Three-Way ANOVA and Post-Hoc Comparisons

Optimization of Protoplast Isolation and Regeneration Protocls in Model Monocot Maize

Methodology

This document provides comparative analysis using t-test and ANOVA. In Week 0 data, cells and clumps concentration were compared by digestion time (5 hours and 20 hours) using t-test. From Weeks 1-4, cells and clumps concentrations were compared by digestion time, variety, and treatment using three-way ANOVA per week data.

For the t-test, results show if there are significant difference in cells and clumps concentration of IPB Var 6 under different digestion times.

For the three-way ANOVA, the ANOVA table shows if which order of interaction is significant.

- If three-way interaction is significant (V x D x T), ignore lower order interaction in the ANOVA table and pairwise two-way ANOVA will be done using every level of the other variable as post-hoc testing.
- If any two-way interaction is significant, ignore lower order interaction in the ANOVA table and post-hoc pairwise t-test will include the involved variables and the cells/clumps concentrations will be compared using the first variable grouped by the levels of the second variable, and vice versa, to see the behavior of the interaction. For example, if V x D is significant, the dependent variables will be compared by V at every level of D, and vice versa.
- If one-way is significant, the post-hoc test will be a pairwise t-test on the levels of the significant variable.
- If none is significant, no post-hoc test is required as the results show that the cells/clumps concentration does not depend on the three independent variables.

All p-values of post-hoc tests were adjusted using Bonferroni method. Results will be summarized using means. For parameters with significant differences across groups, letters are superscripted to define their grouping classification. If groups share at least one letter, they are not significantly different. All unit is in hundred thousand.

The tests were performed in RStudio using the stats and rstatix package, and the results were exported and summarized in an Excel file.

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Week 0

The table below shows the result of the t-test done for cells/clumps concentrations comparing digestion time.

Table. Summary of cells and clumps concentrations by digestion time at week 0.

Concentration	5H	20H	Statistic	p-value
Cells	59.4 ± 6.6	84.7 ± 8.9	-3.23	0.0927
Clumps	81.7 ± 15.0	88.5 ± 0.6	-0.64	0.6390

Results show that at week 0, IPB Var 6 do not have significantly different cells and clumps concentrations between digestion times.

Week 1 – Three-Way ANOVA

The table below shows the summary of the Three-Way ANOVA for week 1.

Table. ANOVA table for cells and clumps concentration for week 1.

Concentration	Source of Variation	F-statistic	p-value
cells	D	6.83	0.0110*
	V	0.51	0.6020
	T	3.63	0.0300*
	D x V	1.78	0.1750
	D x T	5.74	0.0040*
	V x T	1.80	0.1350
	D x V x T	1.02	0.4000
clumps	D	0.07	0.7870
	V	2.25	0.1110
	T	0.70	0.5010
	D x V	0.83	0.4400
	D x T	1.56	0.2160
	V x T	1.69	0.1580
	D x V x T	1.04	0.3910

* Significant at $\alpha = 0.05$

The three-way ANOVA shows that difference in cells concentrations is affected by interaction of digestion time and treatment, while none of the independent variables affect difference differences in clumps concentrations.

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The tables below show the post-hoc summary for cells concentrations for week 1.

Week 1 – Cells Post-Hoc D x T

Table. Post-hoc comparison for Week 1 cells concentrations by levels of treatment within digestion time.

Digestion Time	PI0	PI1	PI2
5H	10.7 ± 4.0	18.0 ± 21.7	11.0 ± 4.5
20H	13.4^{a} ± 2.3	16.5^{a} ± 7.6	26.5^{b} ± 13.8

Table. Post-hoc comparison for Week 1 cells concentrations by levels of digestion time within treatment.

Treatment	5H	20H
PI0	10.7^{a} ± 4.0	13.4^{b} ± 2.3
PI1	18.0 ± 21.7	16.5 ± 7.6
PI2	11.0^{a} ± 4.5	26.5^{b} ± 13.8

Significant difference is not present in 5H, but in 20H, PI2 have significantly higher cells concentrations than the other two treatments. Additionally, PI0 and PI2 have significant difference in cells concentrations across digestion times.

Week 2 – Three-Way ANOVA

The table below shows the summary of the Three-Way ANOVA for week 2.

Table. ANOVA table for cells and clumps concentration for week 2.

Concentration	Source of Variation	F-statistic	p-value
cells	D	1.48	0.2270
	V	0.28	0.7540
	T	0.57	0.5660
	D x V	0.28	0.7550
	D x T	1.58	0.2110
	V x T	2.12	0.0850
	D x V x T	1.08	0.3740
clumps	D	26.28	<0.0001*
	V	12.36	<0.0001*
	T	3.28	0.0420*
	D x V	5.06	0.0080*
	D x T	4.04	0.0210*
	V x T	1.17	0.3280
	D x V x T	1.04	0.3930

* Significant at $\alpha = 0.05$

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The three-way ANOVA shows that difference in clumps concentrations is affected by the separate interaction of digestion time with both variety and treatment, while none of the independent variables affect difference differences in cells concentrations.

The tables below show the post-hoc summary for clumps concentrations for week 2.

Week 2 – Clumps Post-Hoc D x V

Table. Post-hoc comparison for Week 2 clumps concentrations by levels of variety within digestion time.

Digestion Time	IPB Var 6	Lagkitan	CML 161
5H	$2.4^{a} \pm 0.8$	$3.5^{b} \pm 1.3$	$3.7^{b} \pm 0.8$
20H	$3.8^{a} \pm 0.9$	$5.4^{b} \pm 1.4$	$3.9^{a} \pm 1.7$

Table. Post-hoc comparison for Week 2 clumps concentrations by levels of digestion time within variety.

Variety	5H	20H
IPB Var 6	$2.4^{a} \pm 0.8$	$3.8^{b} \pm 0.9$
Lagkitan	$3.5^{a} \pm 1.3$	$5.4^{b} \pm 1.4$
CML 161	3.7 ± 0.8	3.9 ± 1.7

Significant difference is present in both 5H and 20H. In 5H, clumps concentration in IPB Var 6 is significantly lower than the other two, while in 20H, clumps concentration in Lagkitan is significantly higher than the other two. These two varieties also have significant differences in clumps concentrations across digestion time.

Week 2 – Clumps Post-Hoc D x T

Table. Post-hoc comparison for Week 2 clumps concentrations by levels of treatment within digestion time.

Digestion Time	PI0	PI1	PI2
5H	3.4 ± 0.9	3.0 ± 1.1	3.2 ± 1.3
20H	$3.7^{a} \pm 1.1$	$4.2^{ab} \pm 1.4$	$5.1^{b} \pm 1.8$

Table. Post-hoc comparison for Week 2 clumps concentrations by levels of digestion time within treatment.

Treatment	5H	20H
PI0	3.4 ± 0.9	3.7 ± 1.1
PI1	$3.0^{a} \pm 1.1$	$4.2^{b} \pm 1.4$
PI2	$3.2^{a} \pm 1.3$	$5.1^{b} \pm 1.8$

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Significant difference is not present in 5H, but in 20H, PI2 have significantly higher clumps concentrations than PI0. Consequently, only PI0 has no significant difference in terms of clumps concentrations across digestion times.

Week 3 – Three-Way ANOVA

The table below shows the summary of the Three-Way ANOVA for week 3.

Table. ANOVA table for cells and clumps concentration for week 3.

Concentration	Source of Variation	F-statistic	p-value
cells	D	24.35	<0.0001*
	V	1.87	0.1610
	T	2.00	0.1410
	D x V	2.94	0.0580
	D x T	3.41	0.0370*
	V x T	0.82	0.5190
	D x V x T	1.54	0.1980
clumps	D	4.20	0.0430*
	V	6.55	0.0020*
	T	2.95	0.0570
	D x V	8.77	0.0003*
	D x T	1.48	0.2330
	V x T	1.22	0.3080
	D x V x T	0.12	0.9760

* Significant at $\alpha = 0.05$

The three-way ANOVA shows that difference in cells concentrations is affected by the interaction of digestion time and treatment, while clumps concentration is affected by the interaction of digestion time and treatment.

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The tables below show the post-hoc summary for clumps concentrations for week 3.

Week 3 – Cells Post-Hoc D x T

Table. Post-hoc comparison for Week 3 cells concentrations by levels of treatment within digestion time.

Digestion Time	PI0	PI1	PI2
5H	20.7 ± 7.6	26.7 ± 22.0	22.4 ± 10.3
20H	35.8^{ab} ± 19.3	32.8^{a} ± 19.0	49.0^{b} ± 20.5

Table. Post-hoc comparison for Week 3 cells concentrations by levels of digestion time within treatment.

Treatment	5H	20H
PI0	20.7^{a} ± 7.6	35.8^{b} ± 19.3
PI1	26.7 ± 22.0	32.8 ± 19.0
PI2	22.4^{a} ± 10.3	49.0^{b} ± 20.5

Significant difference is not present in 5H, but in 20H, PI2 have significantly higher cells concentrations than PI1. Consequently, only PI1 has no significant difference in terms of cells concentrations across digestion times.

Week 3 – Clumps Post-Hoc D x V

Table. Post-hoc comparison for Week 3 clumps concentrations by levels of variety within digestion time.

Digestion Time	IPB Var 6	Lagkitan	CML 161
5H	1.9^{a} ± 0.6	4.6^{b} ± 2.0	4.2^{b} ± 1.8
20H	4.3 ± 1.5	4.3 ± 1.8	3.9 ± 1.5

Table. Post-hoc comparison for Week 3 clumps concentrations by levels of digestion time within variety.

Variety	5H	20H
IPB Var 6	1.9^{a} ± 0.6	4.3^{b} ± 1.5
Lagkitan	4.6 ± 2.0	4.3 ± 1.8
CML 161	4.2 ± 1.8	3.9 ± 1.5

Significant difference is not present in 20H, but in 5H, IPB Var 6 has significantly higher clumps concentrations than the other two variety. Additionally, only IPB Var 6 has significantly higher clumps concentrations across digestion time.

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Week 4 – Three-Way ANOVA

The table below shows the summary of the Three-Way ANOVA for week 4.

Table. ANOVA table for cells and clumps concentration for week 4.

Concentration	Source of Variation	F-statistic	p-value
cells	D	26.56	<0.0001*
	V	5.85	0.0040*
	T	5.59	0.0050*
	D x V	3.85	0.0250*
	D x T	7.96	0.0007*
	V x T	4.04	0.0050*
	D x V x T	1.49	0.2110
clumps	D	8.41	0.0050*
	V	3.34	0.0400*
	T	6.09	0.0030*
	D x V	3.36	0.0390*
	D x T	0.04	0.9620
	V x T	1.42	0.2330
	D x V x T	0.77	0.5470

* Significant at $\alpha = 0.05$

The three-way ANOVA shows that difference in cells concentrations is affected by the two-way interactions, while clumps concentration is affected by the interaction of digestion time and variety.

The tables below show the post-hoc summary for cells concentrations for week 4.

Week 4 – Cells Post-Hoc D x T

Table. Post-hoc comparison for Week 4 cells concentrations by levels of treatment within digestion time.

Digestion Time	PI0	PI1	PI2
5H	20.2 ± 3.8	21.8 ± 4.3	22.1 ± 5.9
20H	34.0^{b} ± 11.8	21.6^{a} ± 5.4	31.1^{b} ± 14.4

Table. Post-hoc comparison for Week 4 cells concentrations by levels of digestion time within treatment.

Treatment	5H	20H
PI0	20.2^{a} ± 3.8	34.0^{b} ± 11.8
PI1	21.8 ± 4.3	21.6 ± 5.4
PI2	22.1^{a} ± 5.9	31.1^{b} ± 14.4

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Significant difference is not present in 5H, but in 20H, PI2 have significantly lower cells concentrations than the other two. Additionally, only PI1 has no significant difference in terms of cells concentrations across digestion times.

Week 4 – Cells Post-Hoc D x V

Table. Post-hoc comparison for Week 4 cells concentrations by levels of variety within digestion time.

Digestion Time	IPB Var 6	Lagkitan	CML 161
5H	19.8 ± 3.5	21.7 ± 3.9	22.7 ± 6.2
20H	28.4^{ab} ± 7.5	23.8^{a} ± 10.0	34.5^{b} ± 15.8

Table. Post-hoc comparison for Week 4 cells concentrations by levels of digestion time within variety.

Variety	5H	20H
IPB Var 6	19.8^{a} ± 3.5	28.4^{b} ± 7.5
Lagkitan	21.7 ± 3.9	23.8 ± 10.0
CML 161	22.7^{a} ± 6.2	34.5^{b} ± 15.8

Significant difference is not present in 5H, but in 20H, CML 161 has significantly higher cells concentration than Lagkitan. Additionally, only Lagkitan has no significantly different cells concentration across digestion time.

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Week 4 – Cells Post-Hoc V x T

Table. Post-hoc comparison for Week 4 cells concentrations by levels of variety within treatment.

Treatment	IPB Var 6	Lagkitan	CML 161
PI0	27.2 ± 9.6	23.8 ± 11.2	30.4 ± 12.4
PI1	23.0 ± 3.4	21.9 ± 5.7	20.3 ± 5.0
PI2	22.0^{a} ± 6.6	22.7^{a} ± 4.8	35.0^{b} ± 16.0

Table. Post-hoc comparison for Week 4 cells concentrations by levels of treatment within variety.

Variety	PI0	PI1	PI2
IPB Var 6	27.2 ± 9.6	23.0 ± 3.4	22.0 ± 6.6
Lagkitan	23.8 ± 11.2	21.9 ± 5.7	22.7 ± 4.8
CML 161	30.4^{ab} ± 12.4	20.3^{a} ± 5.0	35.0^{b} ± 16.0

For treatment, significant difference is only present in PI2 where CML 161 has the highest cells concentration among the three. On the other hand, for variety, only CML 161 has significant difference across treatments, where PI2 is higher than PI1.

Week 4 – Clumps Post-Hoc D x V

Table. Post-hoc comparison for Week 4 clumps concentrations by levels of variety within digestion time.

Digestion Time	IPB Var 6	Lagkitan	CML 161
5H	2.2^{a} ± 0.8	3.3^{b} ± 1.6	3.5^{b} ± 1.4
20H	3.9 ± 1.1	4.7 ± 3.2	3.2 ± 1.3

Table. Post-hoc comparison for Week 4 clumps concentrations by levels of digestion time within variety.

Variety	5H	20H
IPB Var 6	2.2^{a} ± 0.8	3.9^{b} ± 1.1
Lagkitan	3.3 ± 1.6	4.7 ± 3.2
CML 161	3.5 ± 1.4	3.2 ± 1.3

Significant difference is not present in 20H, but in 5H, IPB Var 6 has significantly lower clumps concentrations than the other two variety. Consequently, only IPB Var 6 has significantly higher clumps concentrations across digestion time.

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Summary of Effects

Week	Cells	Clumps
Week 0	Non-significant	Non-significant
Week 1	D (20H) x T (PI0, PI2)	Non-significant
Week 2	Non-significant	D (20H) x T (PI1, PI2) D (5H, 20H) x V (IPB, LAG)
Week 3	D (20H) x T (PI0, PI2)	D (5H) x V (IPB)
Week 4	D (20H) x V (IPB, CML) D (20H) x T (PI0, PI2) V (CML) x T (PI2)	D (5H) x V (IPB)

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