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APPENDIX

Appendix 1 Statistical analysis of nodule numbers by F test with completely randomized design (unequal of N)

Table 1.1 Data taken from Table 1, summation of nodule numbers was listed as follows:

number of replication	Number of nodules per plant					
	TAL 113	P_{19}	P_1	P_5	P_{21}	
1	12	100	70	40	6	
2	90	230	60	20	20	
3	15	190	26	20	10	
4	60	80	75	25	15	
5	40	47	7	20	8	
6	35	47		7	15	
7	166				3	
8	171					
Total = T.j	589	694	238	132	77	1730= Tt
Mean	73	115	48	22	11	

Total of all observations, Tt = 589 + 694 + --- + 77 = 1730

Correction term, $CT = \frac{Tt^2}{n}$

$= \frac{(1730)^2}{32} = 93528.125$

$SS_{total} = \sum_{i=1}^{n} \sum_{j=1}^{k} X^2_{ij} - CT$

$= (12)^2 + (90)^2 + --- + (3)^2 - 93528.125$

$= 107363.88$

$$SStreatment = \sum_{j=1}^{k} \frac{T_{\cdot j}^2}{nj} = CT$$

$$= \left(\frac{589}{8}\right)^2 + \cdots + \left(\frac{77}{7}\right)^2 - 93528.125$$

$$= 45189.467$$

$$SSrediual = SStotal - SStreatment$$

$$= 62174.41$$

Table 1.2 ANONA table for the randamized complete block design of number of nodules.

Source of Variation	Degree of freedom(df.)	Sum of Square (SS.)	Mean Square (MS.)	F-ratio
treatments	4	45189.467	11297.37	5.09
residual	27	62174.41	2220.51	
Total	31	107363.88		

Mean Square = Sum of Square/degree of freedom

$$\text{F-ratio} = \frac{\text{MS treatments}}{\text{MS residual}}$$

Let's, Null Hypothesis, Ho = There are no significant difference compared between treatments

AT $\alpha = 0.05$, the crical value of F, df (4,27) = 2.73

Since our computed F-ratio, 5.09 > 2.73

∴ The nodule numbers were significantly different compared among strains of mutants. ($p < 0.005$)

Data were paired and were subjected to further step of calculation using Duncan's new multiple range test.

Standard error of the mean, $S\bar{x}$ = $\sqrt{\text{error mean square}/ri}$

$$\text{error mean square}/_{ri} = \frac{\text{error sum square}}{\text{df.of error}X\, ri}$$

$$\text{error sum square}/_{ri} = \sum_{j=1}^{k}\left[\sum_{i=1}^{n} X^2ij = \frac{(\sum_{i=1}^{n} Xij)^2}{ri}\right] /ri$$

$$= \{(12)^2 + (90)^2 + \cdots + (171)^2 - (\tfrac{589}{8})^2\}88 + \cdots$$

$$+ \{(6)^2 + (20)^2 + \cdots + (3)^2 - (\tfrac{77}{7})^2\}/7$$

$$= 9254.48$$

$$\therefore S\bar{x} = \sqrt{\frac{9294.48}{27}} = 18.5537$$

AT $\alpha = 0.05$, df. of error = 27, the significant studentized ranges(SSR.) were as follows:

	p = number of means for range being tested			
SSR.	2	3	4	5
	2.9	3.04	3.13	3.2
LSR.	53.8057	56.4032	58.0731	59.3718

Least significant range (L.S.R.) = SSR. x $S\bar{x}$

Summation of the range of minimal to maximal values of data.

Strain	P_{21}	P_5	P_1	TAL 113	P_{19}
Mean	11	22	48	73	115

Table 1.3 Statistical test for different pairs of mean by Duncan's new multiple range test.

pair-being tested	difference of mean	P	LSR.	Interpretation
P_{19} : P_{21}	104.67	5	59.37	SD+
P_{19} : P_5	93.67	4	58.07	SD+
P_{19} : P_1	68.07	3	56.4	SD+
P_{19} : TAL 113	42.04	2	53.81	SD-
TAL 113 : P_{21}	62.63	4	58.07	SD+
TAL 113 : P_5	51.63	3	56.4	SD-
TAL 113 : P_1	26.03	2	53.81	SD-
P_1 : P_{21}	36.6	3	56.4	SD-
P_1 : P_5	25.6	2	53.81	SD-
P_5 : P_{21}	11.0	2	53.81	SD-

SD+ = significant difference, SD- = no significant difference

c	bc	bc	ab	a
P_{21}	P_5	P_1	TAL 113	P_{19}
			a	
		b		
	c			

From Table 1.3, Line drawn under group of strains indicated no statistical difference decided from the Duncan's new multiple range test. Common letters denoted the same sign, ie no significant difference (SD-), but for different alphabet signified the statistical different of treated data.

Appendix 2 The F test of acetylene reduction activity with completely randomized design. (unequal of N)

Table 2.1 Data taken from Table 1.; the values of acetylene reduction activity were as follows:

number of replications	Acetylene reduction activities (ηmoles C_2H_4/hr/g of nodules				
	TAL 113	P_{19}	P_1	P_5	P_{21}
1	31.3	17.5	7.4	8.5	5.0
2	13.0	8.2	5.6	12.5	21.4
3	10.0	13.4	1.2	13.3	13.3
4	12.0	16.4	8.6	25.0	12.5
5	6.1	28.6	10.3	16.7	80.0
6	35.5	20.0		17.0	33.0
7	17.5				5.0
8	13.0				

$$C.T. = \frac{(538.8)^2}{32} = 9072.045$$

$$SStotal = (31.3)^2 + (13.0)^2 + ---+(5.0)^2 - 9072.045$$

$$= 6329.32$$

$$SStreatment = \frac{(138.4)^2}{8} + \frac{(104.1)^2}{6} + ---+\frac{(170.2)^2}{7} - 9072.045$$

$$= 927.32$$

$$SS.residual = 6329.46 - 927.91 = 5401.99$$

Table 2.2 ANOVA table for the completely randomized design of acetylene reduction activity.

Source of variations	df	SS	MS	F-ratio
treatments	4	927.32	231.83	1.16
residual	27	5401.95	200.07	
total	32	6329.32		

At $\alpha = 0.05$, the critical value of F, df = (4,27) = 2.73

Since, F-ratio = 1.16 < 2.73

$\therefore$ The values of acetylene reduction activity were not significantly different, compared among strains of mutants. ($p > 0.1$)

Appendix 3 The F test of plant wet weight with completely randomized design (unequal of N)

Table 3.1 Data taken from Table 1, summation of plant wet weight was as follows:

	Plant wet weight (g/plant)						
	TAL 113	P_{19}	P_1	P_5	P_{21}	control	
	2.3	3.0	4.4	5.0	3.1	3.2	
	3.6	5.8	6.2	6.2	4.0	2.9	
	3.4	5.0	5.1	4.8	3.1	3.3	
	4.0	6.2	4.6	5.5	3.9	3.9	
	5.8	4.2	4.6	4.4	6.1	3.0	
	4.6	5.8		5.0	7.0	4.4	
	6.1				6.2		
	4.8						
Total	34.6	30.0	24.9	30.9	33.4	20.7	= 174.5
Mean	4.32	5.00	4.98	5.15	4.77	3.45	

$$\text{C.T.} = \frac{(174.5)^2}{38} = 801.322$$

$$\text{SStotal} = (2.3)^2 + (3.6)^2 + \cdots + (4.4)^2 - 801.322$$

$$= 52.3076$$

$$\text{SStreatment} = \frac{(34.6)^2}{8} + \frac{(30.0)^2}{6} + \cdots + \frac{(20.7)^2}{6} - 801.322$$

$$= 12.24$$

$$\therefore \text{SS residual} = 52.3076 - 12.24 = 40.0676$$

Table 3.2 ANOVA table for completely randomized design of plant wet weight.

Source of Variation	df	SS.	MS.	F-ratio
treatments	5	12.24	2.448	1.96
residual	32	40.0676	1.2521	
total	37	52.3076		

At α = 0.05, the critical value of F, df = (5, 32) = 2.51

Since, F-ratio = 1.96 < 2.51

∴ The values of plant wet weight were not significantly different, compared among strains of WT and mutants. ($p > 0.1$)

Appendix 4. The F test of plant height with completely randomized design (unequal of N)

Table 4.1 Data taken from Table 1., summation of plant height was as follows:

	Plant height (cm)						
	TAL 113	P_{19}	P_1	P_5	P_{21}	control	
	30	40	38	42	32	40	
	33	40	39	30	35	37	
	35	39	37	42	33	30	
	38	35	37	30	44	37	
	39	38		40	40	30	
	33				42	30	
total	208	192	151	184	226	204	= 1165
mean	34.7	38.4	37.8	36.8	37.7	34.0	

$$\text{C.T.} = \frac{(1165)^2}{32} = 42413.281$$

$$\text{SStotal} = (30)^2 + (33)^2 + \cdots (30)^2 - 42413.281$$

$$= 551.7180$$

$$\text{SStreatments} = \frac{(208)^2}{6} + \frac{(192)^2}{5} + \cdots \frac{(204)^2}{6} - 42413.281$$

$$= 90.299$$

$$\therefore \text{SSresidual} = 551.7180 - 90.299 = 461.42$$

Table 4.2 ANOVA table for completely randomized design of plant height.

Source of variation	df	SS.	MS.	F-ratio
treatments	5	90.299	18.0598	1.02
residual	26	461.42	17.7469	
total	31	551.7188		

AT $\alpha = 0.05$, the critical value of F, df = (5, 26) = 2.59

Since; F-ratio = 1.02 < 2.59

$\therefore$ The values of plant height were not significantly different, compared among strains of WT and mutants.

Appendix 5 Statistical analysis of the values of O_2 consumption using F test with randomized complete block design.

Table 5.1 Data taken from Table 4., the data analysis of O_2 consumption of mutants was summarized :

Mutant strain (j)	O_2 consumption (µmoles/min/mgprotein)		Ti
	Cell grown in YM	Cell grown in YM + 0.3 M NaCl	
P_1	6.2	13.3	19.5
P_5	4.8	11.5	16.3
P_{19}	4.8	10.0	14.8
P_{21}	4.1	13.4	17.5
T.j	19.9	48.2	68.1 = Tt

The total of ith block, $T_{i.} = \sum_{j=1}^{2} Xij$

ie. $T1, = 6.2 + 13.3 = 19.5$

The total of jth column, $T.j = \sum_{i=1}^{4} Xij$

ie. $T.1 = 6.2 + 4.8 + 4.8 + 4.1 = 19.9$

The grand total, $Tt = \sum_{j=1}^{2} T.j = \sum_{i=1}^{4} Ti.$

$= 19.9 + 48.2 = 19.5 + 16.3 + 14.8 +$

$17.5 = 68.1$

The computing formulas for sums of squares:

$$SStotal = \sum_{j=1}^{2}\sum_{i=1}^{4} X^2_{ij} - CT$$

$$SSblocks = \sum_{j=1}^{4} \frac{T^2_i}{2} - CT$$

$$SStreatments = \sum_{j=1}^{2} \frac{T^2_{.j}}{4} - CT$$

when CT = correction term $= \frac{T^2_t}{n} = \frac{(68.1)^2}{8} = 579.7013$

$$\therefore SStotal = (6.2)^2 + (13.3)^2 + \_\_ + (13.4)^2 - 579.7013$$

$$= 110.3287$$

$$\therefore SSblocks = \frac{(19.5)^2 + (16.3)^2 + \_\_ + (17.5)^2}{2} - 579.7013$$

$$= 5.9137$$

$$\therefore SStreatments = \frac{(19.9)^2 + (48.2)^2}{4} - 579.7013$$

$$= 110.1112$$

$$\therefore SSresidual = SStotal - (SSblocks + SStreatments)$$

$$= 4.3038$$

Table 5.2 ANOVA table for the randomized complete block design of O_2 consumption.

Source of variations	Degree of freedom (df.)	Sum of Square (S.S.)	Mean Square (M.S.)	F-ratio
treatments	1	100.1112	100.1112	69.78
blocks	3	5.9137	1.9712	1.37
residual	3	4.3038	1.4346	
total	7	110.3287		

d.f. (treatments) = k-1 (k - numbers of treatments)

df. (blocks) = n-1 (n = numbers of blocks)

df. (residual) = (n-1)(k-1)

Mean Square = Sum of Square /df.

$$\text{F-ratio of treatments)} = \frac{\text{MS treatments}}{\text{MS residual}}$$

$$\text{F-ratio of block} = \frac{\text{MS blocks}}{\text{MS residual}}$$

1) Compared between treatments.

Ho (null hypothesis) : No effect of treatment on O_2 consumption

At $\alpha = 0.05$, the critical value of F with df. (1, 3) = 10.13

Since F-ratio of treatment (69.78) > the critical value of F (10,13)

∴ The values of O_2 consumption were significantly different compared between treatment with and without salt ($p < 0.005$)

2) Compared among strains.

Ho : The values of O_2 consumption were not significantly different among strains.

At α = 0.05, the critical value of F, df.(3, 3) = 9.28

Since, F-ratio of blocks (1.37) < the critical value of F (9.28)

∴ The values of O_2 consumption were not significantly different among strains of mutants. ($p > 0.1$)

Appendix 6 Statistical analysis of ATPase activities by F test with randomized complete block design.

Table 6.1 Data taken from Table 4., summation of the ATPase activity was as follows:

Mutant strain	ATPase activities (μmoles/min/mg protein)		Total
	YM	YM + 0.3 M NaCl	
P_1	2.7	3.4	6.1
P_5	2.7	3.5	6.2
P_{19}	2.7	2.7	5.4
P_{21}	2.6	2.3	4.9
Total	10.7	11.9	22.6

$$C.T = \frac{(22.6)^2}{8} = 63.845$$

$$SS.total = (2.7)^2 + (3.4)^2 + (2.7)^2 + \ldots + (2.3)^2 - 63.845 = 1.175$$

$$SS.blocks = \frac{(6.1)^2 + (6.2)^2 + \ldots + (4.9)^2}{2} - 63.845 = 0.565$$

$$SS.treatments = \frac{(10.7)^2 + (11.9)^2}{4} - 63.845 = 0.18$$

$$SS.residual = 1.175 - (0.565 + 0.18) = 0.43$$

Table 6.2. ANOVA table for the completely randomized design of ATPase activity.

Source of variation	df.	SS.	MS.	F-ratio
treatments	1	0.18	0.18	1.26
blocks	3	0.565	0.565	1.20
residual	3	0.43	0.1433	
total	7	1.175		

1) Compared between treatments

At $\alpha = 0.05$, the critical value of F, df. (1, 3) = 10.13

F-ratio of treatments, $1.26 < 10.13$

$\therefore$ The values of ATPase activity were not significantly different, compared between treatments with and without salt, $p > 0.1$

2) Compared among strains.

At $\alpha = 0.05$, the critical value of F, df. (3, 3) = 9.28

F-ratio of blocks, $1.20 < 9.28$

$\therefore$ The values of ATPase activity were not significantly different, compared among strains, $p > 0.1$

Appendix 7. Preparation and procedure of electron microscope technique

7.1 Scanning electron microscope (SEM.)

Cell sample in mid-log phase was first prefixed as cell suspension. An equal volume of cell sample was mixed with an equal volume of a mixture of 8% and 4% paraformaldehyde and glutaraldehyde, dissolved in 0.1 M phosphate buffer pH 7.3, used as the fixative agent. The fixation was allowed at room temperature for 2 hrs. Cells were collected into a millipore filtering pad (0.22 μ) and washed once with 0.1 M phosphate buffer pH 7.3. The samples were dehydrated sequentially in a series of grading ethanol, 30%, 50%, 70% and 95% respectively. Soaking in each grading ethanol was allowed at room temperature of 10 min. The final step of dehydration was performed by soaking twice with absolute ethanol for 15 min. the dehydrated sample was then subjected to dry in Samdri Critical Dryer, model 780 (Tousimis USA). After the drying process, sample on filtering pad was mouthed on a brass-stub and subsequently coated with gold by using JEOL. Ion Sputter, model JFC-11000, Japan. Finally, the sample was ready for a visualization by JEOL Scanning Electron Microscope JSM-35 CF, Japan.

7.2 Transmission electron microscope(TEM.)

The cell pellet, collected from a culture of mid-log phase, was used as sample. The sample was subjected to a primary fixation with 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.3 for 24 hrs. The fixative agent was removed by a centrifugation and washed 4 times with 0.1 M phosphate buffer pH 7.3. The secondary fixation was subsequently performed by suspending the sample in 1% OsO_4 at room temperature for 2 hrs. Decantation, washing the fixative agent and dehydration were performed in the same manner as did in the primary

fixation process. The dehydrated sample was embedded in Liquid Spurr Resin, (a mixture of 11.5 g vinyl cyclohexene dioxide, 7 g Dow epoxy resin, 31 g nonenyl succinic anhydride and 0.5 g Dimethyl amino ethanol) and the resin was allowed to polymerize at 65^{o}C for 2 days. Thereafter, the sample was subjected to a thin sectioning on the LKB Ultratome V 2208, Sweden. The 60-90 nm thickness of the sections were selected and placed on the grid. The sample was first stained with 5% uranyl acetate. After washing the staining agent with distilled water, the sample was post-stained with lead acetate solution (1.33 g lead acetate, 1.76 g sodium citrate in 50 ml of distilled water.). The stained sections were dried and were ready for a visualization under the JEOL Transmission Electron Microscope JEM-200 CX, Japan.

BIOGRAPHY

Miss Patcharee Jearanaikoon was born on September 4, 1960 and graduated with the degree of Bachelor of Science in Medical Technology from Mahidol University in 1982.