Barley trials, ICARDA, Rabat, Morocco (2015)

Basics of Design and Analysis of Crop Variety Experiments in Randomized Complete Blocks

M. Singh

Textbook resources on Design of Experiments

These days:

D. C. Montgomery (2020) *Design and Analysis of Experiments*, 10th Ed., Wiley (John Wiley & Sons, Inc.)

C. F. Jeff Wu and M. S. Hamada (2009). *Experiments: Planning, Analysis, and Optimization.* Wiley.

K. Hinkelmann and O. Kempthorne (2005, 2007) *Design and Analysis of Experiments.* Vol 1 and Vol 2. Wiley.

Cox, D. R. and Reid, N. (2000). The theory of the design of experiments, Chapman & Hall/CRC.

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Those days:

O. Kempthorne (1952). The Design and Analysis of Experiments. Wiley

W. G. Cochran and G.M. Cox (1957). *Experimental Designs*. Wiley

D. R. Cox (1958). *Planning of Experiments.* Wiley.

W. T. Federer (1967). *Experimental Design: Theory and Application.* Oxford & IBH Publishing Company.

K. A. Gomez & A. A. Gomez (1984). *Statistical procedures for agricultural research.* Wiley

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Prof. JNR Jeffers *Checklist – Design of Experiments*

http://www.sawleystudios.co.uk/jnrj/StatisticalCheck/Design.htm

Plan of presentation

1. Basic Principles of Design of Experiments

- Set the scene: an example
- Basic elements of an experimental design
- Requirements of a Good Experiment
- Fisher's 3R s

2. Design of an experiment in RCB

- An illustration
- Experimental Process

3. Statistical analysis of experiments in RCB

- Analysis of Data from Designed Experiments
- Assumptions
- Block structure concept
- ANOVA
- GenStat/SAS/R codes for statistical analysis

Introduction

What is an experimental design?

What is an analysis of experimental design?

Is the experimental design a big deal?

- Experimental design is a technique for planned generation of (statistically) valid and reliable pieces (components) of the evidence on the effect of the factors under investigation in relation to a specific population.

- Statistical analysis is a procedure to draw inferences on the factors of interest by searching pattern in those pieces [or components of the evidence] and assessing the strength of the pattern relative to the deviation from the pattern (noise).

- Power of the evidence on the factors can be enhanced by incorporating any features inherited in the experimental material at the design and analysis stages.

- There has always been a need to
  " … differentiate the Real from the unreal"
- "One must continually separate truth from illusion."

Sir Ronald Aylmer Fisher

FRS (17 February 1890 – 29 July 1962), British Statistician, evolutionary biologist, and geneticist

"To consult the statistician after an experiment is finished is often merely to ask him to conduct a post mortem examination. He can perhaps say what the experiment died of."

> Presidential Address to the First Indian Statistical Congress, 1938. [RA Fisher]

The experimental design is a big deal.

PART 1
Basic Principles of Experimental Designs

Is there a purpose in doing experiments?

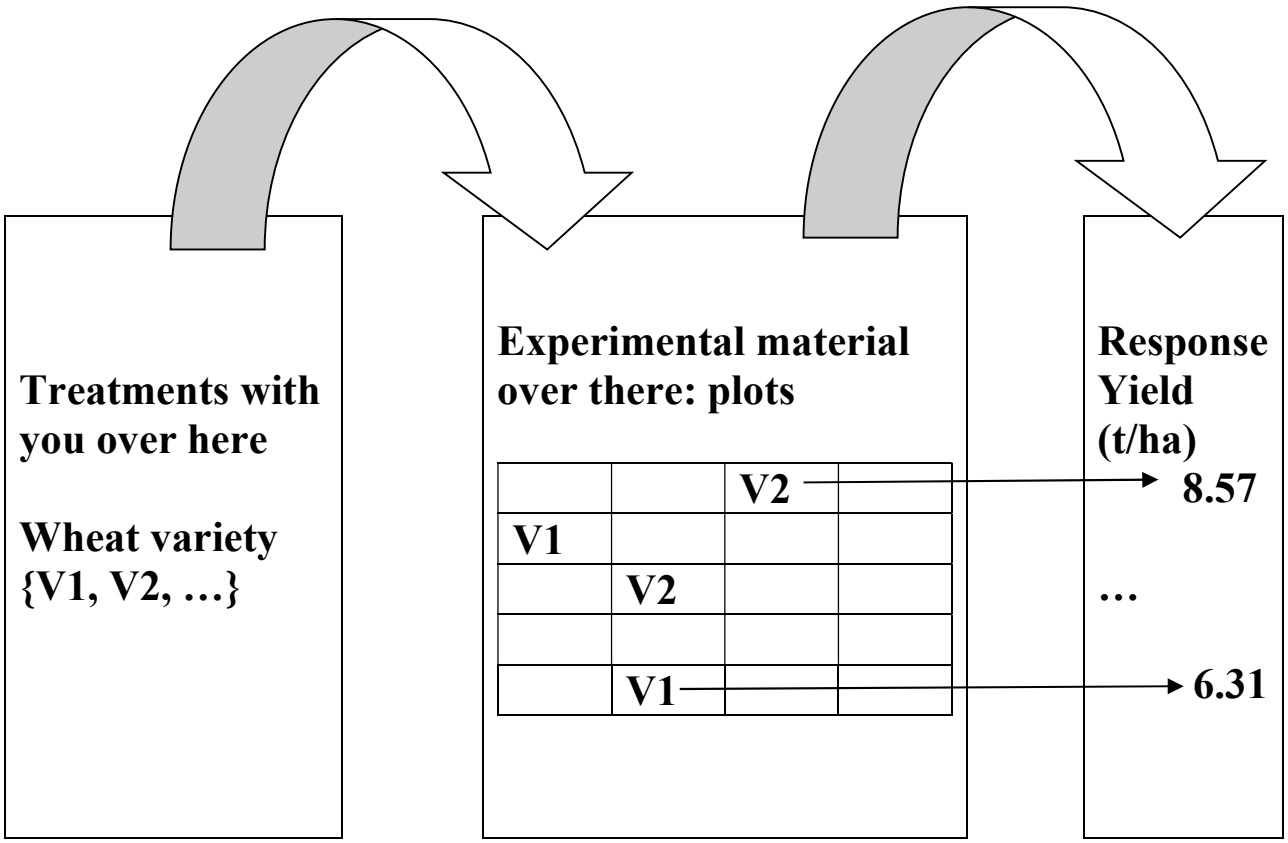
Examples

Context: Agricultural field trials

- An **objective** may be to assess or compare **several varieties of wheat**, or a few fertilizer treatments, or several systems of land and water management, or several disease control methods etc.
- **Experimental units**: Plots of land
- The crop response: yield of the plot.

Context: Industrial -- An integrated circuit manufacturing: Plasma Etching Experiment (Montgomery 2020)

- **Effect of RF (radio frequency) power** (4 levels: 160,180, 200, 220 W) on etch rate (Å/min)
- **Experimental units:** wafers coated with a layer of material (silicon dioxide or a metal)
- **Objective:** to model the relationship between etch rate and RF power, and specify the power setting for getting desired etch rate.



Purpose: Infer on the Varieties using the evidence generated.

Q_1: Should experiment units have any requirements?

Q_2: Which Exp. unit should receive which variety?
Variety - plot correspondence requirement?
How?

Carry out the experiment.

Q_3: How to relate yield to varieties?

Basic elements of experimental design

Treatments:
Experimental Unit:
Experimental Material:

Variability in the response due to sources of variation

Need: Experimental procedures are needed for precisely separating the treatment effects/differences from uncontrolled variation.

Requirements of a Good Experiment

- Comparison of treatments free from systematic errors,
- Estimates should have high precision,
- Uncertainties in the comparisons/inferences assessable,
- Conclusions/inferences should be broadly valid, and
- Experimental arrangement should be simple and operationally convenient.

Experimental Technique

- Size of the experimental unit matters in designing the experiment.

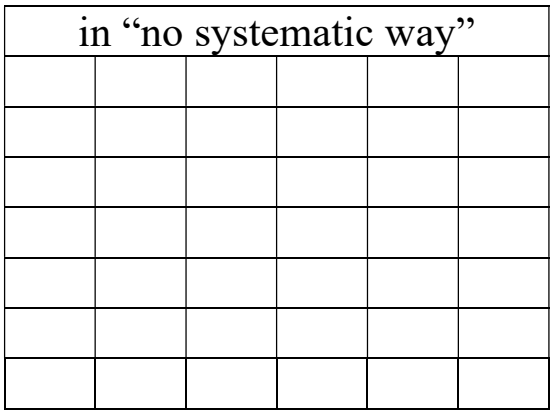
Requirements of a Good Experiment (continued)

(a) Prepare/select the experimental material free from the presence of any non-random pattern in the Expl. units

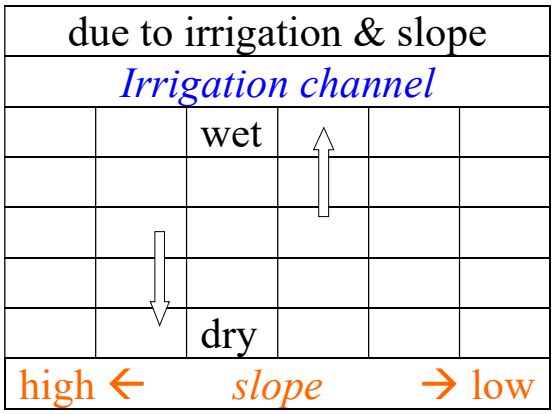
- Uncontrolled or experimental error variability is a reality in real experiments and is needed for drawing statistical inference.

- Notice the variation in your own signatures you made in one go – is not due to any mistake, but due to uncontrolled (cannot be eliminated totally) experimental error.

The experimental units differ



Homogeneous



Heterogeneous

(b) Precision:

Experimental error variance σ^2

- between experimental units within a treatment
- generally **varies with the size** of the expt. Unit (Fairfield Smith's (1938) empirical law)

No. of Experimental units allotted to a treatment /replications

Is there an error of some sort?

On average [Error^2 = (sample mean – population mean)^2]

$= \sigma^2 / r$ **(Apply statistical theory)**

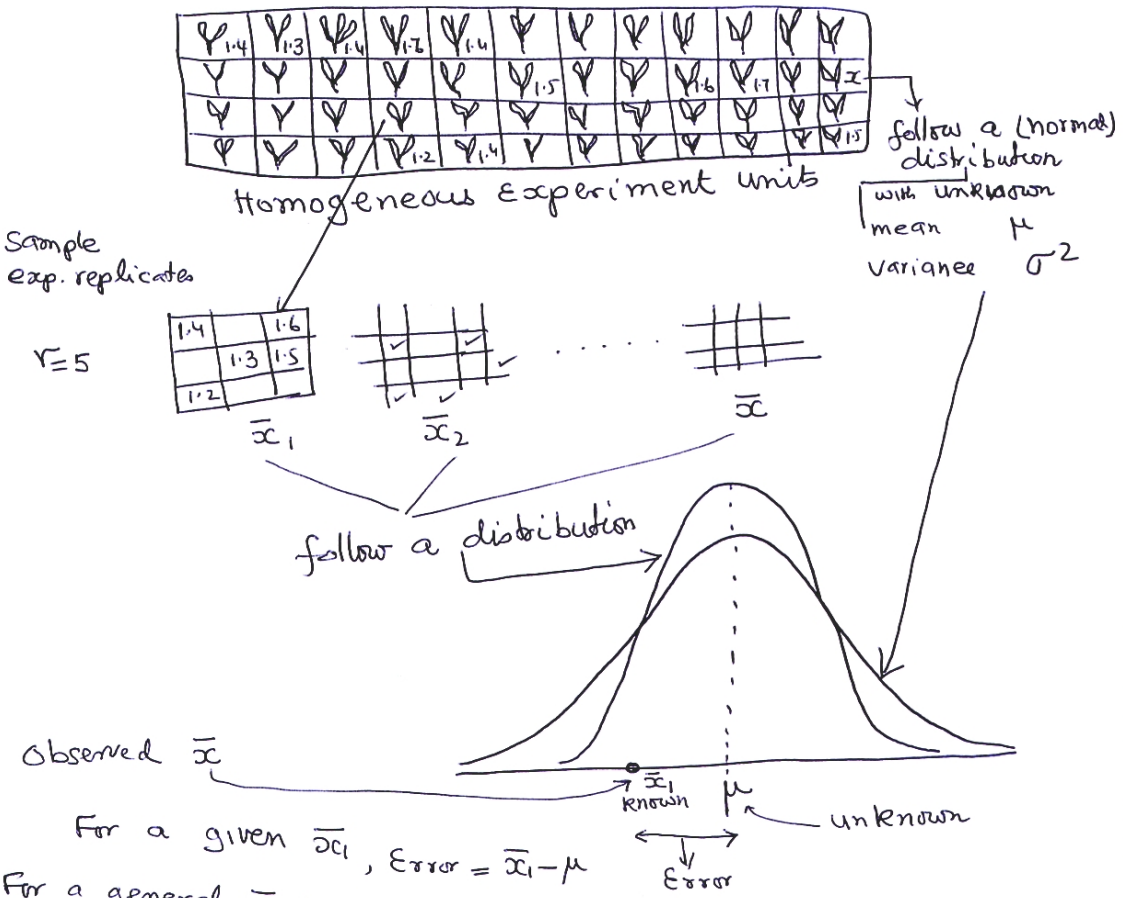
Precision (1/variance)

SEm =SE = $s/\sqrt{r}$

Heterogeneity of experimental material

CV(%)=100 $s/\overline{x}$

Experimental Error



For a given $\bar{x}_1$, $\text{Error} = \bar{x}_1 - \mu$

For a general $\bar{x}$ (r reps)

$\text{Error} = \bar{x} - \mu$

On average $(\text{Error}^2) = \text{mean squared error} = \frac{\sigma^2}{r} = \text{Var}(\bar{x})$ (Statistical Theory)

On original unit scale

$$SEm = SE = \sqrt{\frac{\sigma^2}{r}} = \frac{\sigma}{\sqrt{r}}$$

Note 1. SEm decrease with r, so $\Rightarrow$ Error $|\bar{x} - \mu|$ is likely to be smaller for a larger r.

2. r values $x_1 \ldots x_r$ $(\bar{x}, s^2)$ $s^2 = \sum (x - \bar{x})^2/(r-1)$

Estimate $\mu = \bar{x}$,

Estimate $\sigma^2 = s^2$

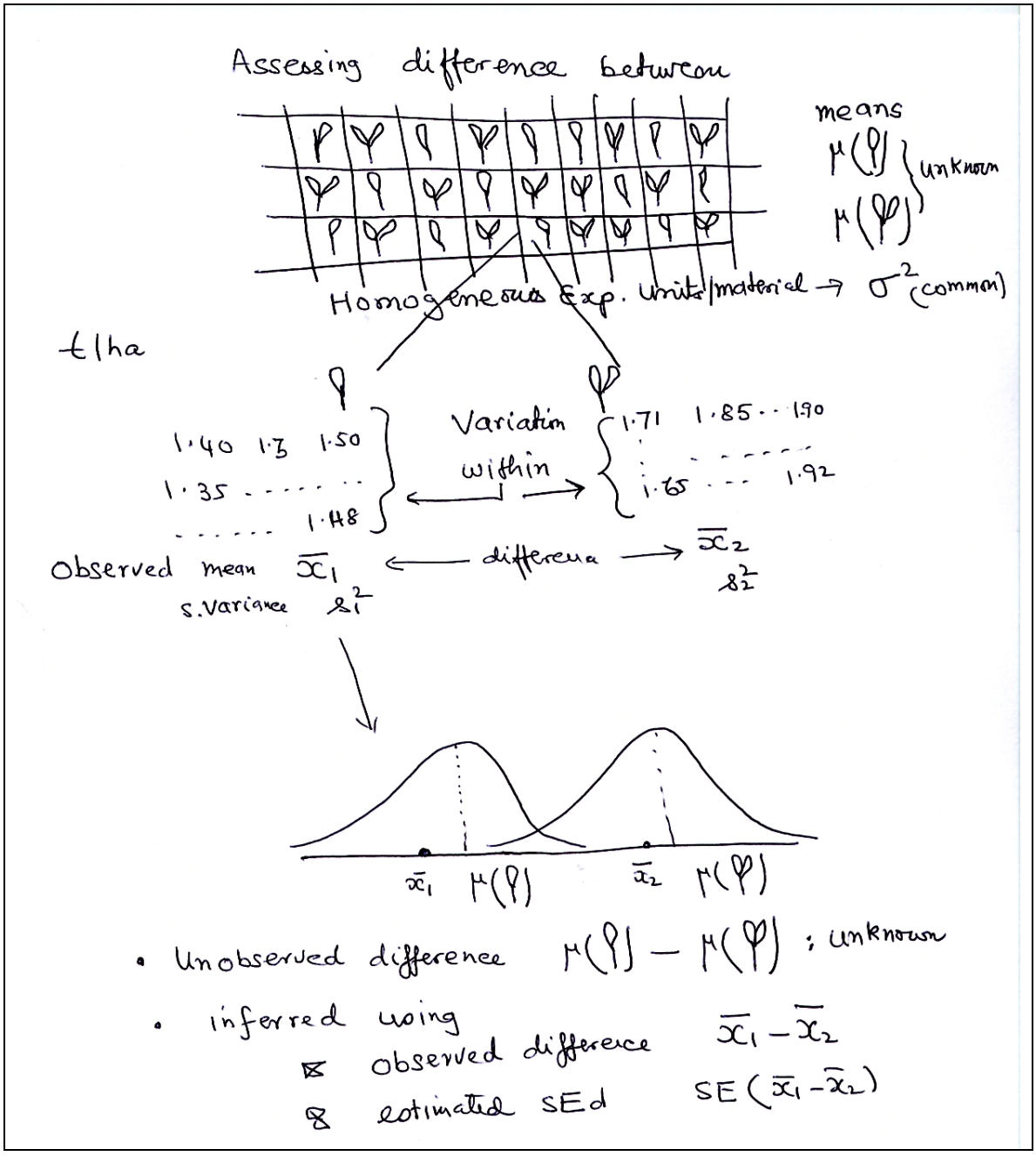
Estimate $SEm = \frac{s}{\sqrt{r}}$.

Est. Heterogeneity $CV(\%) = \frac{s}{\bar{x}} \times 100$.

(c) Uncertainty

The experiment should

- provide a valid (statistical) estimate of error (or standard error of the differences),
- provide confidence limits for the difference of a pair of treatment effects, and
- a test of statistical significance.



(d) Range of Validity

- Conclusions drawn from one or few experiments are normally intended for generalization (out-scaling) to some new conditions.

- Therefore, the experiment must include the wide range of conditions representing the target environment.

(e) Operational Convenience

- Easy operation for field preparation,
- preparing field books,
- seed packets preparation,
- sorting packets for machine sowing, and
- measurements, etc.

‘Preaching is fine, but how to practice it?’

Use

R. A. Fisher's Principles of Experimentation

- randomization,
- replication, and
- local control (reduction of error)

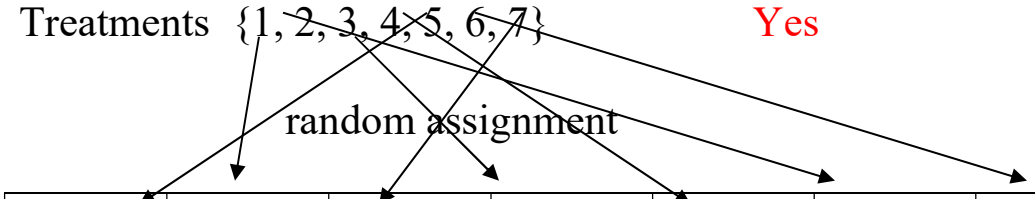
also called Fisher's 3R s for experimentation.

Fisher's 3 R s

1. Randomization

- random allotment of the treatments to the experimental units

Treatments {1, 2, 3, 4, 5, 6, 7} Yes



Plot101	Plot102	Plot103	Plot104	Plot105	Plot106	Plot107
5	1	7	3	4	2	6

- Representative response (over the plots under randomization)
- Validity of estimates of effects and errors
- Minimizes bias (effect of systematic errors) in presence of replications
- Test of significance (enhances random order in uncontrolled errors of any patterns)

No

Treatments {1, 2, 3, 4, 5, 6, 7}

(Non random) Systematic assignment

Plot101	Plot102	Plot103	Plot104	Plot105	Plot106	Plot107
1	2	3	4	5	6	7

Fisher's 3 R s…

2. Replication

- The application of a treatment to more than one unit

- No replication

T1	T2	T3

- replications

T1	T2	T3	T1	T2
T3	T2	T1	T3	T1
T1	T3	T2	T1	T3

Homogeneous experimental material

- Replication is a must for measuring experimental error variance
  - Variation in responses on the experimental units under the same treatment

- Experimental error variance is a must
  - to measure precision of an estimate of a given treatment

    SE(mean) = $\frac{\sigma}{\sqrt{r}}$

  - to compare two or more treatments

- Determining number of replications
  - experimental error variance
  - specified level of error acceptable for treatment effect

Fisher's 3 R s…

3. Local Control or Reduction of Error

An accounting of systematic variation in the experimental material at

- design stage
  - Environment Design

- Covariates (Exp. unit info) /analysis stage
  - Analysis of Covariance

Practical Reality: Consider a situation where the experimental material is neither completely homogeneous nor completely heterogeneous.

- possible to group in the experimental units in homogeneous groups called **blocks**

- allocation of treatments to the units within groups is made through randomization

- account for variation due to groups/blocks

- this is likely to reduce the experimental error

Recommended reading: "Design of Experiments"

- 74 questions in the checklist by Professor JNR Jeffers
  http://www.sawleystudios.co.uk/jnrj/StatisticalCheck/Design.htm

PART 2

Designing of an Experiment

- Environment design (e.g., Blocks in)
- Treatment design (e.g., factorials in)

Randomized Complete Block Design (RCBD, RCB)

Fertility gradient

High	Medium	Low

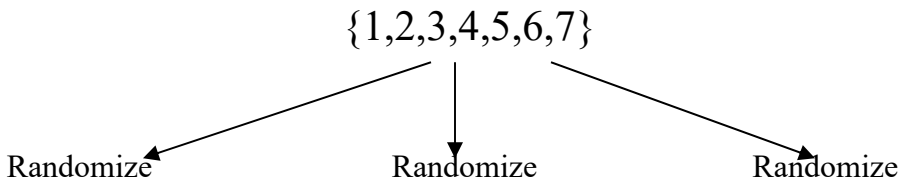
Block 1	**Block-2**	**Block-3**
Plot101:	Plot201:	Plot301:
Plot102:	Plot202:	Plot302:
Plot103:	Plot203:	Plot303:
Plot104:	Plot204:	Plot304:
Plot105:	Plot205:	Plot305:
Plot106:	Plot206:	Plot306:
Plot107:	Plot207:	Plot307:

Field-plot preparation

- Field
- Prepare/mark the blocks (Complete blocks are also called replicates)
- Prepare/mark the plots within each block

Randomization

- Randomly assign treatments to the plots within each block
- Carry out independent randomizations over the blocks



Block 1		**Block-2**		**Block-3**	
Plot101:	3	Plot201:	7	Plot301:	3
Plot102:	7	Plot202:	6	Plot302:	4
Plot103:	1	Plot203:	3	Plot303:	2
Plot104:	4	Plot204:	2	Plot304:	7
Plot105:	6	Plot205:	4	Plot305:	1
Plot106:	2	Plot206:	1	Plot306:	5
Plot107:	5	Plot207:	5	Plot307:	6

Experimental process

Experiment Planning & Management

- Planning
- Planting
- Crop husbandry
- etc.

Data collection

Data Entry

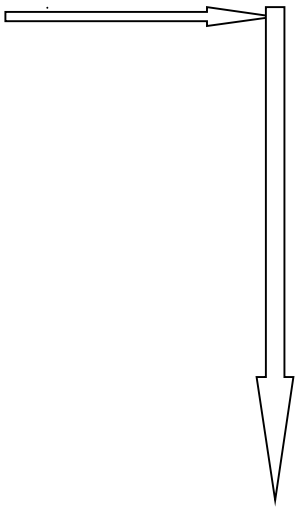
Data Management

Transformation to units for analysis

Statistical Analysis

Presentation of results

Publication



PART 3

Analysis of Data from Designed Experiments

Consider a certain response (y) being obtained on experimental units under the treatments applied according to a block design.

- **Variability in the response** generally forms the basis of analysis of treatment differences and the estimation of experimental error variation.

- **A response model is needed**
  Response (data) = general mean
  + effect of blocking factor(s)
  + effect of treatment applied
  + experimental error

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

  . . .

- ANOVA (**Analysis of variance**, AOV)
  A method which partitions the total variation in the response into the variation due to various sources in the model is called the analysis of variance (ANOVA).

- ANOVA assumptions:
  (i) additivity of factors effects
  (ii) constancy of error variance
  (iii) normality of experimental errors
  (iv) independence of experimental errors

- Design ⟹ Data model ⟹ Design

CONCEPT OF **'BLOCK STRUCTURE '**

J. A. Nelder (1965). The two papers in Proc. Royal Society.

The analysis of randomized experiments with orthogonal block structure:

I. Block structure and the **null analysis of variance**

II. Treatment structure and the **general analysis of variance**

G. N. Wilkinson (1971) … recursive procedure for...in *Biometrika*.

Sum of Squares (SS) and Degrees of Freedom (DF)

Suppose you have $x_1, x_2, \ldots, x_n$ observations which are independent and identically distributed normal variables (e.g., yields from the *r* plots, or means of *v* treatments, etc.)

Degrees of Freedom, DF = number of independent ways/dimensions in which their SS can vary.

$$\text{Sum of squares (from their mean, } \bar{x}=\sum_{i=1}^{i=n} x_i/n)$$

$$SS=(x_1-\bar{x})^2+(x_2-\bar{x})^2+\ldots+(x_n-\bar{x})^2$$

SS is sum of n terms, but note:

$$(x_1-\bar{x})+(x_2-\bar{x})+\ldots+(x_n-\bar{x})=0$$ is always true.

ONE (linear) restriction self imposed

The number of independent terms = n – # restrictions = n-1

‘CONCEPT OF ‘**BLOCK STRUCTURE** ’(continued)

- Field stratified into blocks; each block stratified into plots.

Field

Block 1	**Block-2**	**Block-3**
Plot101:	Plot201:	Plot301:
Plot102:	Plot202:	Plot302:
Plot103:	Plot203:	Plot303:
Plot104:	Plot204:	Plot304:
Plot105:	Plot205:	Plot305:
Plot106:	Plot206:	Plot306:
Plot107:	Plot207:	Plot307:

1. BLOCK STRUCTURE (i.e., no treatments): **NULL ANOVA**

Example: 3 Complete blocks, 7 plots each, 7 treatments
3 x 7 =21 observations

	Field [1] →	Blocks [3] →	Plots[7][Blocks]
DF	0	2	6+6+6 =18
Heterog-eneity	0	CV% (Block size)	CV% (Plot size)

- Seeds, in all the 21 plots, were from the same genotype

Sources	Degrees of freedom
Blocks	3-1=2
Plots within blocks	(7-1) + (7-1) + (7-1) =18
Total	20

Model: data = general mean + Block effects + error

Estimates:		1	3	21
DF	:	-	2	18

NULL ANOVA codes

GENSTAT codes

```
BLOCKSTRUCTURE          BLOCK + BLOCK.PLOTS
ANOVA                   Yield
```

SAS Model statements in PROC ANOVA, PROC GLM

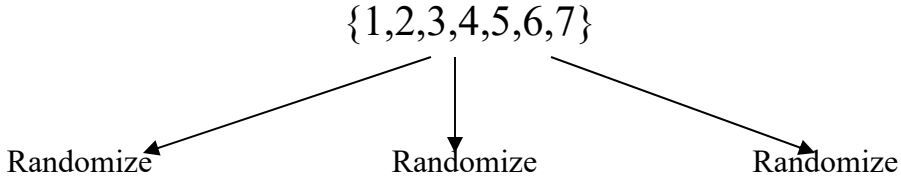
```
Model  Yield = Rep ;
```

R function: aov

```
fit <- aov(Yield ~ Rep, data = wheat)
```

2. TREATMENT STRUCTURE : General **ANOVA**

- Randomly assign treatments to the plots within each block
- independent randomizations over the blocks



Block 1		**Block 2**		**Block 3**	
Plot101:	3	Plot201:	7	Plot301:	3
Plot102:	7	Plot202:	6	Plot302:	4
Plot103:	1	Plot203:	3	Plot303:	2
Plot104:	4	Plot204:	2	Plot304:	7
Plot105:	6	Plot205:	4	Plot305:	1
Plot106:	2	Plot206:	1	Plot306:	5
Plot107:	5	Plot207:	5	Plot307:	6

Sources Of variation

Field [1] → Blocks [3] → Plots[7] [Blocks]

NULL-ANOVA

DF 0 3-1 =2 6 + 6 + 6 = 18

Field [1] → Blocks [3] → Plots[7] ← allot(treatments G1…G7)

Here you randomly allotted the genotypes to plots within blocks:

ANOVA

DF 0 3-1 =2 Genotypes : 7-1 =6

Residual : 18 – 6 =12

⇩

Sources	Degrees of freedom
Blocks	2
Plots within blocks	18
Genotype	6
Residual	12
Total	20

Model: data = general mean + Block effects + treatments + error				
Estimates:	1	3	7	21
DF	-	2	6	12

GENERAL ANOVA codes

GENSTAT codes

BLOCKSTRUCTURE	BLOCK + BLOCK.PLOTS
TREATMENTSTRUCTURE	GENO
ANOVA	Yield

SAS Model statements in PROC ANOVA, PROC GLM

```
Model  Yield = Rep  Genotype ;
```

R function: aov

```
fit <- aov(Yield ~ Rep + Geno, data = wheat)
```

An experiment with two sizes of experimental units

Split-plot Experiment in Complete blocks

Environment design: Complete blocks
Treatment design: Two-factor factorial

Experimental material: Two sizes of experimental units.

Field **Split plots in RCB**

	REP I	REP II	REP III
Large size plot 1	1	1	1
	2 small size plots	2 small size plots	2 small size plots
	3	3	3
	4	4	4
	5	5	5
Large size plot 2	1	1	1
	2 small size plots	2	2 small size plots
	3	3	3
	4	4 small size plots	4
	5	5	5
Large size plot 3	1	1	1
	2 small size plots	2	2 small size plots
	3	3	3
	4	4 small size plots	4
	5	5	5
Large size plot 4	1	1	1
	2 small size plots	2	2 small size plots
	3	3	3
	4	4 small size plots	4
	5	5	5

Large size plots =: Whole plots
Small size plots =: Subplots

	N
Field	**1**
REP s	**3**
Whole plots	**3x4 =12**
Subplots	**3x4x5=60**

Null ANOVA

Source of Variation	**DF**
REP s	**3-1=2**
Whole plots	**3x(4-1) =9**
Subplots	**3x4x(5-1)=48**
Total	**59**

Field — **Split plots in RCB**

REP I	REP I	REP II	REP III
Large size plot 1	1 W1	1	1
	2 W3	2 small size plots	2 small size plots
	3 W5 D_1 (Oct 5)	3 D_2 (Oct 12)	3 D_4 (Oct 26)
	4 W4	4	4
	5 W2	5	5
Large size plot 2	1 W5	1	1
	2 W2	2	2 small size plots
	3 W4 D_4 (Oct 26)	3 D_1 (Oct 5)s	3 D_2 (Oct 12)
	4 W1	4	4
	5 W3	5	5
Large size plot 3	1 W1	1 W5	1
	2 W5	2 D_3 (Oct 19) W1	2 small size plots
	3 W3 D_2 (Oct 12)	3 W4	3 D_3 (Oct 19)
	4 W4	4 small size p W2	4
	5 W2	5 W3	5
Large size plot 4	1 W3	1 W1	1 W4
	2 W1	2 D_4 (Oct 26) W4	2 small size p W2
	3 W5	3 W3	3 W1
	4 W4 D_3 (Oct 19)	4 small size p W2	4 D_1 (Oct 5)
	5 W2	5	5 W5

Large size plots =: Whole plots
Small size plots =: Subplots

	N
Field	**1**
REP s	**3**
Whole plots	**3x4 =12**
Subplots	**3x4x5=60**

General ANOVA

Source of variation	DF
REP s	**3-1=2**
Whole plots	**3x(4-1) =9**
Pl. Date	**4-1 =3**
Residuals (Ea)	**6**
Subplots	**3x4x(5-1)=48**
Variety	**5-1=4**
Pl. Date x Variety	**(5-1)x(4-1)=12**
Residual (Eb)	**(5-1)(3-1)4=32**
Total	**59**

An experiment with three sizes of expt. Units:

An experiment with 5 factors (Planting date, planting depth, plant density, fungicide, lentil genotypes)

Three sizes of plots

Field…..> [REP1 | REP 2 | REP 3| REP 4]

1.Rep stratum DF =4-1=3

2.Each Replicate was divided into **Whole plots**
Rep.Date.Depth stratum DF=4(2x2-1)=12

3.Each Whole plot was divided into **Subplots**
Rep.Date.Depth.Density.Fungicide stratum
DF=4(2x2)(3x3-1)=128

4. Each Subplot was divided into **Sub-subplots**
Rep.Date.Depth.Density.Fungicide.Genotype stratum
DF=4(2x2)(3x3)(4-1)=432

N = 3 x (2 x2) x (3 x3) x 4 =

```
 "General Analysis of Variance."

BLOCK Rep/Date.Depth/Density.Fungicide/Genotype
TREATMENTS Date*Density*Depth*Fungicide*Genotype
ANOVA BY

Over years, it leads to interaction with Year.
```

A little bit for current and in near future:

Singh, M. (2014). **Statistical Research Issues in Crop and Cropping System Experiments for Enhancing Food Security Support**. Journal of Indian Society of Agricultural Statistics, 68(1):1-10.

https://www.ssca.org.in/media/26_19_1_2021_SA_Murari_Singh_FINAL.pdf

Thank you