Analysis of Variance



Analysis of Variance / D Ledesma AVRDC – The World Vegetable Center Kamphaeng Saen, Thailand – Dec 2015

















Statistical hypotheses

Null hypothesis (Ho) states that there are no differences among treatment means

-- Objective is to reject Ho so that we can conclude that differences exist among treatments.

Alternative hypothesis (Ha) states the opposite of Ho







Objective: To compare the shelf life (sl) in days of 4 chili pepper varieties (V1, V2, V3, V4)

<u>Null hypothesis</u> Ho: $sl_1 = sl_2 = sl_3 = sl_4$

Alternative hypothesis Ha: $sl_1 \neq sl_2 \neq sl_3 \neq sl_4$







Objective: To compare the firmness of tomato fruits with and without packaging

Null hypothesis Ho: with p = w/out pAlternative hypothesis Ha: with $p \neq w/out p$







Objective: To assess the effect of 2 irrigation types (drip, furrow) on fruit weight (fwt) of chili pepper

Null hypothesis

Ho: fwtdrip = fwtfurrow

Alternative hypothesis Ha: fwtdrip \neq fwtfurrow





Test of significance

<u>Objective</u> is to reject 'Ho' so that it can be concluded that significant differences exist among treatments.

F = TrMS/EMS

- If F-test is significant, reject Ho and accept Ha.
- If F-test is nonsignificant, we do not accept Ho, rather, we say that the test failed to detect significant differences





Completely Randomized Design (CRD) 5 tomato varieties (V1, V2,....V5), 2 replications Experimental unit = one plot, each measuring 3m x 4m. Yield is measured on each plot

Y1	Y2	Y3	Y4	Y5
V1	V4	V1	V3	V4
Y6	Y7	Y8	Y9	Y10
V2	V3	V5	V2	V5

Y1, Y2, Y3, Y4, Y5, Y6, Y7, Y8, Y9, Y10 = plot yields





Case 1



$$Y_1 = Y_2 = Y_3 = Y_4 = Y_5 = Y_6 = Y_7 = Y_8 = Y_9 = Y_{10} = '4'$$

Mean = 4 Variance = 0 (no variation)





Case 2









The <u>total variation</u> (*variance=2.2*) among these 10 quantities is called the **total sum of squares (TSS)** and can be computed through the <u>analysis of variance</u> (ANOVA).





Measures of Central Tendency

Describe the middle or center of distribution

- Mean sum of the observations divided by the number of observations
 (6+5+4+4+3+3+2+2+1) ÷ 10 = 3.3
- Median number where half of the observations are above and half are below it.

6 5 4 4 3 3 3 2 2 1 = (3+3) ÷ 2 = 3

• Mode – value that occurs most frequently = 3



Measures of Dispersion

Describe how data spreads out or cluster around the middle or center of distribution

• Variance or standard deviation

Range





Sample mean and variance

40	40	Mean = $\frac{\sum X_i}{\sum X_i}$
	10	<i>n</i>
40	40	$= 40 + 40 + \dots + 40 = 40$
40	40	12
40	40	Var = $\Sigma (X_i - \overline{X})^2$
40	40	n-1
40	40	$= (40-40)^2 + \dots + (40-40)^2 = 0$

Standard error of the mean =
$$\sqrt{\frac{\text{var}}{n}} = \sqrt{\frac{0}{12}} = \mathbf{0}$$

(Sem)





Sample mean and variance

40
40
40
40
40
40

Mean =
$$\frac{\sum X_i}{n}$$

= $\frac{36+36+....+40}{12}$ = 38

Var =
$$\frac{\sum (X_i - \overline{X})^2}{n-1}$$

= $\frac{(36-38)^2 + (40-38)^2 \dots + (40-38)^2}{11}$ = 4.36

Sem =
$$\sqrt{\frac{\text{var}}{n}} = \sqrt{\frac{4.36}{12}}$$
 = **0.60**

 $\mu = \overline{X} \pm \text{Sem} = 38 \pm 0.60 = (37.4, 38.60)$





Sample mean and variance

47
40
49
38
42
41

ŀ

Mean =
$$\frac{\sum X_i}{n}$$

= $\frac{35+38+....+42+41}{12}$ = **39.1**
Var = $\frac{\sum (X_i - \overline{X})^2}{n-1}$
= $\frac{(35-39.1)^2 + (38-39.1)^2...+(41-39.1)^2}{11}$ = **28.3**
Sem = $\sqrt{\frac{\text{var}}{n}} = \sqrt{\frac{28.3}{12}}$ = **1.54**

$$u = \overline{X} \pm Sem = 39.1 \pm 1.54 = (37.6, 40.6)$$





Analysis of Variance

The analysis of variance (ANOVA)

- identifies sources of variability
- tests if variability due to treatments is real and not due to random variations





The different *sources of variation* are dictated by the experimental design used:

Completely randomized design (CRD) *TSS = treatment + exptal error*

Randomized complete block design (RCBD) TSS = treatment + block + exptal error













ANOVA format

t = no. of treatments r = no. of reps

Completely Randomized Design (CRD)				
SV	df	SS	MS	F
Treatment	t-1	TrSS	TrMS = TrSS / (t-1)	TrMS / EMS
Error	t(r-1)	ESS	EMS = ESS / t(r-1)	
Total	tr-1	TSS		

TrMS - estimate of variation among treatments

EMS - estimate of the inherent variations within treatments.

- The null hypothesis of "no treatment difference" can be done by comparing TrMS and EMS
- If there are no differences in treatment means, TrMS and EMS should be very similar.



One-way ANOVA (CRD)

The objective is to identify how much of this variation is explained by treatment and how much is unexplained.

Trt1	Trt2
40	40
40	40
40	40
40	40
40	40
40	40

Source of variation	df	SS	MS
Treatment	t-1 =1	0	0
Error	t(r-1)=2(6-1)=10	0	0
Total	tr-1 = 2(6)-1 = 11	0	0

One-way ANOVA (CRD)

The objective is to identify how much of this variation is explained by treatment and how much is unexplained.

Trt1	Trt2	_			
36	40	Source of	df	SS	MS
36	40	variation			
36	40	Treatment	t-1 = 2-1 = 1	48	48
36	40	Error	t(r-1)=2(6-1)=10	0	0
36	40	Total	tr-1 = 2(6)-1 = 11	48	4.36
36	40				

One-way ANOVA (CRD)

The objective is to identify how much of this variation is explained by treatment and how much is unexplained.

Trt1	Trt2				
35	47	Source of	df	SS	MS
38	40	variation			
36	49	Treatment	t-1 =1	169.8	169.8
20	20	Error	t(r-1)=2(6-1)=10	142.2	14.2
30	38	Total	tr-1 = 2(6)-1 = 11	310.9	28.3
39	42				
34	41	le the year	ciability botwoon	trootm	onte

Is the variability between treatments different from random error ?

TSS = TrSS + BlockSS + ESS

TSS = TrSS + BlockSS + ESS

Analysis of Variance

- The ANOVA procedure partitions the total variation (TSS) into components
- Each component represents a different source of variation (SV) depending on the experimental design

Hypothesis testing

Variety	Rep	Fruit wt
V1	1	39.4
V2	1	40.4
V3	1	26.2
V1	2	42.5
V2	2	41.6
V3	2	25.9
V1	3	37.0
V2	3	41.1
V3	3	26.8
V1	4	43.4
V2	4	41.5
V3	4	32.2

<u>Ho:</u> No differences among varieties (i.e., variability among varieties not different from random error)

<u>Objective</u>: To reject *Ho* so that it can be concluded that differences among varieties exist.

ANOVA Example - RCBD

ANOVA for fruit weight (kg/plot)

SV	df	SS	MS	F	Pr > F
Rep	3	30.4733	10.1578	2.67 ^{ns}	0.1410
Variety	2	457.4150	228.7075	60.21**	0.0001
Error	6	22.7917	3.7986		
Total	11	510.6800			
C.V. = 5.3 %					

** - significant at 1% level

^{ns} – not significant

Significance level What it means

1% (**) chances are < 1 in 100 that all observed differences among the treatments are due to chance

5% (*) chances are < 5 in 100 that all observed differences among the treatments are due to chance

Standard error of the mean

Estimate of the treatment mean: $\overline{\mathbf{X}}$

Standard error of the treatment mean (SEM):

$$= \sqrt{\frac{\text{var}}{n}} = \sqrt{\frac{\text{EMS}}{r}}$$
$$\text{SEM} = \sqrt{\frac{3.7986}{4}} = 0.97$$

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Standard error of the difference

Estimate of the difference between 2 treatment means:

$$X_i - X_j$$

Standard error of the difference between 2 means (SED)

$$= \sqrt{\frac{2EMS}{r}}$$

SED = $\sqrt{\frac{2(3.7986)}{4}}$ = 1.38

Least Significant Difference (LSD)

t-table

	t-v	alue
df	5%	1%
1 2 3 4 5 6 7 8	$ \begin{array}{r} 12.70600 \\ 4.30297 \\ 3.18212 \\ 2.77626 \\ 2.57049 \\ 2.44689 \\ 2.36465 \\ 2.30606 \\ \end{array} $	63.65699 9.92509 5.84016 4.60521 4.03249 3.70711 3.49886 3.35469
9	2.26223	3.24918
10	2.22823/	3.16871
11	2.20108	3.10536
12.	- 2.17891 -	

$$t_{(0.05, 6)} = 2.45$$

 $t_{(0.01, 6)} = 3.71$

Least Significant Difference (LSD)

LSD_{.05} = t x SED = 2.45 x 1.38 = 3.38LSD_{.01} = t x SED = 3.71×1.38 = 5.12

If the difference between 2 treatment means is greater than the LSD value, then the means are significantly different.

LSD example

LSD_{.05} = t x SED
= 2.45 x 1.38
=
$$3.38$$

LSD_{.01} = t x SED
= 3.71×1.38
= 5.12

Mean difference between varieties:

V1 = 5.32
V2 = 9.01
$$\longrightarrow$$
 Difference: 9.01 - 5.32 = 3.69

Since treatment mean difference (3.69) is greater than the LSD.₀₅=3.38, then the treatment means are significantly different at 5%, but not at 1% level.

Trt	Mean		Trt	Mean
T1	33.5		T4	42.5
T2	38.4	LSD _{.05} = 7.3	T5	39.6
Т3	33.0		T2	38.4
T4	42.5		T1	33.5
T5	39.6		Т3	33.0
Т6	31.2		Т6	25.9

1. Arrange means in descending order.

2. Subtract the LSD from the highest mean: 42.5 - 7.3 = 35.2

Trt	Mean		Trt	Mean
T1	33.5		T4	42.5
T2	38.4		T5	39.6
Т3	33.0	$LSD_{.05} = 7.3$	T2	38.4
T4	42.5		T1	33.5
T5	39.6		Т3	33.0
Т6	31.2		Т6	31.2

3. All means less than the difference (35.2) are significantly different from the highest mean. T1,T3 and T6 are significantly different from T4.

4. Draw a vertical line connecting the means that are not significantly different from the largest mean.

Trt	Mean		Trt	Mean
T1	33.5		T4	42.5
T2	38.4		T5	39.6
Т3	33.0	$L3D_{.05} - 7.3$	T2	38.4
T4	42.5		T1	33.5
T5	39.6		Т3	33.0
Т6	31.2		Т6	31.2

5. Repeat process using the 2nd highest mean. \rightarrow 39.6 – 7.3 = 32.3

6. Only T6 is significantly different from T5. Draw another vertical line connecting the means that are not significantly different from T5.

7. Repeat process using the 3rd highest mean. \rightarrow 38.4 – 7.3 = 31.1

Trt	Mean		Trt	Mean	a
T1	33.5		T4	42.5	b
T2	38.4	→	T5	39.6	(
Т3	33.0	$LSD_{.05} = 7.3$	T2	38.4	
T4	42.5		T1	33.5	
T5	39.6		Т3	33.0	
Т6	31.2		Т6	31.2	

 Since no means are lower than the difference, there are no treatments significantly different from T2. Draw another vertical line connecting T2 and the rest of the means. Assign a letter to each vertical line.

Trt	Mean		Trt	Mean]
T1	33.5		T4	42.5	a
T2	38.4		T5	39.6	ab
Т3	33.0		T2	38.4	abc
T4	42.5	$L_{.05} - 7.5$	T1	33.5	bc
T5	39.6		Т3	33.0	bc
Т6	31.2		T6	31.2	c

9. Use of letters, without the vertical line.

Example of mean comparison using LSD

Packaging	No of reps	Weight loss (%)	Mean difference
MAP (M)	3	41.2	
PE (P)	3	40.6	(M-P) 41.2 – 40.6 = 0.6 ^{ns}
Open (O)	3	27.8	(M-O) 41.2 – 27.8 = 13.4 **
			(P-O) 40.6 – 27.8 = 12.8 **
LSD (0.05) = 3.9			
LSD (0.01) = 5.6			

Non-significant F-test

What does a nonsignificant (ns) F-test mean?

ANOVA for tomato fruit firmness from a postharvest trial in CRD involving 6 treatments and 3 replications

SV	df	SS	MS	F	Pr > F		
Treatment	5	2.9625	0.5925	2.48 ^{ns}	0.1034		
Error	12	2.8692	0.2391				
Total	17	5.8317					
C.V. = 12.2 %							
ns - not signif	ns - not significant						

Non-significant F-test

the experiment fails to detect **significant** differences among treatments or among blocks

Non-significant F-test

Two-way ANOVA - RCBD

Ho: C32 = C99 = Ck Ha: C32 ≠ C99 ≠ Ck

Block			
1	33	12	52
	C32	Ck	C99
2	13	48	30
	Ck	C99	C32
3	30	15	58
	C32	Ck	C99
4	53	34	18
	C99	C32	Ck

Chili		Fruit weight
Variety	Block	(g)
C32	1	33
Ck	1	12
C99	1	52
Ck	2	13
C99	2	48
C32	2	30
C32	3	30
Ck	3	15
C99	3	58
C99	4	53
C32	4	34
Ck	4	18

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Ho: C32 = C99 = Ck	Ha: C32 ≠ C99 ≠ Ck
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ANOVA for fruit weight (g) of chili from a trial involving 3 varieties and 4 replications							
SV	df	SS	MS	F	Pr > F		
Replication	3	40.0000	13.3333	1.80 ^{ns}	0.2477		
Variety	2	2935.5000	1467.7500	971.90 **	<0.0001		
Error	6	44.5000	7.4167				
Total	11	3020.0000					
C.V. = 8.3 %							
** significant a	** significant at P<0.01; ^{ns} - not significant						

ANOVA based on RCBD

(6 bacterial strains and 3 reps)

ANOVA for disease incidence					
SV	df	SS	MS	F	Pr > F
Replication	2	0.3534	0.1767	39.54**	<0.0001
Strains	5	0.5356	0.1071	23.97 **	<0.0001
Error	10	0.0447	0.0045		
Total	17	0.9336			
C.V. = 15.7 %	1				
** significant a	at P<0.01				

Factorial experiments

• Two or more factors are tested simultaneously in a single trial

• Objective is to assess interaction between test factors

Factorial experiments (RCBD)

Objective: To determine the effect of fertilizer application on yield of chili peppers

Treatments: 3 Nrates = 0, 60, 90 kg/ha, 2 chili varieties (V1, V2), 4 reps

Trt No.	Trt combinations	
	Nitrogen (kg/ha)	Variety
1	0	V1
2	60	V1
3	90	V1
4	0	V2
5	60	V2
6	90	V2

Source of variation	df	
Block(rep)	r-1	4-1= 3
Factor A	a-1	3-1= 2
Factor B	b-1	2-1= 1
АхВ	(a-1)(b-1)	(3-1)(2-1)= <mark>2</mark>
Error	(r-1)(ab-1)	(4-1)(6-1)= 15
Total	rab-1	(4)(3)(2)-1= 23

Factorial experiments

Two or more factors are tested simultaneously in a single trial

Example: To determine the effect of packaging on firmness (kg) of two tomato varieties, a 2x3 factorial experiment in RCBD was conducted.

Block						
1	w/P	Wo/P	Wo/P	w/P	Wo/P	w/P
	Ck	CLN	TLCV	CLN	Ck	TLCV
	1.15	1.28	1.40	2.10	1.70	1.63
2	Wo/P	w/P	Wo/P	Wo/P	w/P	w/P
	TLCV	Ck	Ck	CLN	TLCV	CLN
	1.80	1.03	1.65	1.37	<i>1.53</i>	2.15
3	w/P	Wo/P	w/P	Wo/P	w/P	Wo/P
	CLN	CLN	TLCV	Ck	Ck	TLCV
	2.65	1.48	1.58	1.55	1.09	1.85

Factorial experiments

Packaging	Variety	Rep 1	Rep 2	Rep 3
with	TLCV	1.63	1.53	1.58
with	CLN	2.10	2.15	2.65
with	Ck	1.15	1.03	1.09
w/out	TLCV	1.40	1.80	1.85
w/out	CLN	1.28	1.37	1.48
w/out	Ck	1.70	1.65	1.55

1 A A A A A A A A A A A A A A A A A A A	
Interaction	-
meeraction	

SV	df	SS	MS	F	Pr > F
Replication	2	0.0151	0.0076	0.50 ^{ns}	0.6223
Packaging (P)	1	0.4020	0.4020	26.49 **	0.0004
Variety (V)	2	0.4588	0.2294	15.12 **	0.0009
V x P	2	0.1506	0.0753	4.96 *	0.0319
Error	10	0.1518	0.0152		
Total	17	1.1783			
C.V. (%) = 29.7	,				
**, * significan	t at P<0.01	and P<0.05,	respectively;		
^{ns} not significa	int				

• Interaction is the change in the effect of one factor when another factor varies.

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

Interaction

Multi-location trial: 6 varieties, 4 reps, 2 locations

Individual analysis			
	Degrees of	freedom (DF)	
SV	Location1	Location2	
Rep	3	3	
Variety	5	5	
Error	15	15	
Total	23	23	

Combined analysis over 2 locations

SV	DF
Location (L)	1
Reps w/in location	6
Variety (V)	5
V x L	5
Pooled error	30
Total	47

Types of GxE Interaction

No interaction: varieties have the same performance for a given trait in both locations; ranking of variety is unchanged between locations.

Types of GxE Interaction

With interaction - (Case 1): Yield varies greatly between locations; ranking of variety is unchanged

Types of GxE Interaction

With interaction - (Case 2): Carotenoid content varies greatly between seasons; ranking of varieties changes with season.

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Split Plot Design

F1	F3	F3	F2
F3	F2	F1	F1
F2	F1	F2	F3
Block1	Block2	Block3	Block4

Source of variation	df	
Mainplot:		
Block	r-1	4-1= 3
Fertilizer (F)	f-1	3-1= 2
Error (a)	(r-1)(f-1)	(3)(2) = 6
Subplot:		

3 fertilizer trts (MP), 2 varieties (SP), in 4 reps

Split Plot Design

Source of variation	df	
Mainplot:		
Block	r-1	4-1= 3
Fertilizer (F)	a-1	3-1= 2
Error (a)	(r-1)(a-1)	(3)(2) = 6
Subplot:		
Variety (V)	b-1	2-1 = 1
FxV	(a-1)(b-1)	(1)(2) = 2
Error (b)	a(r-1)(b-1)	3(3)(1) = 9
Total	rab-1	4(3)(2)-1 = 23

3 fertilizer trts (MP), 2 varieties (SP), in 4 reps

Split-split Plot Design : Layout

Split-split Plot Design: ANOVA

Source of variation	df	
Mainplot:		
Block	r-1	4-1= 3
Fertilizer (F)	a-1	3-1= 2
Error (a)	(r-1)(a-1)	(3)(2) = 6
Subplot:		
Variety (V)	b-1	2-1 = 1
FxV	(a-1)(b-1)	(1)(2) = 2
Error (b)	a(r-1)(b-1)	3(3)(1) = 9
Sub-subplot:		
Plant spacing (P)	c-1	2-1 = 1
FxP	(a-1)(c-1)	(3-1)(2-1) = 2
V x P	(b-1)(c-1)	(2-1)(2-1) = 1
FxVxP	(a-1)(b-1)(c-1)	(3-1)(2-1)(2-1) = 2
Error (c)	ra(b-1)(c-1)	4(3)(2-1)(2-1) = 12
Total	rabc-1	4(3)(2)(2)-1 = 47

Coefficient of variation (CV)

$$CV = \left(\frac{\sqrt{EMS}}{\overline{X}}\right) \times 100$$

- indicates the degree of precision with which the treatments are compared
- the higher the CV, the lower the reliability of the experiment

Coefficient of variation (CV)

CVs vary with

- type of expt
- crop
- character measured

Test procedures for pair comparisons

- Least Significant Difference (LSD)
 used for planned pair comparisons
- Multiple Range Tests (Tukey's, Scheffe's)
 applicable to unplanned pair comparisons
- Single DF contrasts (PSS)

Multiple Range Test

 Each treatment mean can be compared with every other treatment mean

 provides different critical values for each pair of treatments being compared.

One- way table of means for factorial treatments

Packaging	Variety	No. of reps	Firmness	Multiple range comparison
with	TLCV	3	1.68	а
with	CLN	3	1.63	а
with	Ck	3	1.58	ab
without	TLCV	3	1.38	b
without	CLN	3	1.13	С
without	Ck	3	1.09	С

Two-way table of means for factorial treatments

Variety	Packa	ging	V_mean	Mean difference
	without	with		(with- without)
TLCV	1.58 ab	1.68 a	1.63	0.10 ^{ns}
CLN	1.13 c	1.38 b	1.26	0.25 *
Ck	1.09 c	1.63 a	1.36	0.54 **
P_mean	1.27	1.56	1.42	0.29

Misuses of multiple comparison procedures

- When treatments have an obvious structure
- When treatments are quantitative such as rates of fertilizer, plant density, seeding rate, or time
- Factorial treatment combinations are compared by multiple range tests without consideration of significant interactions.

Mean Separation Tests

• **Constant LSD.** In this category, a single LSD value is computed and used to compare all pairs of means.

Ex. Fisher's LSD, Tukey's HSD

- Variable critical values. In this category, the means are ranked and the magnitude of the LSD is determined by the number of intervening means between the two means being compared.
 - Ex. Duncan's multiple range test (DMRT), Waller Duncan

