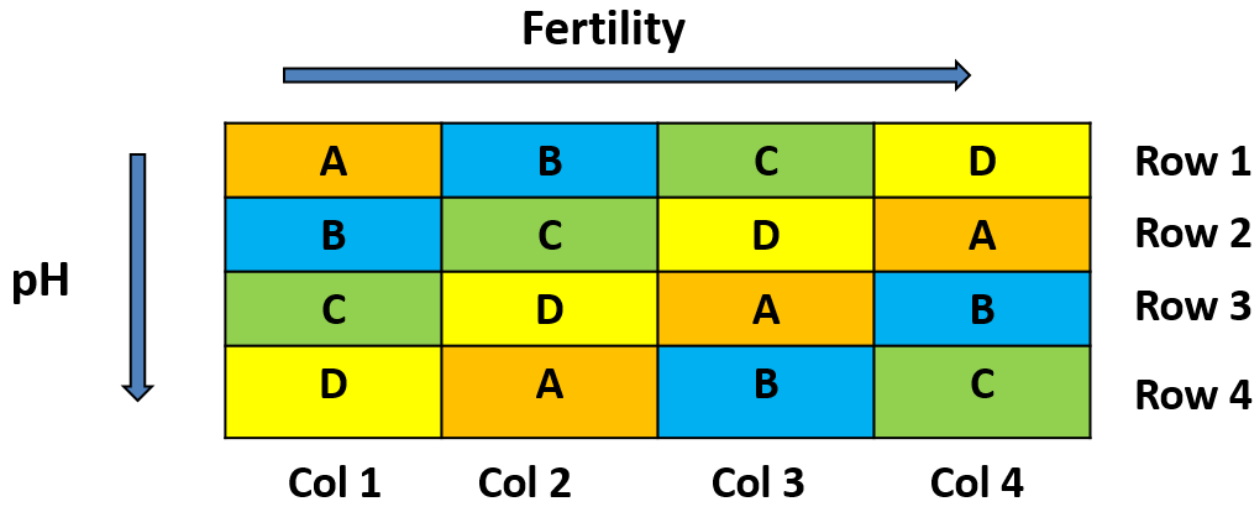
Experimental Designs: Latin Square Designs (LS)

- RCBD allows to block on a single source of variation in the responses
- However, there are experimental situations with more than one source of extraneous variation
- The Latin Square (LS) blocks for two gradients of variability



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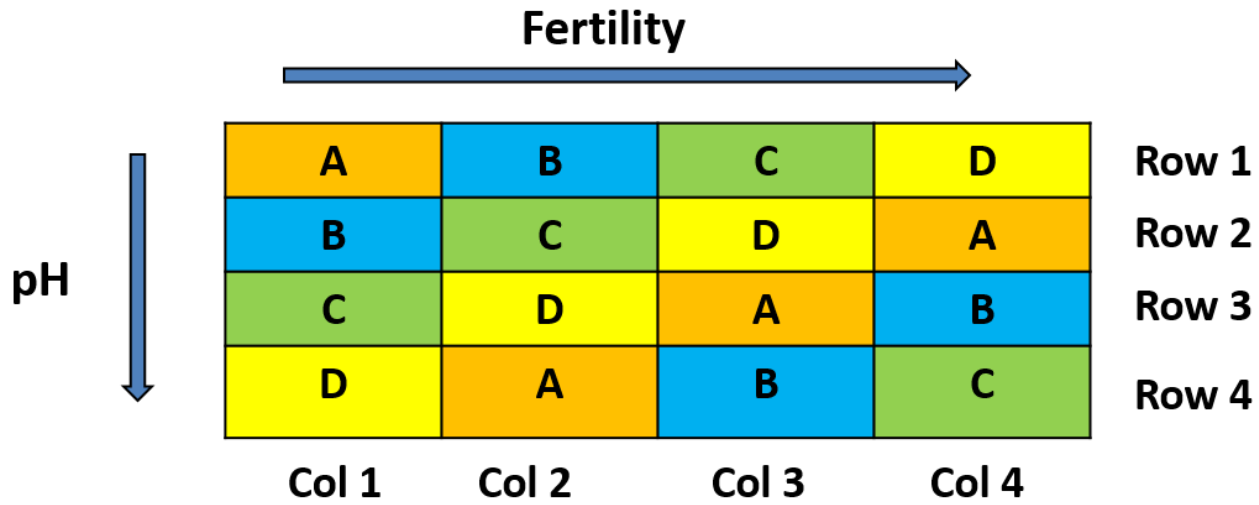

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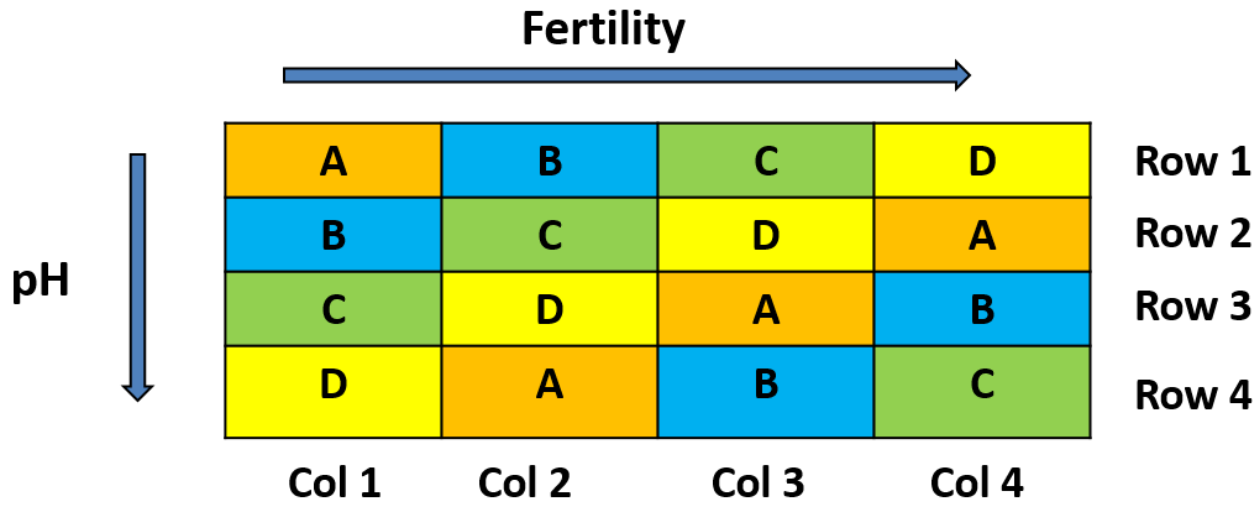

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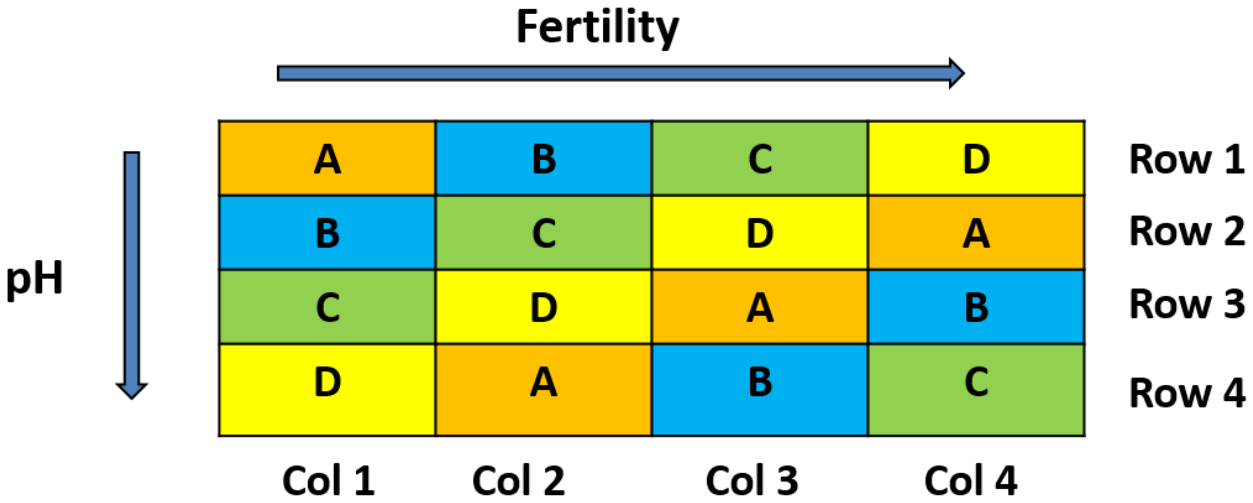

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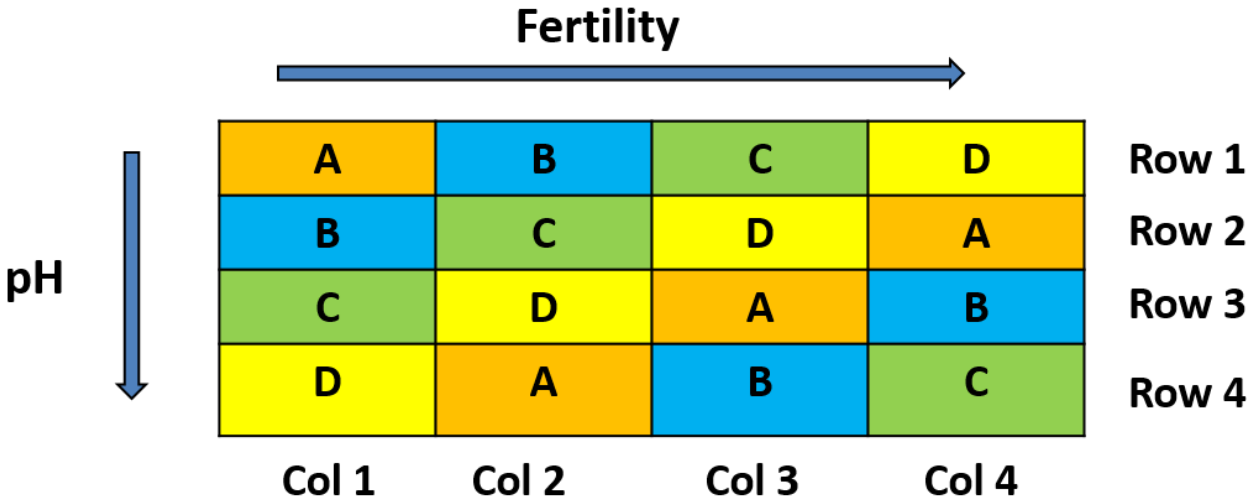

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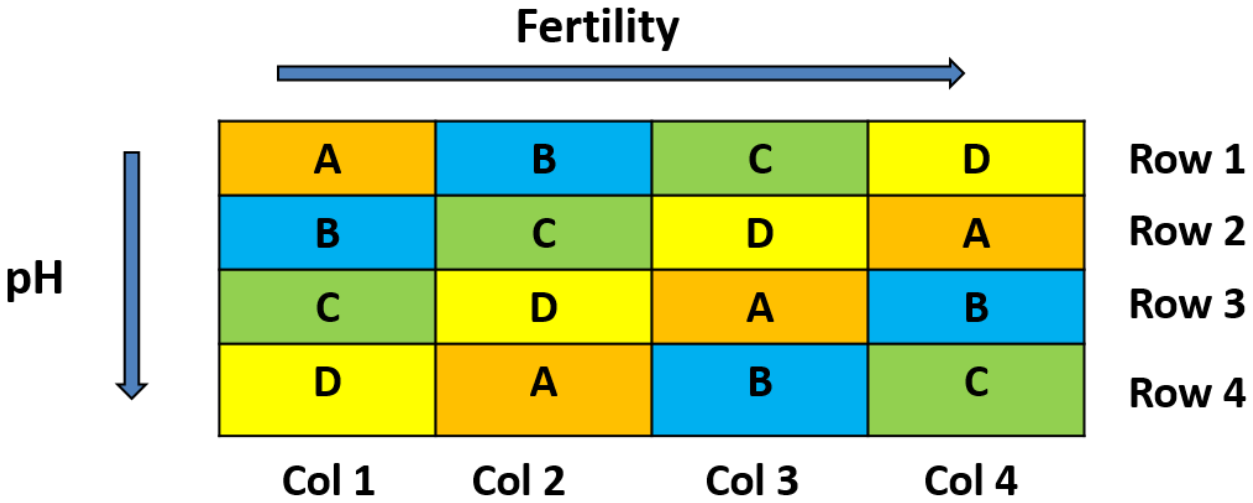

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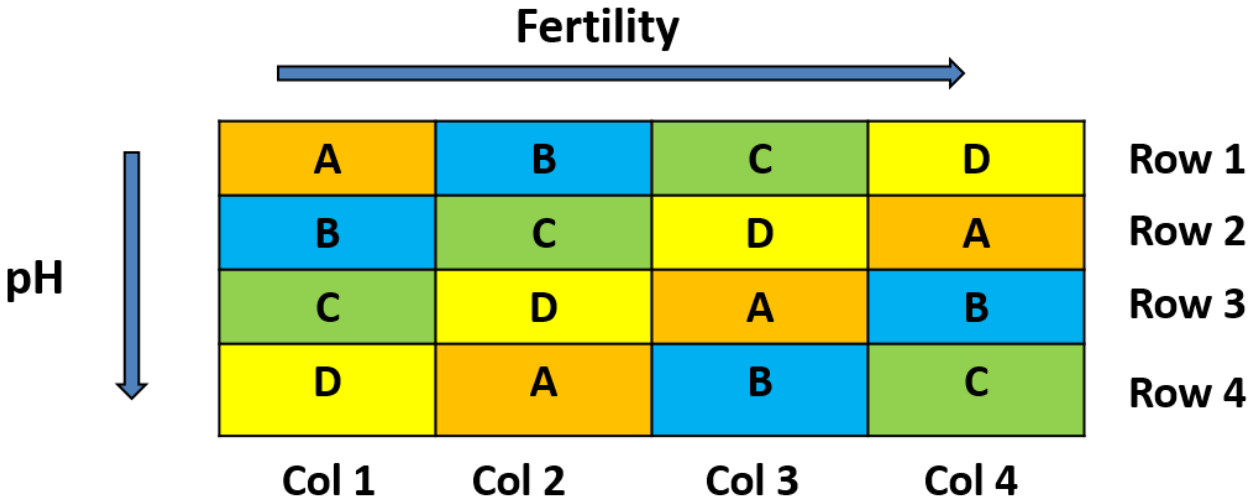

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- We have the null hypotheses
- H_0: $\tau_i = \tau_j$, for all i $\neq$ j
- H_a: $\tau_i \neq \tau_j$; for at least one i $\neq$ j

Source of variation	Df	Sum of squares	mean squares	F value	Pr > F
Treatments	(t-1)	$\sum_{i=1}^{t} \frac{(y_{i..})^2}{t} - \frac{(y_{...})^2}{t\,t}$	$\frac{ss\ treat}{(t-1)}$	$\frac{ms\ treat}{ms\ error}$	
Rows	(t-1)	$\sum_{j=1}^{t} \frac{(y_{.j.})^2}{t} - \frac{(y_{...})^2}{t\,t}$			
Columns	(t-1)	$\sum_{k=1}^{t} \frac{(y_{..k})^2}{t} - \frac{(y_{...})^2}{t\,t}$			
Error	(t-1)(t-2)		$\frac{ss\ error}{(t-1)(t-2)}$		
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Experimental Designs: Factorial Designs

- When there are the suspect that two independent variables are correlated
  - The fertilization level applied to increase the performance of genotypes
  - The susceptibility/resistance of genotypes infested with some pest
  - The performance of genotypes in different environments
- Thus, the independent variables are considered as array of factors
- Therefore, this factors can be included in an classical experimental designs as CRD or RCBD or more sophisticated designs as Lattice or alpha-lattice
- If there are a levels of factor A, and b levels of factor B, then each replicate contains all ab treatment combinations

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- The main effect of a factor is defined to be the change in response produced by a change in the level of a factor
- However, in some experiments we may find that the difference in response between the levels of one factor is not the same at all levels of the other factor. When this occurs, there is an interaction between the factors
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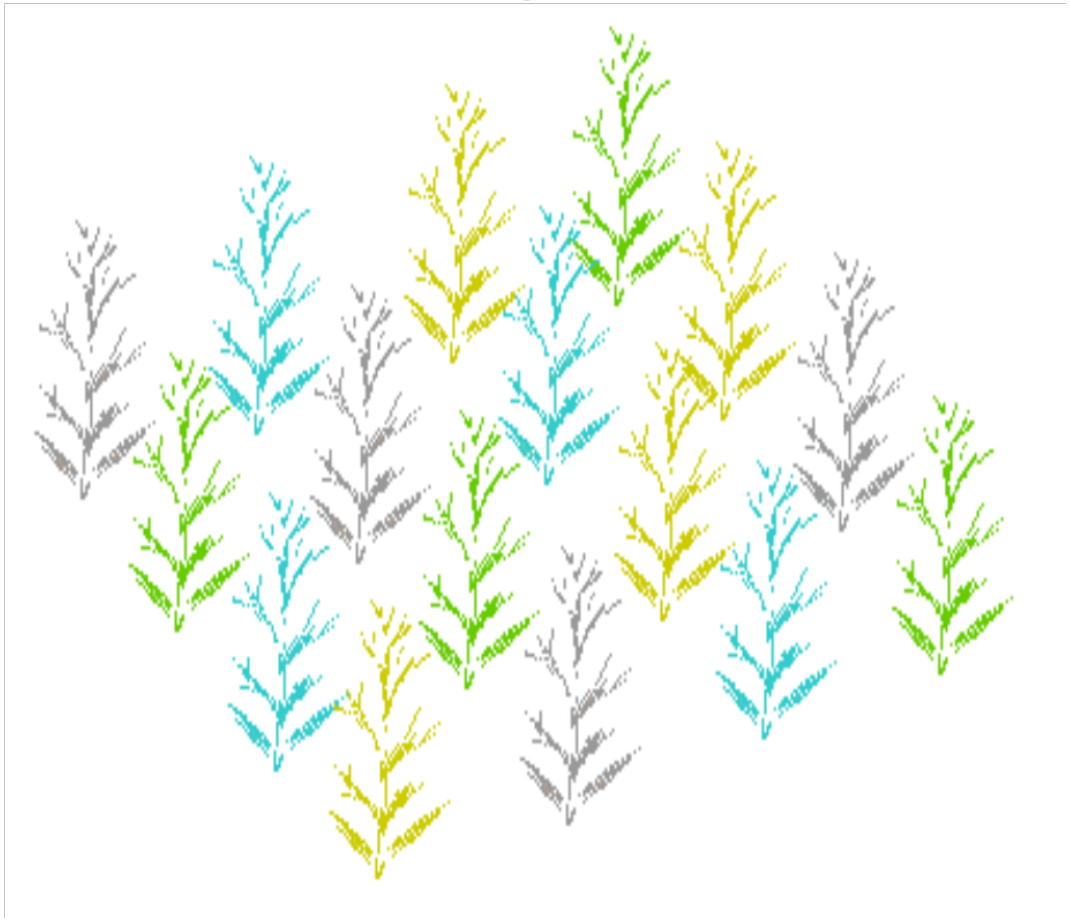
➢ Example: 2 factors at 2 levels each, 4 replications

Ab	aB	ab	AB
AB	Ab	aB	ab
aB	AB	ab	Ab
ab	Ab	aB	AB



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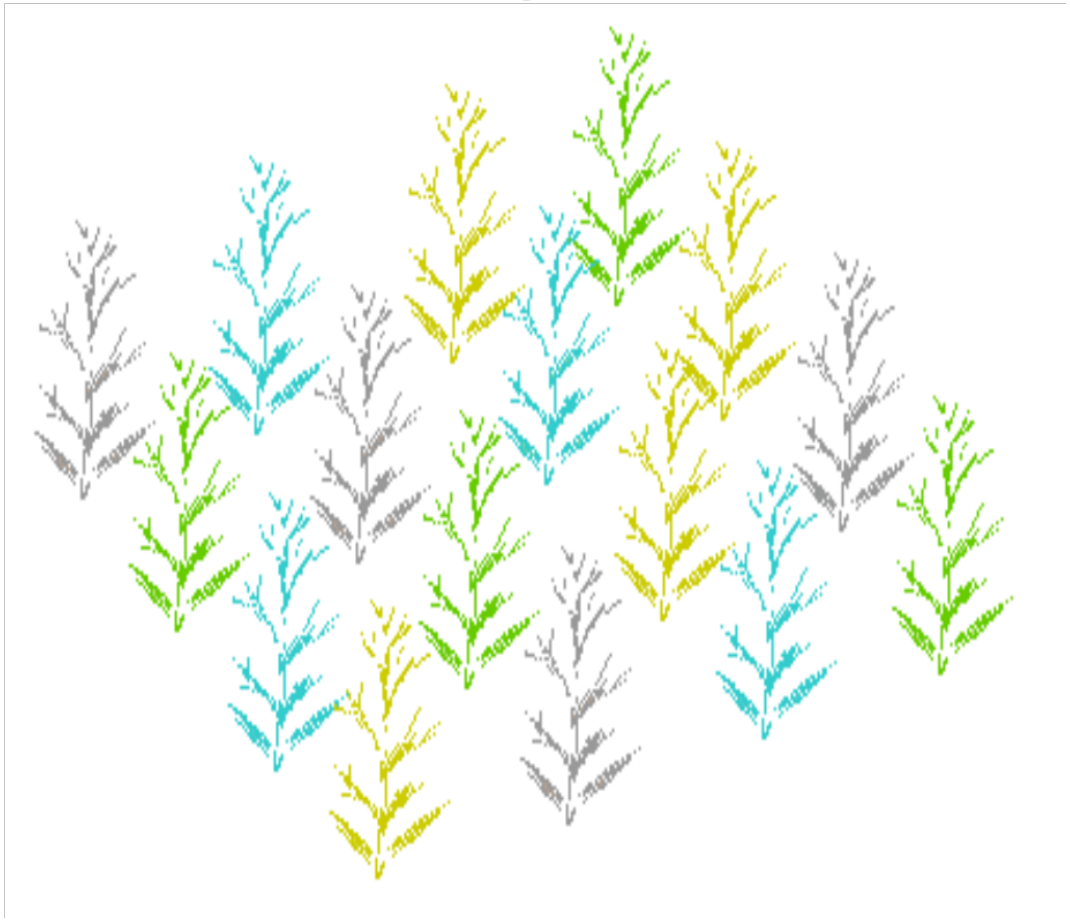

Factorial Designs

- statistical Linear model

$$y_{ijk} = \mu + \tau_i + \delta_j + (\tau\delta)_{ij} + \epsilon_{ijk} \quad (5)$$

- μ: Is the general grand mean common to all experimental units before applying the treatments
- τ_i: Is the effect of the i^{th} level of factor A (i=1,2,.....,n_a)
- δ_j: Is the effect of the j^{th} level of factor B (j=1,2,.....,n_b)
- $(\tau\delta)_{ij}$: represents the interaction effect between A and B
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  - H_a: $\tau\delta_{ij} = \tau\delta_{i'j'}$; for all ij $\neq$ i'j'
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Factorial Designs: Test of hypotheses and analysis of variance

- The test of hypotheses for factor A
  - H_0: $\tau_i = \tau_{i'}$, for all i $\neq$ i'
  - H_a: $\tau_i \neq \tau_{i'}$; for at least one i $\neq$ i'
- The test of hypotheses for factor B
  - H_0: $\delta_j = \delta_{j'}$, for all j $\neq$ j'
  - H_a: $\delta_j \neq \delta_{j'}$; for at least one j $\neq$ j'
- The test of hypotheses for interaction AB
  - H_a: $\tau\delta_{ij} = \tau\delta_{i'j'}$; for all ij $\neq$ i'j'
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Factorial Designs: Test of hypotheses and analysis of variance

Source of variation	Df	Sum of squares	mean squares	F value	Pr > F
A Treatments	(A-1)	$\sum_{i=1}^{t} \frac{(y_{i..})^2}{n_a} - \frac{(y_{...})^2}{t\,t}$	$\frac{ss\ treat}{(A-1)}$	$\frac{ms\ A\ treat}{ms\ AB}$	
B Treatments	(B-1)	$\sum_{i=1}^{t} \frac{(y_{.j.})^2}{n_b} - \frac{(y_{...})^2}{t\,t}$	$\frac{ss\ treat}{(B-1)}$	$\frac{ms\ B\ treat}{ms\ AB}$	
AB Treatments	(AB-1)	$\sum_{j=1}^{n_b}\sum_{i=1}^{n_a} \frac{(y_{ij.})^2}{n_a n_b} - \sum_{i=1}^{n_a} \frac{(y_{i..})^2}{n_a} - \sum_{i=1}^{n_b} \frac{(y_{.j.})^2}{n_b} - \frac{(y_{...})^2}{A\,B}$	$\frac{ss\ treat}{(AB-1)}$	$\frac{ss\ treat}{Error}$	
Error	Tot-AB		$\frac{ss\ error}{(t-1)(t-2)}$		
Total	Tot -1	$\sum_{i=1}^{t}\sum_{j=1}^{t}\sum_{k=1}^{t} (y_{ijk})^2 - \frac{(y_{...})^2}{t\,t}$			

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AB Treatments	(AB-1)	$\sum_{j=1}^{n_b}\sum_{i=1}^{n_a} \frac{(y_{ij.})^2}{n_a n_b} - \sum_{i=1}^{n_a} \frac{(y_{i..})^2}{n_a} - \sum_{i=1}^{n_b} \frac{(y_{.j.})^2}{n_b} - \frac{(y_{...})^2}{A\,B}$	$\frac{ss\ treat}{(AB-1)}$	$\frac{ss\ treat}{Error}$	
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Factorial Design: Test of hypotheses and analysis of variance (ANOVA)

- With decision rule
- if $[Pr > F] \leqslant threshold$ then we reject H_0

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Experimental Designs: Split Plot Designs

- In some experimental situations there is not practical accomodate all treatments of a factorial experiment in one complete block
- Thus, is necessary to use incomplete blocks, no all treatments are included in the blocks
- We can do this by using the split plot designs, where each block is named as whole plot and the subdivisions into the plot are named as small plots
- As example, suppose that we want test the effect of 3 irrigation methods (a1 = gravity, a2=sprinking, and a3=drip) and 4 yield maize varieties b1, b2, b3 and b4
- So, the list of treatments will be: a1b1 a1b2 a1b3 a1b4, a2b1 a2b2 a2b3 a2b4, a3b1 a3b2 a3b3 a3b4, a4b1 a4b2 a4b3 a4b4

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a_3b_4	a_3b_2
a_2b_4	a_1b_4

a_2b_4	a_1b_4
a_1b_1	a_3b_2
a_3b_4	a_2b_3
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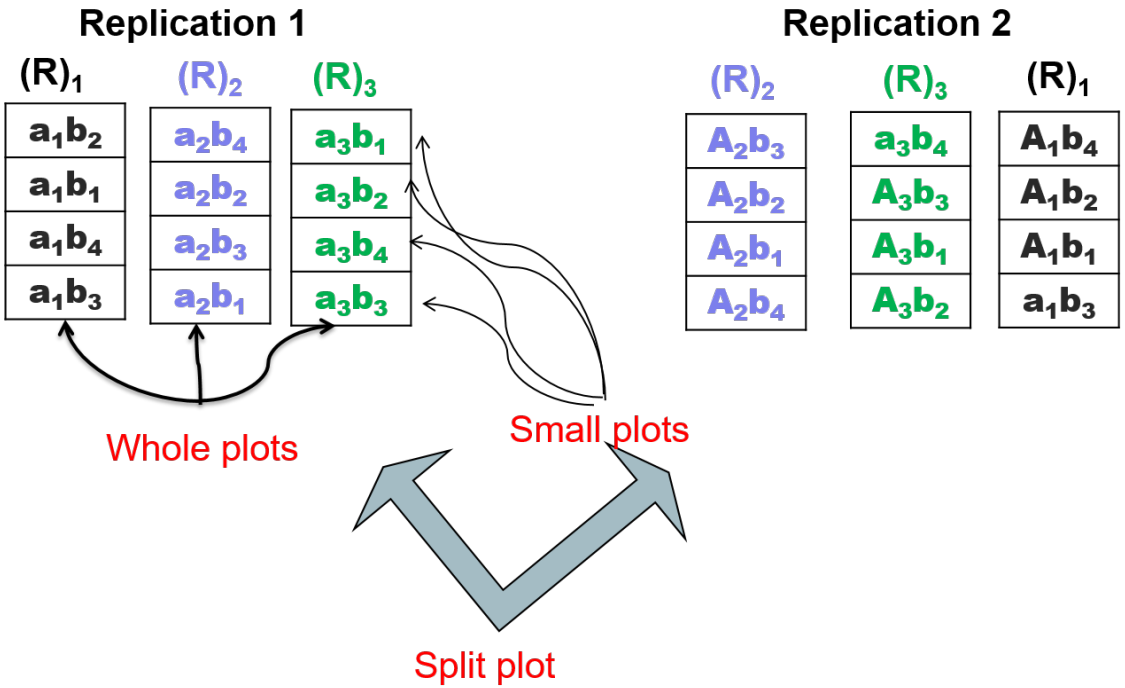
Experimental Designs: Split Plot design

- Therefore, the split plot array could be



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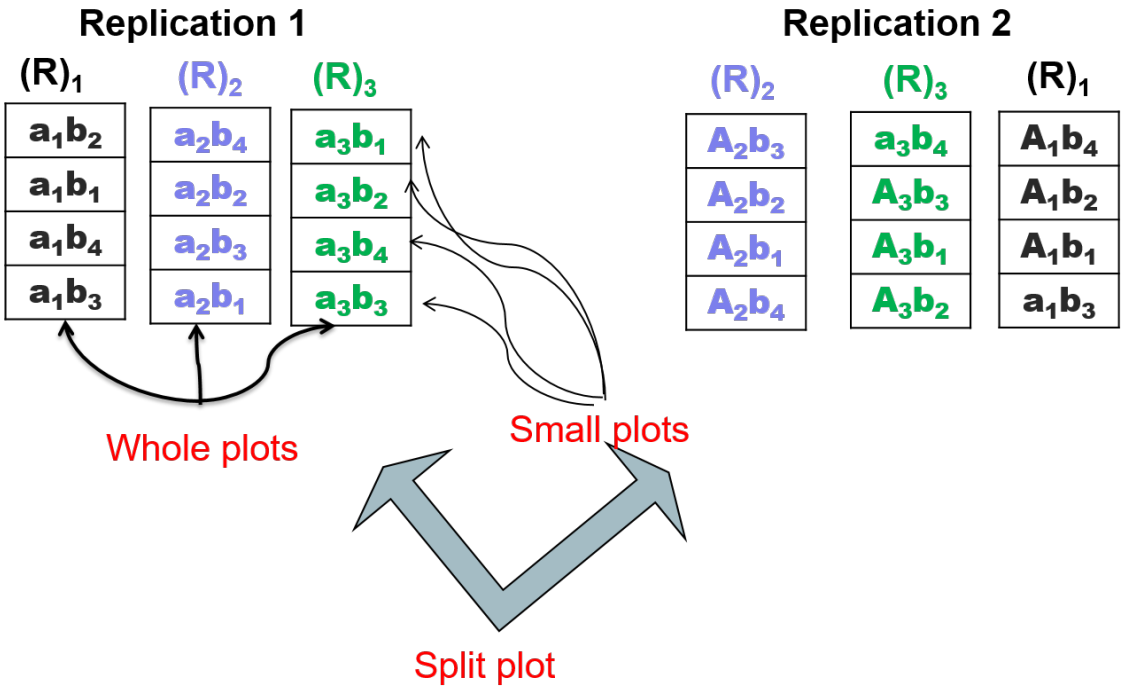

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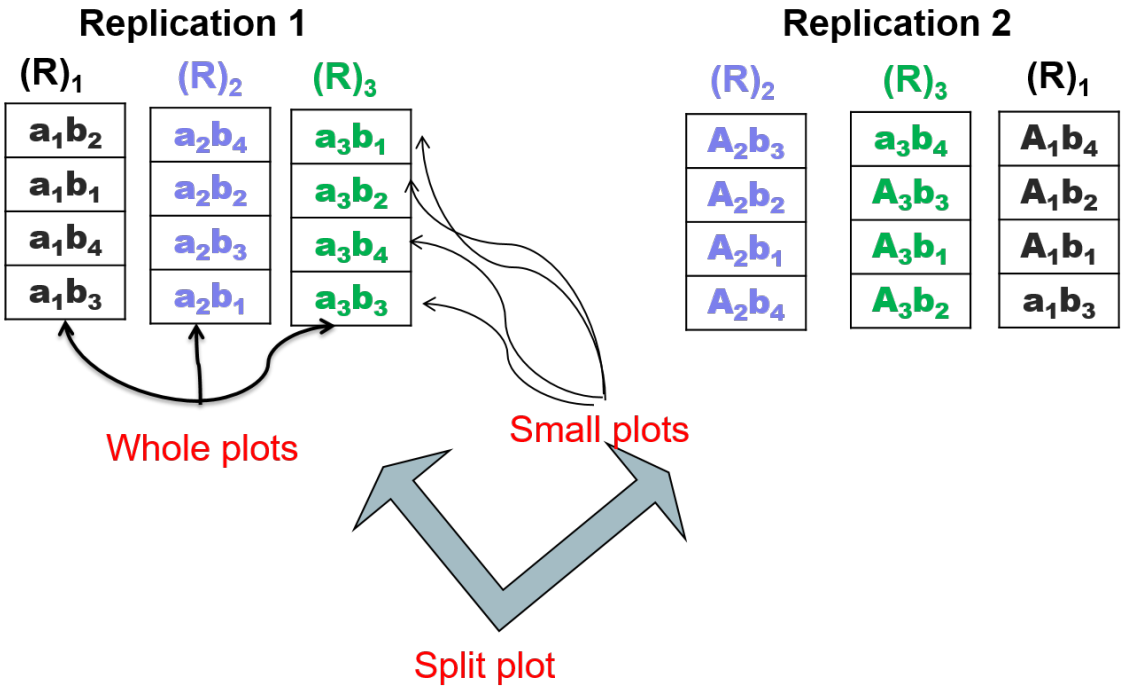

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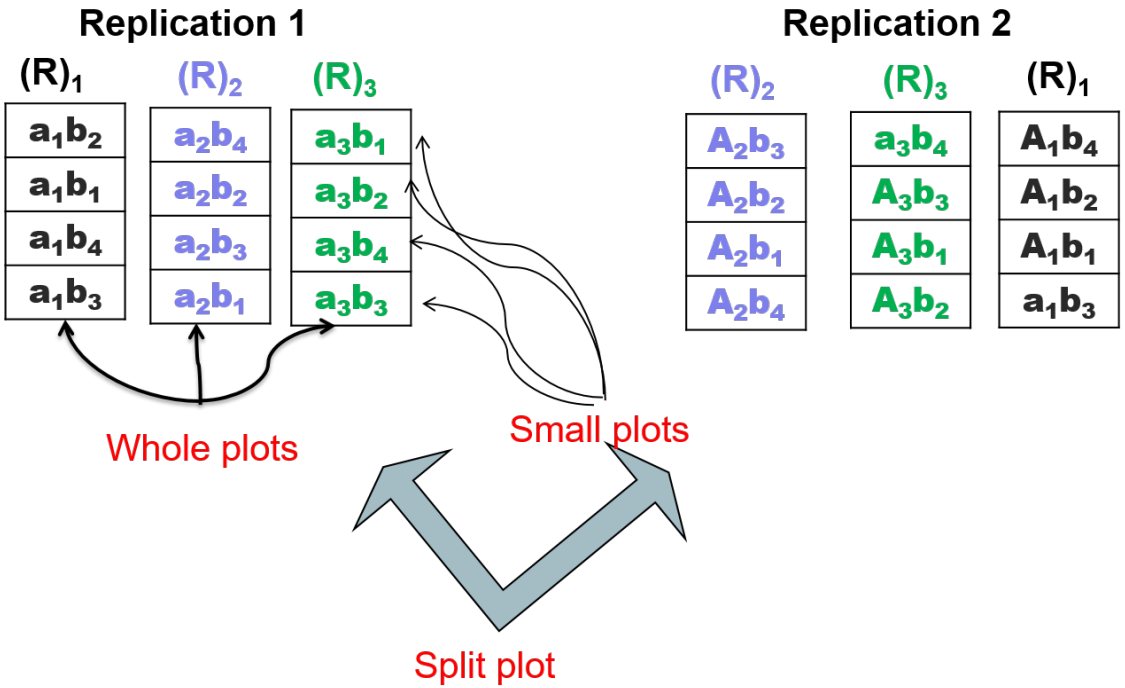

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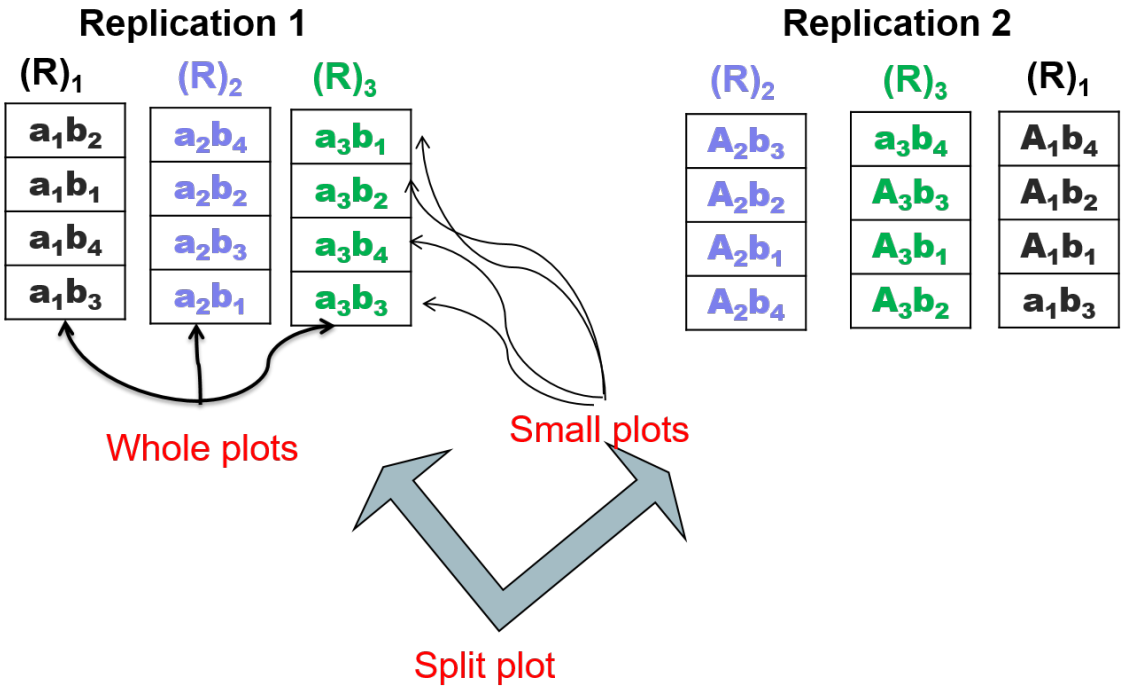

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Split Plot

- statistical Linear model
- The statistical linear model depends of the experimental design in which the whole and small plots are arranged
- For example, when the whole Plot (WP) and the small plots are arranged in a CRD

$$y_{ijk} = \mu + A_i + \epsilon_{a(i)} + B_j + (AB)_{ij} + \epsilon_{(b)ijk} \quad (6)$$

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- y_{ijk}: Is the response for the whole plot i, small plot j, and the replicate k
- μ: Is the general grand mean common to all experimental units before applying the treatments
- A_i: Is the effect of the treatment i over the whole plot i
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$$y_{ijk} = \mu + B_i + A_i + \epsilon_{a(ij)} + B_k + (AB)_{jk} + \epsilon_{(b)ijk} \quad (7)$$

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$$y_{ijkl} = \mu + \gamma_i + \delta_j + A_k + \epsilon_{a(ijk)} + B_l + (AB)_{kl} + \epsilon_{(b)ijkl} \quad (8)$$

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- γ_i: Is the effect of the i^{th} row
- δ_j: Is the effect of the j^{th} column

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$$y_{ijkl} = \mu + \gamma_i + \delta_j + A_k + \epsilon_{a(ijk)} + B_l + (AB)_{kl} + \epsilon_{(b)ijkl} \quad (8)$$

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SPD: ANOVA, PG→ RCBD, PCh→CRD

Source	DF	S.S	MS	Expected mean squares	F
Bloque	**(i-1)**			------	
A	**(j-1)**			$\sigma^2{}_b+(k)\sigma^2{}_a+(ik)\sum\frac{(A_j-(\bar{A}))^2}{(j-1)}$	**$F_{(A)}$**
E(a)=Bloq x A	**(i-1)(j-1)**			$\sigma^2{}_b+(k)\sigma^2{}_a$	
B	**(k-1)**			$\sigma^2{}_b+(ij)\sum\frac{(B_k-(\bar{B}))^2}{(k-1)}$	**$F_{(B)}$**
AB	**(j-1)(k-1)**			$\sigma^2{}_b+(i)\sum\sum\frac{[AB_{jk}-\overline{AB}]2}{(j-1)(k-1)}$	**$F_{(AB)}$**
E(b)=Residual	**(i-1)j(k-1)**			$\sigma^2{}_b$	
Total	**Ijk-1**			-----	

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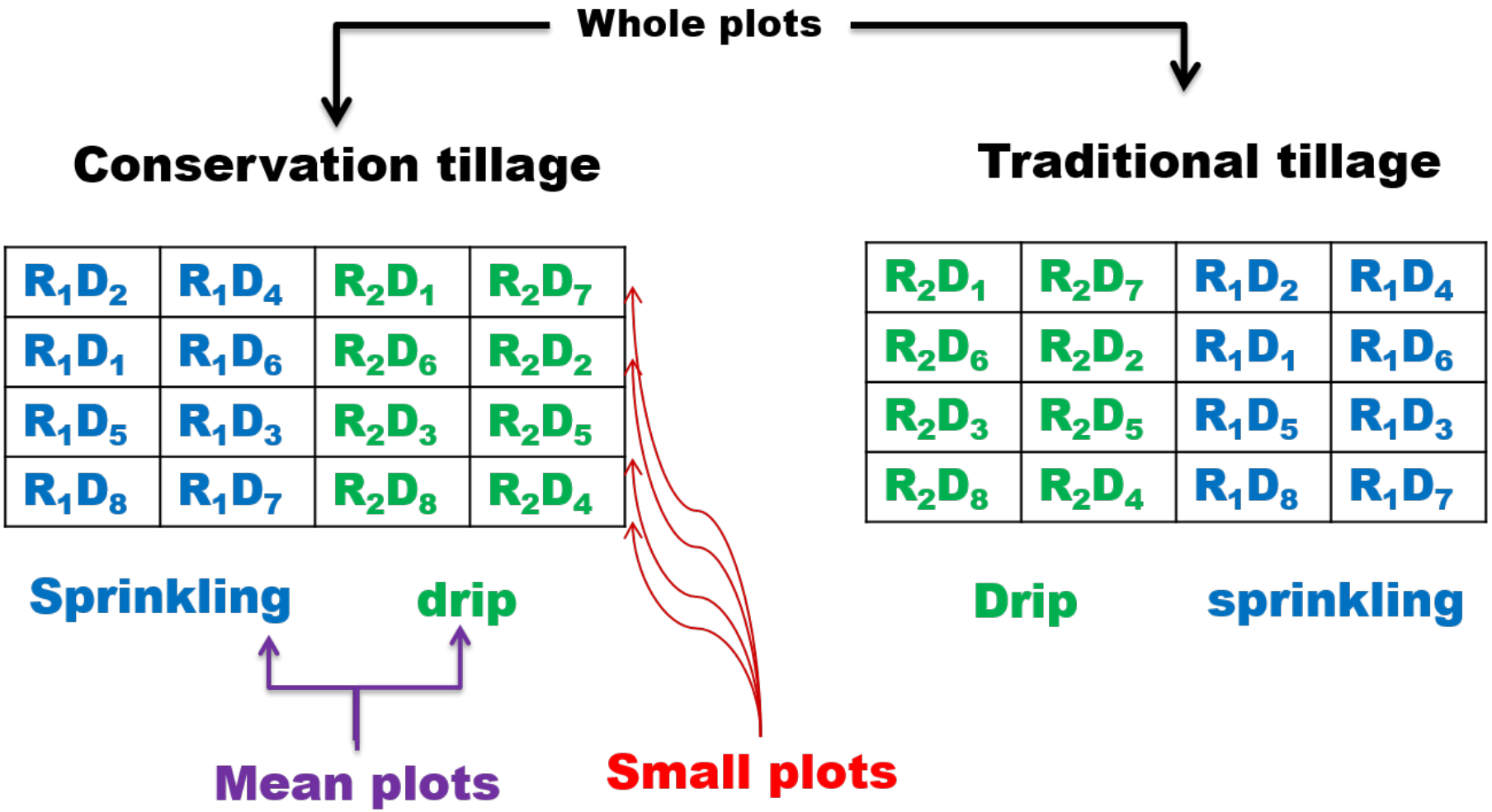
Whole plots
Conservation tillage
Traditional tillage
R1D2 R1D4 R2D1 R2D7
R1D1 R1D6 R2D6 R2D2
R1D5 R1D3 R2D3 R2D5
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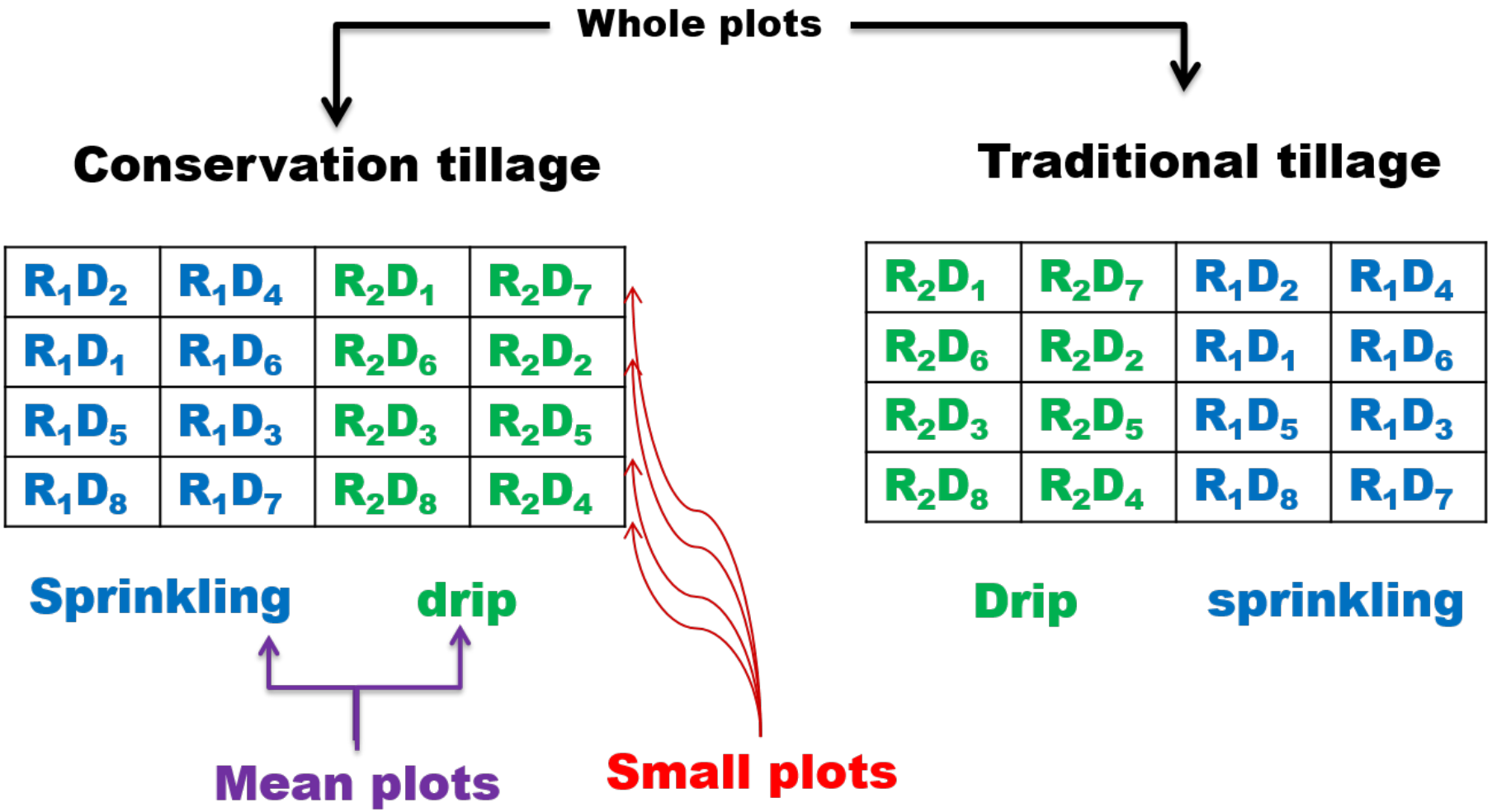
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After the ANOVA analyses what ?

- The ANOVA hypothesis is quite little informative
- If we didn't reject $H_0(\tau)$: $\tau_i = \tau_j \quad \forall\, i \neq j$ then the analysis ends here. In applied research the cheaper, more practical, more available treatments should be recommended
- If the ANOVA H_0 hypothesis is rejected, then that rejection could be due to different situations:

- $\tau_1 = \tau_2 = \ldots = \tau_{t-1}$, but $\tau_{t-1} \neq \tau_t$
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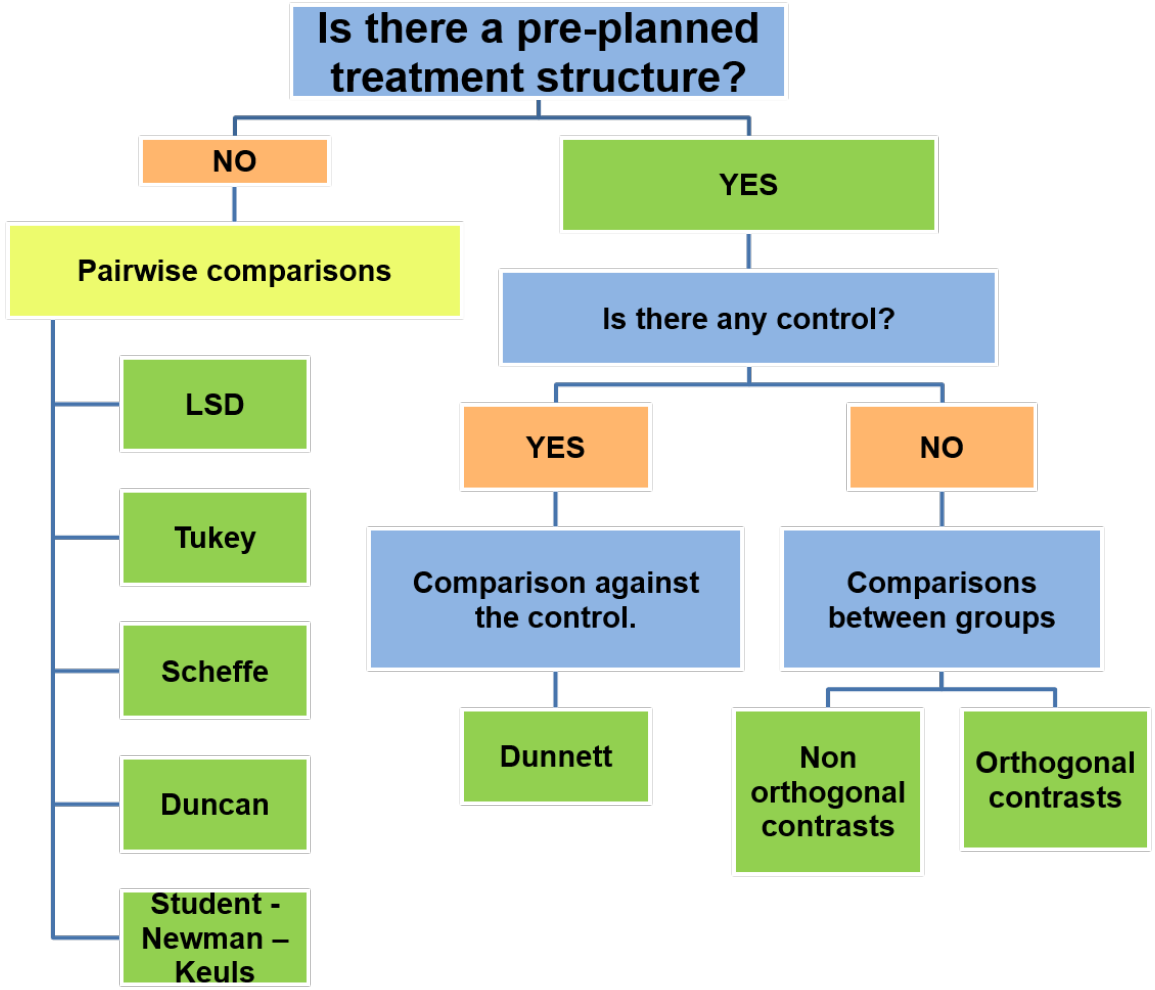

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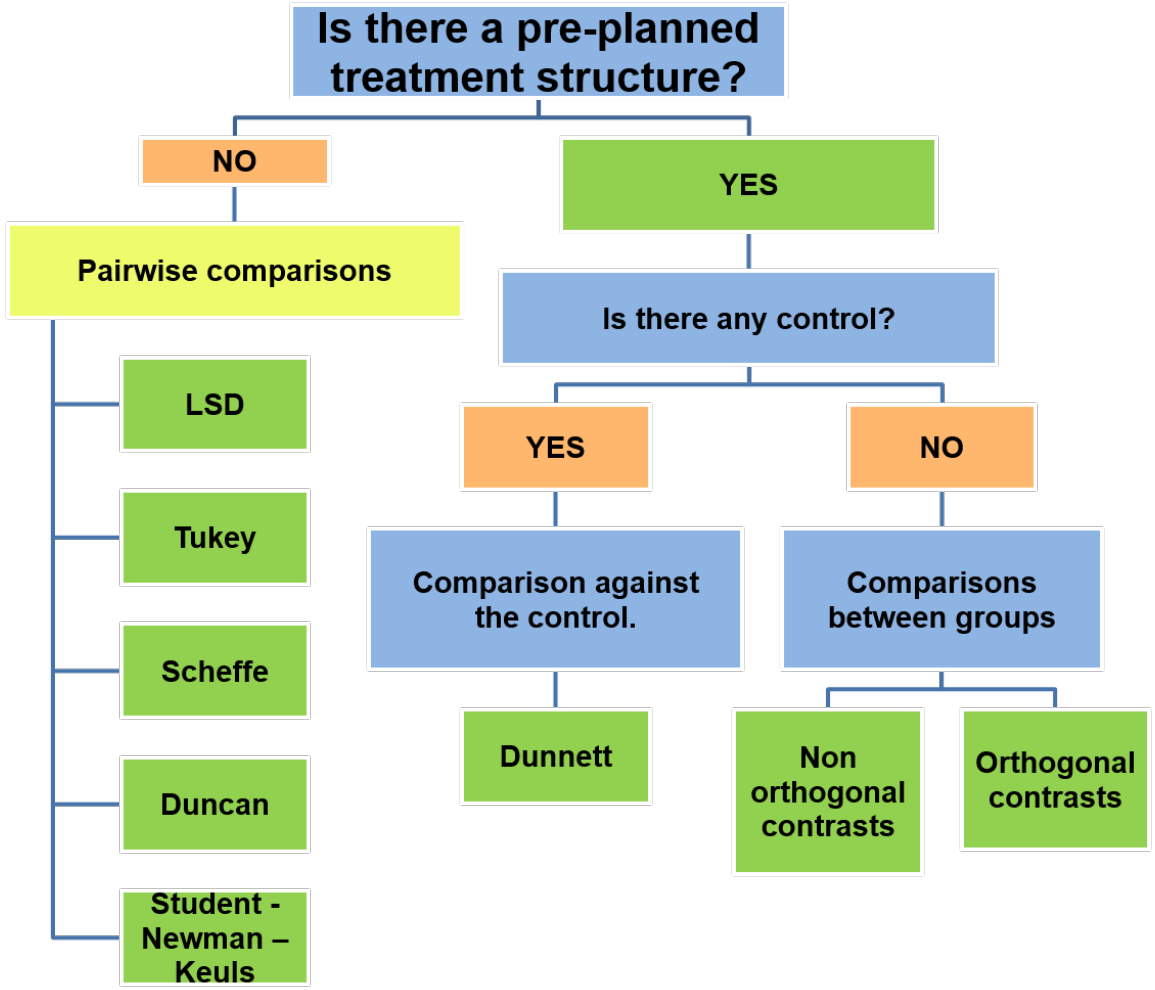

Pairwise comparisons methods: fundaments

- Hypothesis:

$Ho_k: \tau_i = \tau_j$ versus $Ha_k: \tau_i \neq \tau_j$

where $i = 1, 2, \ldots, t-1$; $j = i+1, i+2, \ldots, t$

$k = 1, 2, \ldots, \frac{t(t-1)}{2}$ $\binom{t}{2} = \frac{t!}{2!(t-2)!}$

- Example, for 4 treatments ; the hypotheses to test are:
- $\frac{t(t-1)}{2} = \frac{4(3)}{2} = 6,$

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- **The Test Statistic is:**

$$D_k = \left|\bar{y}_i - \bar{y}_j\right| ; \qquad k = 1, 2, \ldots, \frac{t(t-1)}{2}$$

- **The number of differences to calculate is equal to the number of hypothesis to test, using the means of the treatments involved in the corresponding hypothesis.**
- **So, for the above example its necessary to calculate 6 differences**
- **Critical Value (Threshold):**

$$MSD = [D' n_{dfe,\alpha/2}][\text{Standard Error}]$$

- **With Decision Rule :**

$$\text{If} \quad \left|\bar{y}_i - \bar{y}_j\right| \geq MSD \rightarrow Reject\ Ho_k$$

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Least Significant Difference (LSD) method

- It is based on the Student's t distribution

- For $r_i \neq r_j$ (unbalanced designs)

$$LSD_k = [t_{dfe,\alpha/2}][\sqrt[2]{MSE(\frac{1}{r_i} + \frac{1}{r_j})}]$$

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Comparisonwise error rate vs Experimentwise error rate

Pr (type I error) = Pr (rejecting H0 / H0 is true)
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= Pr (False Positive)

Experimentwise error rate:

$$Pr(False\ Positive) = 1 - (1 - \alpha)^k$$

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For $\alpha = 0.05$

If t = 4; k=6; $Pr(False\ Positive) = 1 - (1 - 0.05)^6 = 0.4012$

t = 5; k=10; $Pr(False\ Positive) = 1 - (1 - 0.05)^{10} = 0.9005$

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Tukey's method (Honestly Significant Difference)

- It is based on the Studentized or Standardized Range Distribution
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Scheffe's method

- Like ANOVA, it is based on the Snedecor's F distribution
- Because is a general method for both pairwise and groups comparisons is more strict than LSD and Tukey
- For $r_i \neq r_j$ (unbalanced designs)

$$S_k = \sqrt[2]{(t-1)\left[F_{dfe,\alpha}^{(t-1)}\right] \quad [MSE\left(\frac{1}{r_i}+\frac{1}{r_j}\right)]}$$

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- For alpha = 0.05 we got:

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