

Dolores Ledesma 'Didit' Biometrician AVRDC HQ









The World Vegetable Center



#### Familiarize • Know • Learn • Enhance • Appreciate

- basic statistical methods useful in experiments
- strengths and limitations of statistical methods
- importance of Statistics in research







#### General steps in a research study

Identifying a question of interest
 -- formulate hypothesis



- 2. Study design
  - -- what are you going to measure, how, under what conditions?
  - -- what are your treatments or comparison groups?
- 3. Actual experimentation including data collection
- 4. Statistical analysis
- 5. Interpretation/Presentation





#### Before the experiment



#### Before carrying out the experiment, the specific questions that the experiment intends to answer must be <u>clearly stated.</u>





#### Hypothesis

- a statement that indicates what you want to verify as true or false
- examples:
  - Hybrid pepper produces higher yields than traditional pepper varieties
  - -- Grafted tomato has lower bacterial wilt infection during the hot-wet season than non-grafted tomato
  - -- Application of organic fertilizer results in higher pakchoi yield





#### NEED: a plan for experimentation

- what varieties to test
- how to assign varieties to experimental units (e.u.s)
- what "effects" to measure
- experimental layout
- replication (how many e.u.s for each treatment?)
- what and how to collect data





#### Creating a list of measurements

- What measurements to take to draw the right conclusions
  - Primary data to collect depends on your objectives
  - Data needed to increase your understanding about what happened in your experiment
- Other types of data to collect
  - Site-characteristics
  - Climatic conditions
  - Cultural practices
  - Experimental unit measurements







Objective of experimental design

To ensure that measurements made on the experimental units are:

- free from bias
- precise
- scientifically valid









#### Principles of Experimental Design

- Replication
- Randomization
- Error Control



AVRDC The World Vegetable Center

#### Review



## **Basic Statistical Concepts**





### Experimental unit

- the experimental material to which a treatment is applied
- examples
  - a plot of land
  - a single leaf
  - 3 plants in a pot





#### Factors and levels of treatments

- Factors variables that the experimenter varies in the experiment
- Levels various quantities or aspects of a given factor







#### Varietal effect

• expected increase or decrease in varietal response







#### Treatment effect

 expected increase or decrease in response to treatment application

Pesticide application

without with

#### Effect on insect population

75
10
treatment effect is the "decrease" in insect population when pesticide is applied





#### Precision

- refers to the closeness of the measurements to the average
  - Two sets of data:

     Set I –
     2
     5
     12
     25

     Set II 9
     13
     12
     10

Mean = 11 for both sets

Which set has a more precise estimate of the mean?





#### Standard deviation (Std)

• a measure of spread or extent to which observations vary in a population gives a small value if the observations cluster closely about the mean and a large one if they are spread widely

								Mean	Standard deviation
Set I	7	8	8	9	10	10	11	9	1
Set II	1	2	3	9	14	16	18	9	7





#### Standard error (SE)

- measures the variation of sample means instead of individual observations

Variety	S1	S2	S3	S4	S5	Mean	STD	SE
V1	20	24	25	30	35	26.8	5.8	2.6
V2	15	16	12	40	55	27.6	19.0	8.5
V3	10	18	30	70	47	35.0	24.0	10.7
V4	18	28	15	30	23	22.8	6.4	2.9
V5	15	13	20	20	16	16.8	3.1	1.4

#### For each variety, mean is the average weight of 5 tomato sample fruits





standard deviation or standard error

Which should you use ?

- standard deviation if you want to describe the within-sample variability
- standard error if you wish to provide information about the reliability of the sample mean



Experimental Design / D Ledesma AVRDC – The World Vegetable Center Kamphaeng Saen, Thailand – Dec 2015





#### Standard error

- describes the variation among sample means

V1: mean=26.8 and SE=2.6  $\longrightarrow$  26.8 ± 2.6  $\longrightarrow$  24.2 ≤ Mean ≤ 29.4

V2: mean=27.6 and SE=8.5  $\longrightarrow$  27.6 ± 8.5  $\longrightarrow$  19.1 ≤ Mean ≤ 36.1

Which variety has larger variation ?





#### Precision

- achieved through the ff:
- 1. Uniform experimental units
- 2. Careful conduct of all operations before and during the experiment
- 3. More reps

#### 4. Appropriate experimental design







- *Difference:* Observed value True value = *Bias*
- Possible causes
  - subjective assignment of treatments to e.u.s
  - subjective scoring
  - measurements using badly calibrated instruments





## Coefficient of Variation (CV)

 a measure of an experiment's precision (whether it is reliable or not)







#### Types of Scientific Studies

- Measurement of a constant
  - -- height of a single tomato plant
  - -- source of error is "measurement error" if more than one person takes the measurement
- Measurement of a population
  - -- one person measuring the mean height of 10 tomato plants --- sampling error
  - -- two persons measuring the mean height of 10 tomato plants (5 plants per person)-- sources of error are "measurement error" and "sampling error" error

experimental error

#### • Controlling the conditions

- -- not measuring what is readily found but measuring
- the "effect" of treatments applied





#### Types of Scientific Studies

- Measurement of a constant
  - -- height of a single tomato plant
  - -- source of error is "measurement error" if

more than one person takes the measurement









- what is it ?
- why is it important ?





• Defined as the difference between experimental units treated alike

 It can be minimized but never tota There will always be some variatic controlled







- The presence of inherent variation in any experiment is a reality one can't escape from.
- Whether big or small, there's always going to be natural variation in what is being measured.
- If variation did not exist, statistical analyses will not be needed.





Experimental error results from :

 natural variation that exists among experimental units that have the same treatments,

#### and/or

from random variations in the procedures used in an experiment





#### Experiment: Compare Tomato A with Tomato B

## Hypothesis: Yield of Tomato A will be higher than Tomato B

#### How do we "prove" or "disprove" the hypothesis?





Objective: To evaluate the yielding capacity of 2 varieties of tomato



**D** = observed yield difference between A and B

D = (varietal effect)

#### **D** = 2

#### **Questions:**

- After observing these results can we recommend variety A to the farmers ?
- How do we know that "D" was due to varietal difference, and not due to random variations occurring in the field ?





Objective: To evaluate the yielding capacity of 2 varieties of tomato



The observed difference in yields of two plots planted to the same variety "A" is due to the natural variations present in the experimental area.

**E** = yield difference between the same variety, A

E= experimental error

E = 1









- The observed yield difference between A and B varieties is due to *confounding effects* of various factors.
- How then can the researcher separate the varietal effect from the effect due to other sources of variation?





Objective: Prove that yield of Tomato A > Tomato B



D = yield difference between A and B

D = 2



E = yield difference between same variety A

E = 1







When is a varietal effect significant?

(When is "D" considered significant)?











# Statistical test

## F-test of significance

## F = D / E $\uparrow \uparrow$




### Experimental Error

- a "deciding factor" in declaring whether a treatment difference is <u>real</u> and not just due to chance
- expt should be designed so that a measure of experimental error can be obtained (only possible if treatments are replicated)





# Experimental Error

• Not an error in the sense of being wrong

 Defined as the differences among experimental units treated alike

It can be minimized but never totally eliminated.
There will always be some variations that cannot be controlled





### What is replication ?

- Independent application of a treatment to more than one experimental unit
- In an experiment where varieties are being evaluated, *replication* means that each variety has been planted to more than one plot





# Why is replication important?

By planting the same varieties in 2 or more plots:

- experimental error can be measured
- more precise estimates of treatment means even if there are uncontrolled variations in the experiment







Recommended	Local
(Mean=26.8)	(Mean=22.8)

Example: Two tomato varieties, a recommended and a local variety, were planted using five plants for each variety, to know which of the two varieties will give higher yield.







Replication allows us to make accurate estimates of varietal effects even if there are uncontrolled variations in the experiment







#### Recommended Local (Mean=26.8) (Mean=22.8)

# Suppose we planted only one local variety and one recommended variety (*no replication*)







Recommended Local (Mean=26.8) (Mean=22.8)

With no replication the recommended variety does not seem to offer a yield advantage over the local variety





### Notes on replications

 With <u>no replications</u>, there is <u>no measure of</u> <u>experimental error</u> against which the observed differences can be tested

• If the recommended variety was truly higher yielding, the average yield of replications will reflect its real worth





- no hard and fast rule to follow
- the greater the variation, the greater the number of reps required (Note: you would not know the amount of variation at the start of the expt)
- depends on the desired precision
- arbitrary criterion: at least three reps but four or five is better (why???)





Suppose, only 12 plots are available, the combinations of number of varieties and reps that can be accommodated:

<u>Varieties</u>	<u>Reps</u>
6	2
4	3
3	4
2	6

#### How do you choose from among the 4 combinations?





Arbitrary criterion: at least three reps but four or five is better. .. (why???)







Arbitrary criterion: at least three reps but four or five is better. .. (why???)



With large number of reps, one questionable figure will only have a small effect on the mean





Arbitrary criterion: at least three reps but four or five is better. .. (why???)



The real worth of a variety will be reflected by the average effect of the replications





# Pseudo replication

#### Fertilizer trial: 4 N trts and 3 reps



#### Are these 'true' reps ?

The subunits are not independent of one another. The experimental unit is a plot and the three subunit measurements taken are only sample values and not replicate values.





# Pseudo-replication

- Incorrect or false "replication"
  - false replicates are not independent
  - provides sample values and not replicate values
- Violates ANOVA's assumption of <u>independent</u> <u>replicates</u>
  - true replicates increase precision





# True replication



Replication refers to different measurements from *independent* experimental units.





 process of assigning treatments to experimental units, in such a way that each treatment is equally likely to be assigned to any e.u.





### No Randomization



### Low Direction of fertility gradient High





### Systematic Randomization



#### Low Direction of fertility gradient High





# Valid Randomization



#### Low Direction of fertility gradient High





### Systematic arrangement in RCB design

Rep1	V1	V4	V7	V10
	V2	V5	V8	V11
	V3	V6	V9	V12
Rep2	V1	V4	V7	V10
	V2	V5	V8	V11
	V3	V6	V9	V12

Example: 12 varieties, 2 reps





### Example of systematic pattern

### Layout of an experiment with 6 varieties and 3 reps



- Avoid systematic arrangement
- The position of a variety relative to another variety may affect their performances.





# Randomization is necessary

- to avoid bias
  - to obtain a valid estimates of treatment means and experimental error







## Notes on randomization

- Simplest method -- using slips of papers
- Table of random numbers
- Use of statistical software, like CROPSTAT







# Notes on randomization

- never arrange varieties in an order that is merely convenient
- keep in mind that you can never have a perfect randomization

 on rare occasions that you happen to obtain a systematic pattern on your first try, do not hesitate to randomize again







-- provides an estimate of experimental error

### **Randomization**

- -- ensures that the estimate obtained is unbiased
- -- ensures that statistical tests are valid







# **Experimental error**

- cannot be totally eliminated
- always present
- can be kept at a minimum



### Main Sources of Variation

- Inherent variability in the experimental units

- Variation due to non-uniformity in the conduct of the experiment







# Controlling Variation

- Increase no. of replications
- Block properly
- Refine measurement and field plot techniques
- Use appropriate experimental design







# Blocking

assigning similar or uniform
experimental units into a "block"









### **Experimental area**





### How to block







# Two ways of blocking

### Conditions of soil and other environmental factors:

1. known

2. not known







# Source of variation is known

An experiment with 8 treatments is to be conducted in 4 reps.









# Source of variation is known

High Direction of fertility gradient				Low				
Т3	Т8	T2	Т6	Т8	T2	т5	T1	
Т6	Т5	Т7	T1	Т3	Т6	Т2	Т7	
T1	Т4	Т3	т8	Т4	Т7	т6	Т8	
Т7	Т2	Т4	T5	Т5	T1	Т3	Т4	
Blo	ck1	Block	x2	Blo	ck3	: Bl	ock4	

Block perpendicular to the source of variation




Blocking parallel to source of variation

High	Direction of fertility gradient	Low
	Block1	
	Block2	
	Block3	
	Block4	

### Blocking along the source of variation Good or bad ?





#### When direction of source of variation is <u>known</u>



#### Block perpendicular to the source of variation





#### When direction of source of variation is <u>unknown</u>

Block1	Block2
Block3	Block4

- Use compact blocks
- Avoid using long and narrow blocks







Two known sources of variation *in different directions* 



Block <u>perpendicular</u> to the source of greater variation (or use the Latin Square Design)





#### How to block (5 trts and 4 reps)







# Blocking



#### Fertility Gradient





# Plot orientation within a block







# Maximize use of blocks

- Manage field gradients
  - Native Weed Population, Fertility, Moisture
- Manage rater-to-rater differences
  - -- Assign single rater to specific block (rep)
  - -- Avoid having multiple raters in a single block
- Manage effects due to time of rating
  - If a single rater is doing evaluations in a field trial, do all ratings within a block before moving to the next block





#### Rater-to-rater differences



**— — — — —** Path of rater





### Differences in rating done over time



- Single rater taking ratings across blocks in time may confound the treatment effects with time effects (afternoon wilted plants VS more vigorous plants in the morning)
- Any issues with T2? (T2 effects may be biased since plants are more vigorous in the morning)
- - - Path of rater

Experimental Design / D Ledesma AVRDC – The World Vegetable Center Kamphaeng Saen, Thailand – Dec 2015





Time

## Differences in rating done over time



The World Vegetable Center

AVRDC – The World Vegetable Center Kamphaeng Saen, Thailand – Dec 2015

### Why is it important to block properly?

- It helps to reduce experimental error (noise), which could lead to results showing real varietal differences, if truly present.
- With proper blocking, the block-to-block variation can be quantified and mathematically removed from the experimental error, thereby reducing the size of the experimental error.
- Field operations when done by block could further reduce the experimental error, because when these field operations (e.g., harvesting, taking measurements) are done by block, this source of variation coincides with the physical blocks, and can be quantified and mathematically subtracted from the experimental error.





Variations in greenhouse or laboratory experiments

- variations may be caused by position of e.u.s relative to the source of light in lab experiments
- variations due to the position of pots relative to alleys between benches or doors in GH experiments







## Variations in greenhouse experiment

• Variation due to direction of sunlight





- Other sources:
  - -- position of pots relative to alleys between benches
  - -- position of pots relative doors





- Variations in greenhouse experiment
  - Blocking will remove variation due to nonuniform light





- Use the direction of light as basis for blocking
- Orient blocks perpendicular to the direction of light





### Variations in laboratory experiment

- Variation is introduced by nonuniform overhead light intensity at different shelf levels
- Use shelves as blocks to remove the effect of light







# Why block ?



#### Experimental error is reduced when variation among "blocks" is removed from the experimental error





# Groups of experimental designs

- Single-factor experiments
  - 1 Completely Randomized Design (CRD)
  - 2 Randomized Complete Block Design (RCBD)
  - 3 Latin Square Design (LS)
  - 4 Incomplete Block Designs
- Multi-factor experiments
  - 1 CRD
  - 2 RCBD
  - 3 Split plot
  - 4 Split-split plot
  - 5 Strip plot







- simplest design
- treatments are replicated but not blocked
- treatments are assigned completely at random
- difference between same treatments is experimental error
- requires homogeneous experimental units thus, is rarely used for field trials
- more commonly used in laboratory and greenhouse experiments
- treatments do not have to be replicated the same number of times.





<u>Main Advantage</u> is its flexibility - any number of replications may be used

Disadvantages:

- suited only for few treatments
- large experimental error when experimental units are not homogeneous





#### 5 treatments and 4 reps







#### 5 treatments and 4 reps







# Randomized Complete Block Design (RCBD)

- most widely used design in agricultural field expts
- experimental area is divided into blocks and plots are allocated within each block
- each block is a replicate and contains complete set of treatments
- treatments are assigned to plots at random, independently and separately in each block
- block difference is not part of experimental error





#### RCBD: 6 trts, 4 reps



#### Experimental area





# RCBD: 6 trts, 4 reps



Experimental area





# • RCBD: 6 trts, 4 reps



**Experimental area** 





# **RCBD:** 6 trts, 4 reps







# RCBD: 6 trts, 4 reps

Rep1	Т3	T1	Т4	Т6	T5	Т2
Rep2	Т6	Т2	T5	<b>T1</b>	Т3	Т4
Rep3	T1	T4	T2	Т3	T5	Т6
Rep4	т2	Т3	Т6	T4	T1	Т5
Experimental area						





#### Increase in efficiency due to elimination of "block" effect

**ANOV - CRD** 

SV	df	SS	MS	F	
Treatm	nents 3	208	69.33	1.29 <sup>ns</sup>	
Error	12	646	) 53.83		
Total	15	854			
	ANOV ·	- RCB			
SV	df	SS	MS	F	
Block	) 3	576	192.00	24.68 **	
Treatme	ents 3	208	69.33	8.91 **	
Error	9	70	7.78		
Total	15	854			





## Advantage of RCB over CRD

 In RCB, the variability among "blocks" can be measured and removed, resulting in smaller experimental error.







#### Factorial Experiments

- in factorial experiments the effects of multiple factors are investigated simultaneously
- treatments consist of all combinations that can be formed from the levels of different factors
- can be conducted using RCBD and many other designs
- as in single-factor experiments, the treatments are assigned randomly to plots, independently and separately in each block





# Example of factorial treatments

<u>NPK (N</u> )	Manure (M) Treatment		
N <sub>0</sub>	M <sub>0</sub>	$T1 = N_0 M_0$	$T3 = N_1 M_0$
N <sub>1</sub>	$M_1$	T2= N <sub>0</sub> M1	$T4 = N_1 M1$

**T4** = with NPK, with manure  $(N_1M_1)$ 

**T3** = with NPK, without manure  $(N_1M_0)$ 

**T2** = without NPK, with manure  $(N_0M_1)$ 

**T1** = without NPK, without manure  $(N_0M_0)$ 





Trt No	Variety	Growth regulator
1	Bcc11	Composted chicken manure (CM)
2	Bcc11	CM + 1 appln of starter solution (CMST1)
3	Bcc11	CM + 2 appln of starter solution (CMST2)
4	Bcc11	Standard inorganic fertilizer (SI)
5	Bcc19	Composted chicken manure (CM)
6	Bcc19	CM + 1 appln of starter solution (CMST1)
7	Bcc19	CM + 2 appln of starter solution (CMST2)
8	Bcc19	Standard inorganic fertilizer (SI)

2 varieties of pakchoi and 4 starter fertilizer treatments





#### Example: 2x2x2 factorial treatments

Trt No	Shelter	Variety	Irrigation
1	Net	WVCT-1	Drip
2	Net	WVCT-1	Traditional
3	Net	WVCT-2	Drip
4	Net	WVCT-2	Traditional
5	Open	WVCT-1	Drip
6	Open	WVCT-1	Traditional
7	Open	WVCT-2	Drip
8	Open	WVCT-2	Traditional

2 shelter, 2 varieties of pakchoi and 2 Irrigation treatments





# "Factorial design" - a misnomer

- Such an experimental design does not exist
- The term "factorial" refers to the way the treatments are arranged
- When two or more factors are tested in an experiment, the combinations of all the levels of these factors are referred to as "factorial treatments"
- If the factorial treatments are tested in an experiment using RCBD, then we say it is a *"factorial experiment in RCBD"*





# Two Types of Experiments

• Single factor - only one factor varies

• Multifactor - two or more factors vary






# Single-factor experiments

- The result of a single factor experiment is applicable only to the levels at which other factors are maintained in the trial
- Often criticized for their limited application
- Examples:
  - -- Variety Trial
  - -- Fertilizer Trial
  - -- Insecticide Trial





# Multi-factor experiments

- Several factors are considered simultaneously
- It is possible to test not only the effect of each factor but also the changes in its effect when the levels of the other factors vary
- Such changes are known as "interaction effects".







## RCBD for 2x2 factorial experiment

	<u>NPK (N</u> ) N <sub>0</sub> N <sub>1</sub>	<u>Manure (M</u> ) M <sub>0</sub> M <sub>1</sub>	<u>Tre</u> T1 = N <sub>0</sub> T2 = N <sub>0</sub>	$\frac{\text{Patment}}{M_0} \text{ M}_3 = \text{N}_1$ $M_1 \text{ T}_4 = \text{N}_1$	M <sub>0</sub> M1
	T4 (N <sub>1</sub> M1 )	T2	T1	Τ4	
	T1 (N <sub>0</sub> M <sub>0</sub> )	T4	Т3	Т2	
	T3 (N <sub>1</sub> M <sub>0</sub> )	T1	T2	Т3	
	T2 (N <sub>0</sub> M1)	Т3	T4	T1	
	Block1	Block2	Block3	Block4	-





# 2x2 factorial experiment

In the 2x2 factorial experiment involving 2 NPK application rates and 2 manure application rates, it is possible to:

- (1) compare 2 NPK appln rates
- (2) compare 2 manure appln
- (3) determine if the relative effect of the NPK changes with varying amounts of manure applied, or vice versa

Conducting two separate single-factor experiments involving each of the factors can provide information for (1) and (2), but (3) is only possible if both factors are simultaneously tested in the same experiment.





### RCBD for 2x2x2 ("two-by-two-by-two") factor experiment

<u>Variet</u>	<u>y (V</u> )	<u>Fert. rate (N</u> )		anure (N	<u>1</u> )	<u>Treatment</u>			
V <sub>1</sub> V <sub>2</sub>	-	N <sub>0</sub> N <sub>1</sub>	M <sub>0</sub> M <sub>1</sub>	)	T1=V T2=V T3=\ T4=\	/ <sub>1</sub> N <sub>0</sub> M <sub>0</sub> / <sub>1</sub> N <sub>0</sub> M <sub>1</sub> / <sub>1</sub> N <sub>1</sub> M <sub>0</sub> / <sub>1</sub> N <sub>1</sub> M <sub>1</sub>	$T5=V_2$ $T6=V_2$ $T7=V_2$ $T8=V_2$	$N_0M_0$ $N_0M_1$ $N_1M_0$ $N_1M_1$	
	T4 V <sub>1</sub> N <sub>1</sub> M <sub>1</sub>	T7 V <sub>2</sub> N <sub>1</sub> M <sub>0</sub>	Т3	T6	Т3	Т6	Т3	Т6	
	T1 V <sub>1</sub> N <sub>0</sub> M <sub>0</sub>	$ \begin{array}{c} T5\\ V_2N_0M_0 \end{array} $	Τ7	Τ4	Τ7	T4	Т7	Τ4	
	T8 V <sub>2</sub> N <sub>1</sub> M <sub>1</sub>	$\begin{array}{c} T3\\V_1N_1M_0 \end{array}$	Т2	Т8	Т2	Т8	Т2	Т8	
	Т2	Т6	T5	T1	Т5	T1	T5	T1	

Block1

 $V_2 N_0 M_1$ 

 $V_1 N_0 M_1$ 

Block2

Block3

Block4





# Interaction effects

• Can be measured only if two or more factors are tested together in the same experiment

• Changes in the effects of a treatment when the levels of the other factors vary







# Interaction (2 Varieties and 2 Locations)





Location

No interaction

With interaction (difference in *magnitude* of response) A B Location With interaction (difference in *direction* of response)

**V2** 

**V1** 





Adding more levels to a factor quickly increases the number of treatments so include only the levels which are important or practical.

Example:

3 fertilizer rates x 4 varieties = 12 treatments

6 fertilizer rates x 4 varieties = 24 treatments





## Other experimental designs for factorial experiments



- Split plot
- Split-split plot
- Split-split-split plot
- Strip plot
- Strip-split







# Split plot design



- The subplot factor and its interaction with the mainplot factor:
  - -- associated with smaller experimental error
  - -- estimated with higher precision than the mainplot factor
- The precision for the mainplot factor is sacrificed to have greater precision for the subplot factor.





# Split plot is useful when --

- a factor requires large plots
- additional factor is needed to increase scope
- higher precision is desired for comparison of certain factors







# Split plot: guidelines in factor assignment

- Management practices
- Degree of precision
  - assign the more important factor and the less important one to the mainplot
  - the mainplot treatments in split plot design are measured with less precision than RCBD
  - if both factors are equally important, use RCBD





# Split Plot Design







# Split- split plot design







AVRDC

The World Vegetable Center

# Split- split plot design

3 fertilizer trts (MP), 2 varieties (SP), 2 mulching types (SSP)



#### fertility gradient





Split-split plot design

• Extension of the split plot design



- Three plot sizes
  - Different precision levels for various effects



