Final Presentation

BITS F421T Thesis

Investigating the Pathogenicity and Host Interactions of *Macrophomina phaseolina*: Insights into Fungal Metabolomics and Cell Wall Immunogenicity

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Thesis Objectives

1. To investigate the temperature-induced metabolic shifts in *Macrophomina phaseolina* (*Mp*) at 28°C and 35°C and identify intracellular and extracellular metabolites as potential biomarkers.
2. To characterize the cell wall composition of *Mp* and study the key monosaccharides, glycosidic linkages, and quantify general sugars, hexoses, uronic acids, and chitin.
3. To assess the immunogenic potential of *Mp* cell wall fractions by studying early pattern-triggered immunity (PTI) markers.

Chapter 1: Metabolomics

Methods:

- Cultures incubated in PDB at 28°C and 35°C for 3 days.
- **Metabolite Extraction:**
  - ICM: Acetonitrile:Water (1:1), freeze-thaw cycles, sonication, and lyophilization.
  - ECM: Chloroform:Methanol:Water (2:5:2), phase separation, and lyophilization.
- **Derivatization:** Methoximation with MeOx; silylation with MSTFA.

	Grams (per 200 mL culture)	
	Myc 28	Myc 35
R1	26.67	15.59
R2	22.99	16.20
R3	25.88	14.32
Avg	25.18	15.37

Data Pre-processing

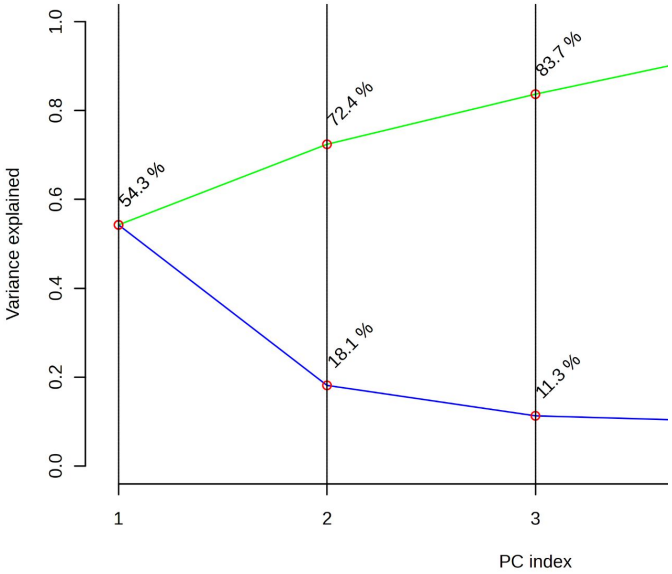
Compound Name		RT			m/z	Area	Height				SI					
Silane, chlorotrimethyl-	0	1.78	0	Target	95	49072101	8175185	ppm	0	Auto(Saturation)	94	892.44	1.33	2.33	ASTM	0
2-Isopropenyl-3,6-dimethylpyra	Ratio of reference ion does not match.		0	Target	148	0	0	ppm	0	Auto	0	0	0	0	ASTM	0
1,2-Butanediol, 2TMS derivative	0	2.191	0	Target	149	42676798	6100337	ppm	0	Auto(Saturation)	79	50.71	1.675	2.675	ASTM	0
1,2-Butanediol, 2TMS derivative	Ratio of reference ion does not match.		0	Target	131	0	0	ppm	0	Auto	0	0	0	0	ASTM	0
Sulfurous acid, dimethyl ester	Ratio of reference ion does not match.		0	Target	79	0	0	ppm	0	Auto	0	0	0	0	ASTM	0
Tetrahydrofuran-D8	0	2.59	0	Target	80	13230051	1480350	ppm	0	Auto(Saturation)	92	1.77	2.07	3.07	ASTM	0
N-Methyltrifluoroacetamide, TM	Ratio of reference ion does not match.		0	Target	184	0	0	ppm	0	Auto	0	0	0	0	ASTM	0
Methylamine, 2TMS derivative	0	4.149	0	Target	160	40592462	7070293	ppm	0	Auto(Saturation)	100	318.89	3.65	4.65	ASTM	0
Methylamine, 2TMS derivative	0	4.26	0	Target	160	33428266	5884845	ppm	0	Auto	99	54.46	3.765	4.765	ASTM	0
2,2,2-Trifluoroethane-1,1-diol, 2	Ratio of reference ion does not match.		0	Target	147	0	0	ppm	0	Auto	0	0	0	0	ASTM	0
Methoxyamine, 2TMS derivative	0	4.965	0	Target	191	42705730	8391236	ppm	0	Auto(Saturation)	99	320.06	4.46	5.46	ASTM	0
Disilathiane, hexamethyl-	0	5.156	0	Target	73	32442973	7405348	ppm	0	Auto(Saturation)	100	432.09	4.655	5.655	ASTM	0
N-(tert-butyldimethylsilyl)-2,2,2	0	5.393	0	Target	77	2237966	1177369	ppm	0	Auto	99	638.93	4.895	5.895	ASTM	0
Lactic Acid, 2TMS derivative	0	6.391	0	Target	147	1268031	482298	ppm	0	Auto	99	83.69	5.895	6.895	ASTM	0
Diglycolic acid, 2TMS derivative	Ratio of reference ion does not match.		0	Target	147	0	0	ppm	0	Auto	0	0	0	0	ASTM	0
Tris(trimethylsilyl)carbamate	0	6.971	0	Target	147	388468	196790	ppm	0	Auto	79	0.51	6.465	7.465	ASTM	0
1-(Trimethylsilyl)-3-[(trimethyls	0	7.583	0	Target	147	14975049	5555315	ppm	0	Auto	100	1557.79	7.08	8.08	ASTM	0
Pentasiloxane, dodecamethyl-	0	7.952	0	Target	281	366488	122181	ppm	0	Auto	92	335.51	7.45	8.45	ASTM	0

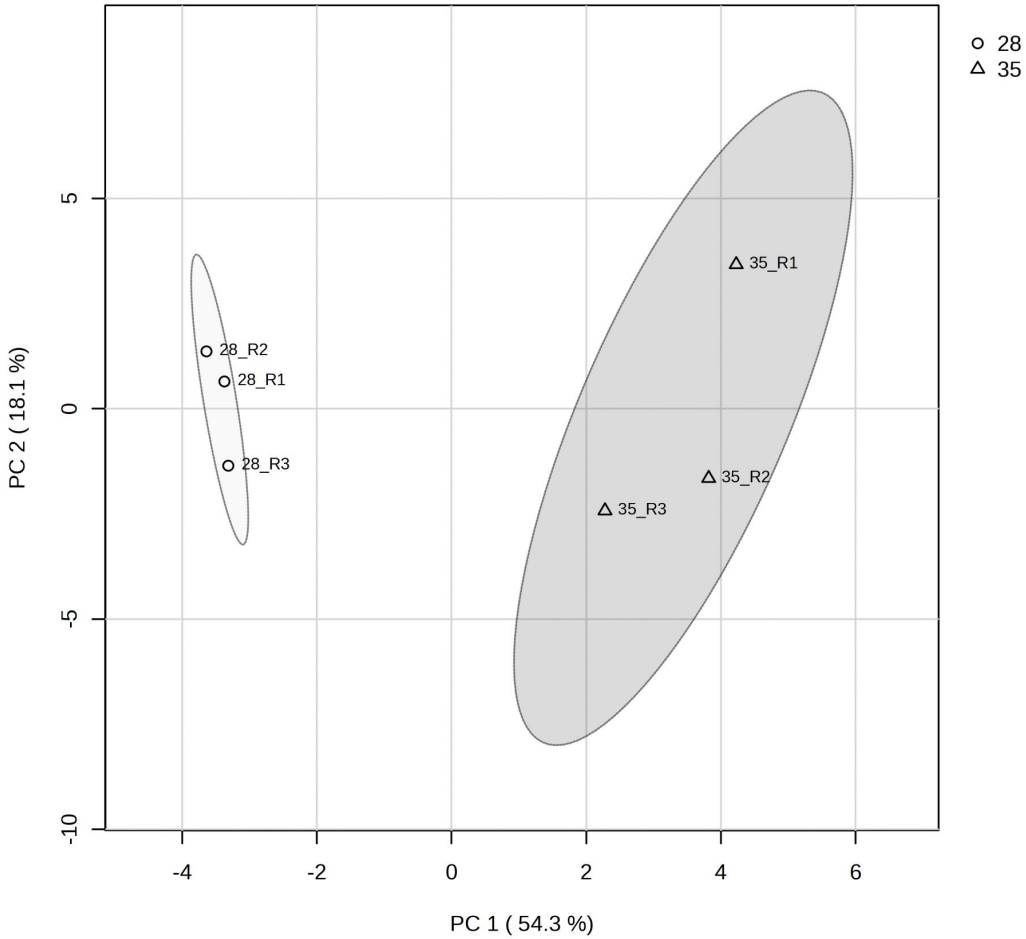
ICM28-1 | ICM28-2 | ICM28-3 | ICM28-4 | ICM35-1 | ICM35-2 | **ICM35-3**

MetaboAnalyst format
(65 metabolites)

Sample	28_R1	28_R2	28_R3	35_R1	35_R2	35_R3
Label	28	28	28	35	35	35
1	2494094	2115479	123673	1731366	2299477	1278848
2	2858675	2036726	3748241	1272235	1571031	809889
3	0	0	0	742626	512866	478331
4	170145	389505	0	0	0	0

ICM Analysis

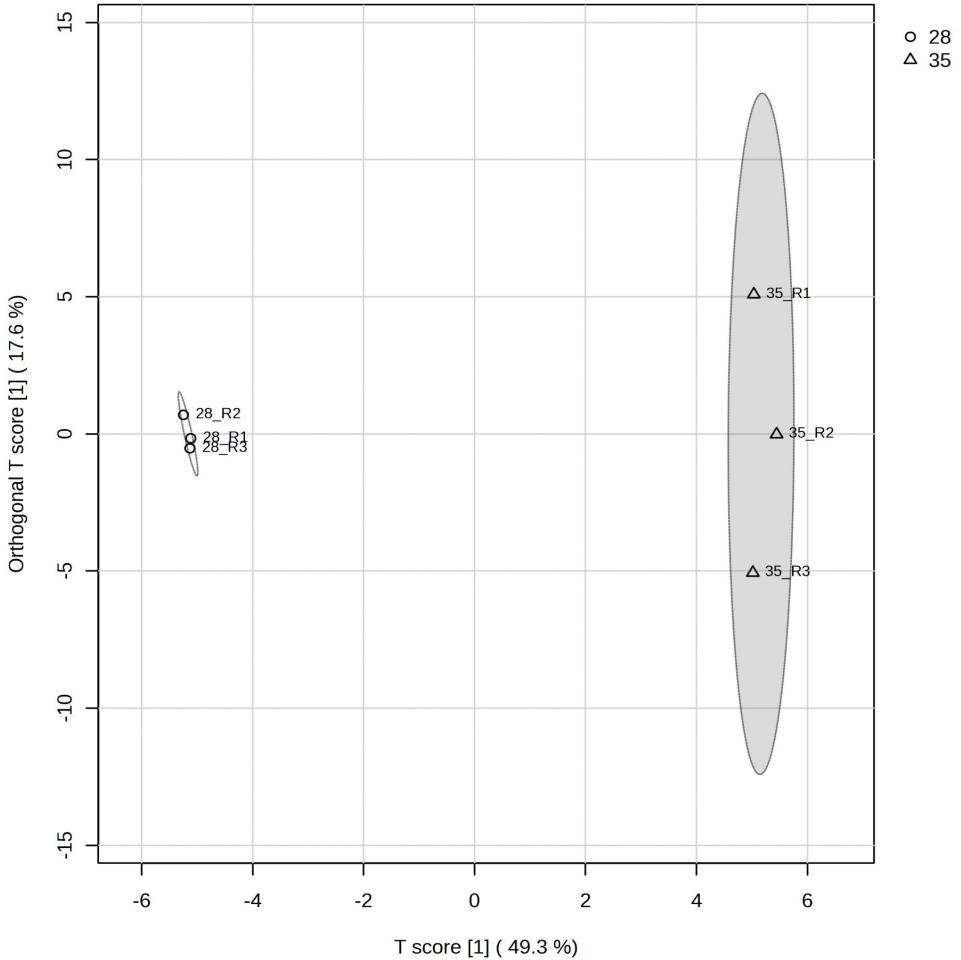




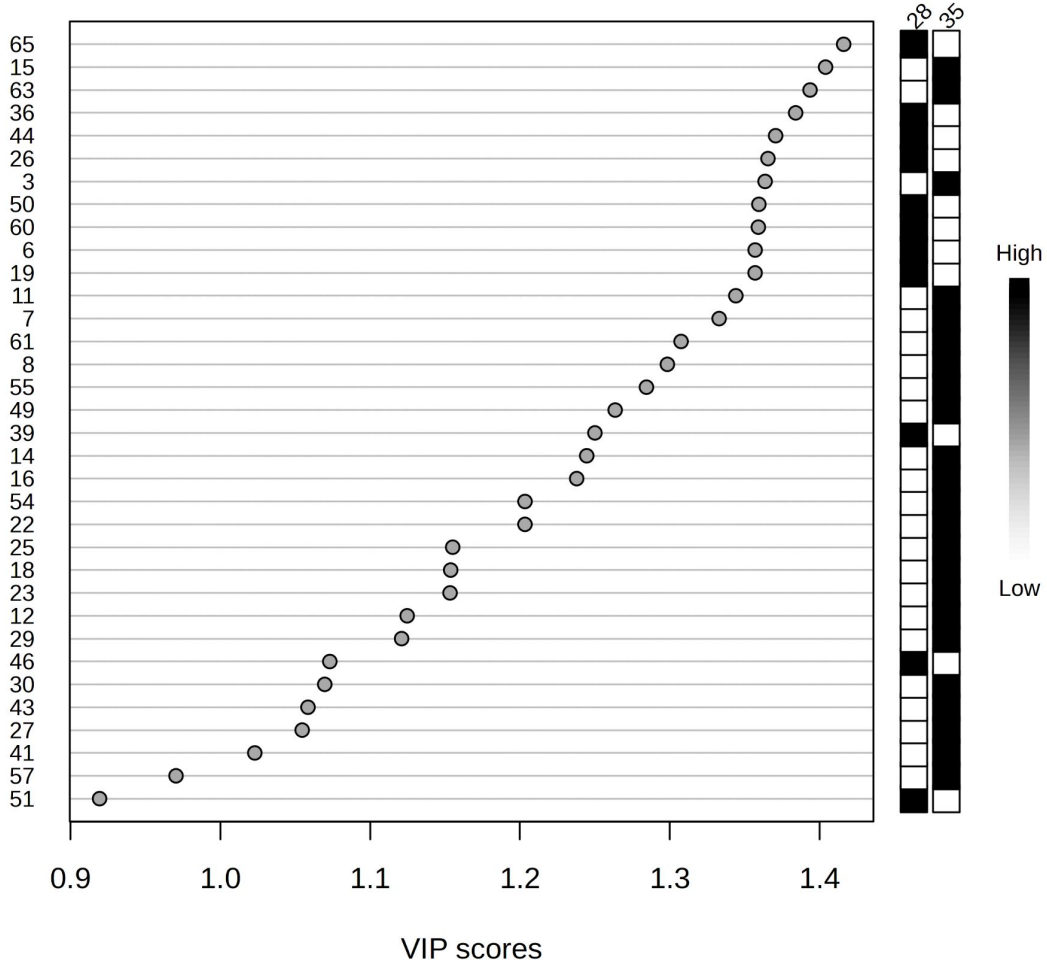
OPLS-DA

	p1	o1
R2X	0.493	0.176
R2Y	0.991	0.0086
Q2	0.917	0.0246

R^2X: 66.9% of total variation in X is explained by the model.
R^2Y: 99.96% of total variation in Y is explained by the model.
Predictive Variation: 49.3% of X correlates directly with 99.96% of Y.
Orthogonal Variation: 17.6% of X is orthogonal and irrelevant to Y.
Q^2: Predictive ability is 0.917

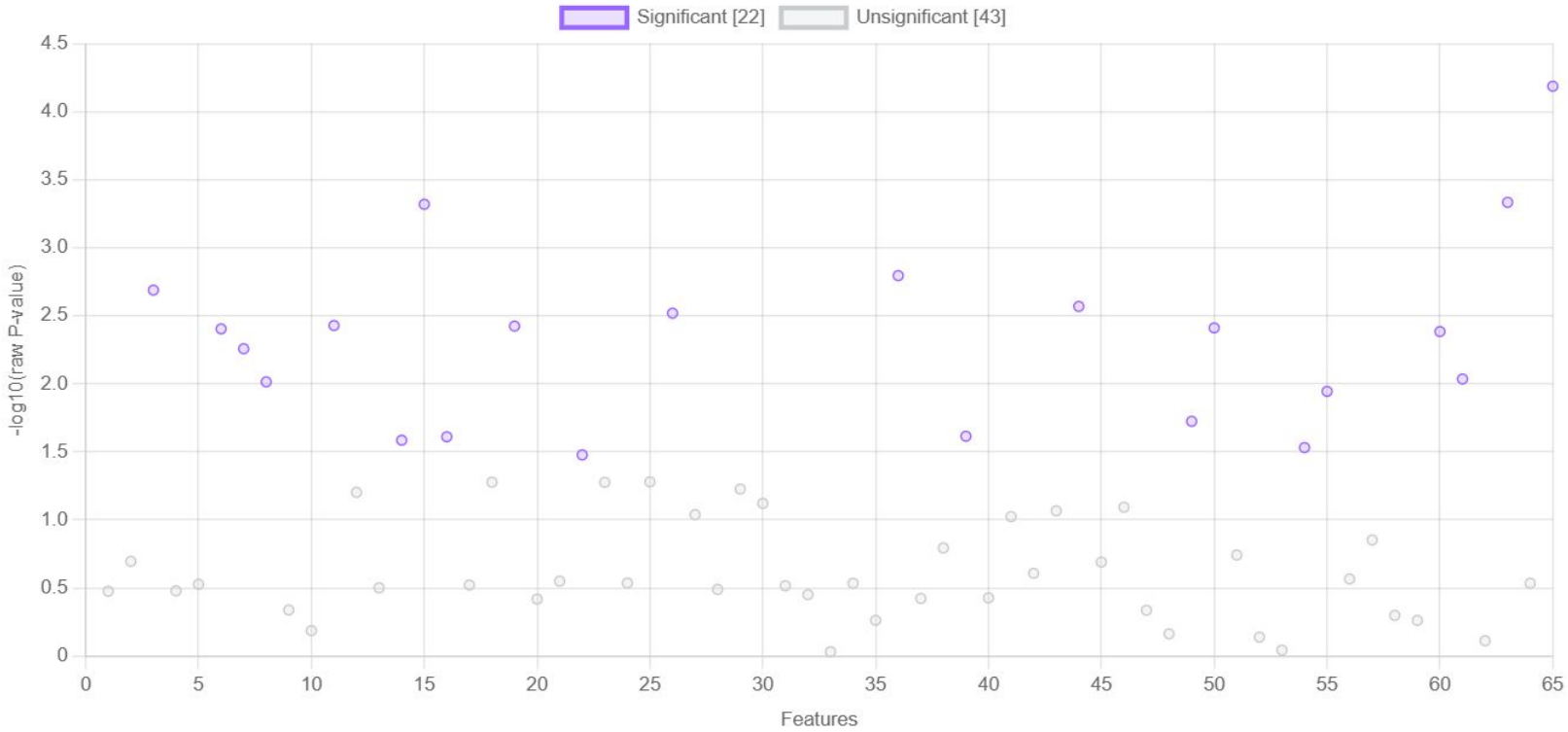


OPLS-DA VIP



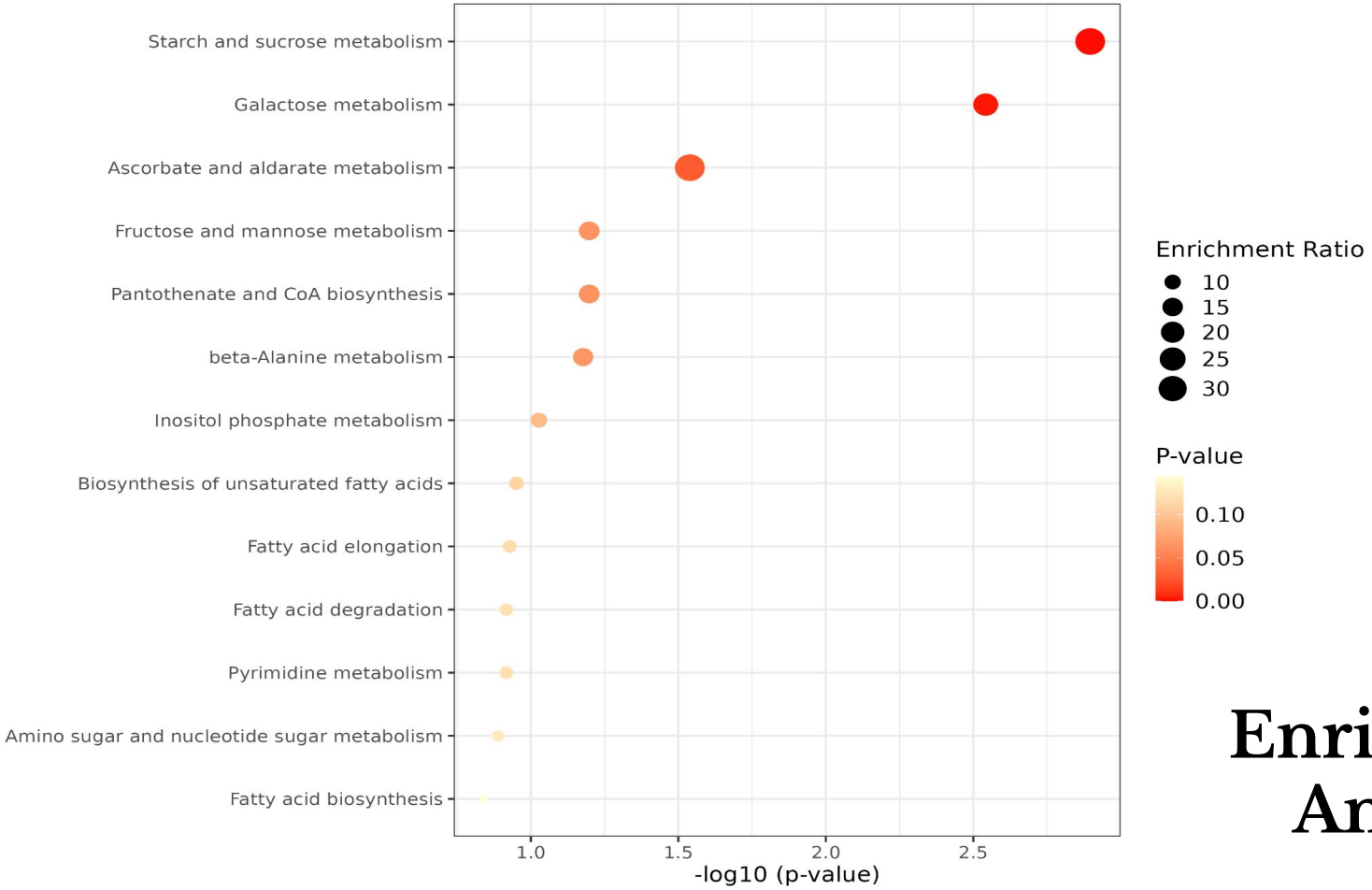
32 variables with VIP > 1

Student's t-test



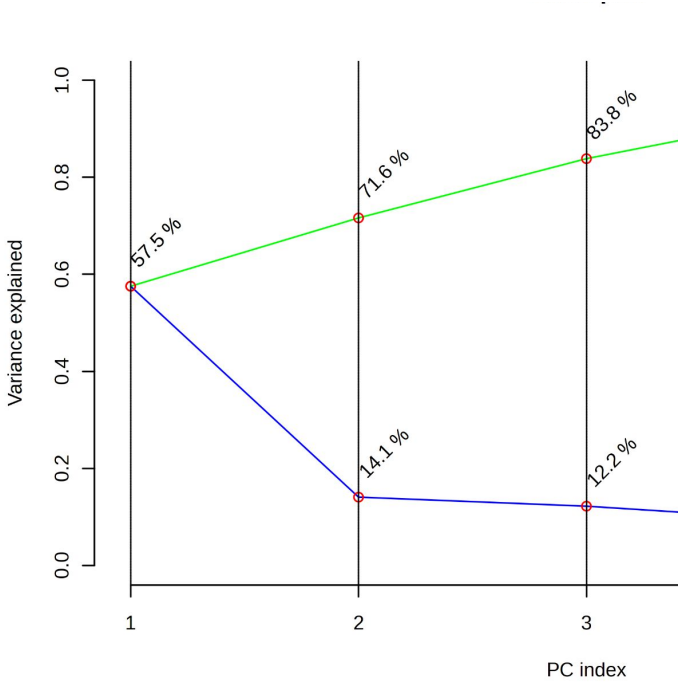
	Compound Name	Type	VIP Score	P-value	Regulation
1	2-Hydroxyglutaric acid	Dicarboxylic acid	1.403898244	0.00047835	Up
2	Uracil	Nucleotide	1.393560738	0.00046314	Up
3	Hydroxy-methylpent-ene naphthalene aldehyde	Aromatic	1.363547047	0.002045	Up
4	10-Heptadecenoic acid	Fatty Acid	1.344036748	0.0037272	Up
5	Dec-2-enoic acid	Fatty Acid	1.332891778	0.0055192	Up
6	5-nitrobarbiturate	Nitro Compounds	1.307482065	0.0092089	Up
7	1-Monolinolein	Mono glycerides	1.298330335	0.0096697	Up
8	Phosphoric acid	Carboxylic acid	1.284389944	0.011358	Up
9	Myo-Inositol	Sugar	1.263519978	0.018852	Up
10	Morpholine-naphthol derivative	Aromatic	1.244523415	0.025966	Up
11	2-linoleoylglycerol	Lipid	1.237826659	0.024491	Up
12	Palmitic Acid	Fatty Acid	1.20332866	0.029423	Up
13	4-Estren-4-chloro-17.beta.-ol-3-one	Steroid	1.20331034	0.033264	Up
14	Xylitol	Sugar Alcohols	1.415975974	6.49E-05	Down
15	D-Fructose	Sugar	1.383958166	0.001601	Down
16	Heptanoic acid	Fatty Acid	1.370587094	0.0026956	Down
17	8-Hexadecyne	Alkynes	1.365487991	0.0030238	Down
18	Aminomethylmethacrylamide	Aromatic	1.359401662	0.0038766	Down
19	Thiophenol	Aromatic	1.359034596	0.0041266	Down
20	Acetylhydroxy-tetramethyl naphthalene propene acid	Aromatic	1.356913056	0.0039417	Down
21	3,4,8-Trihydroxycoumarin	Aromatic	1.356890154	0.0037628	Down
22	D-Trehalose	Sugar	1.249982128	0.024266	Down

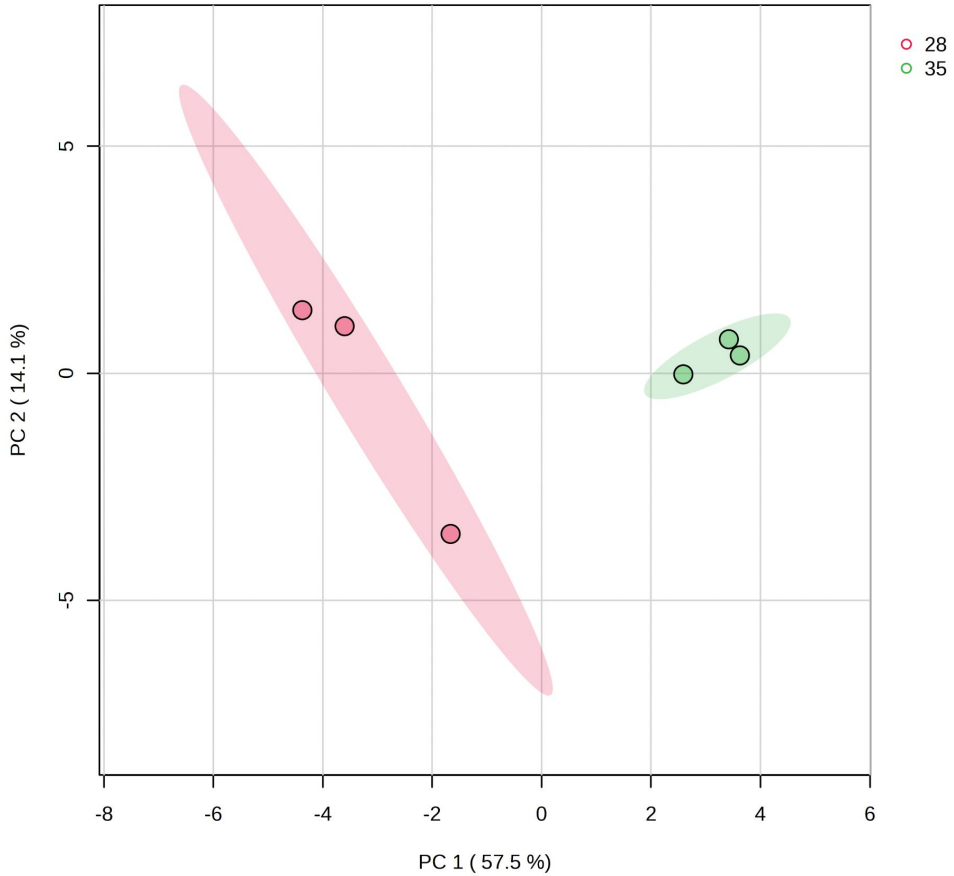
13 differentially upregulated, 9 differentially downregulated

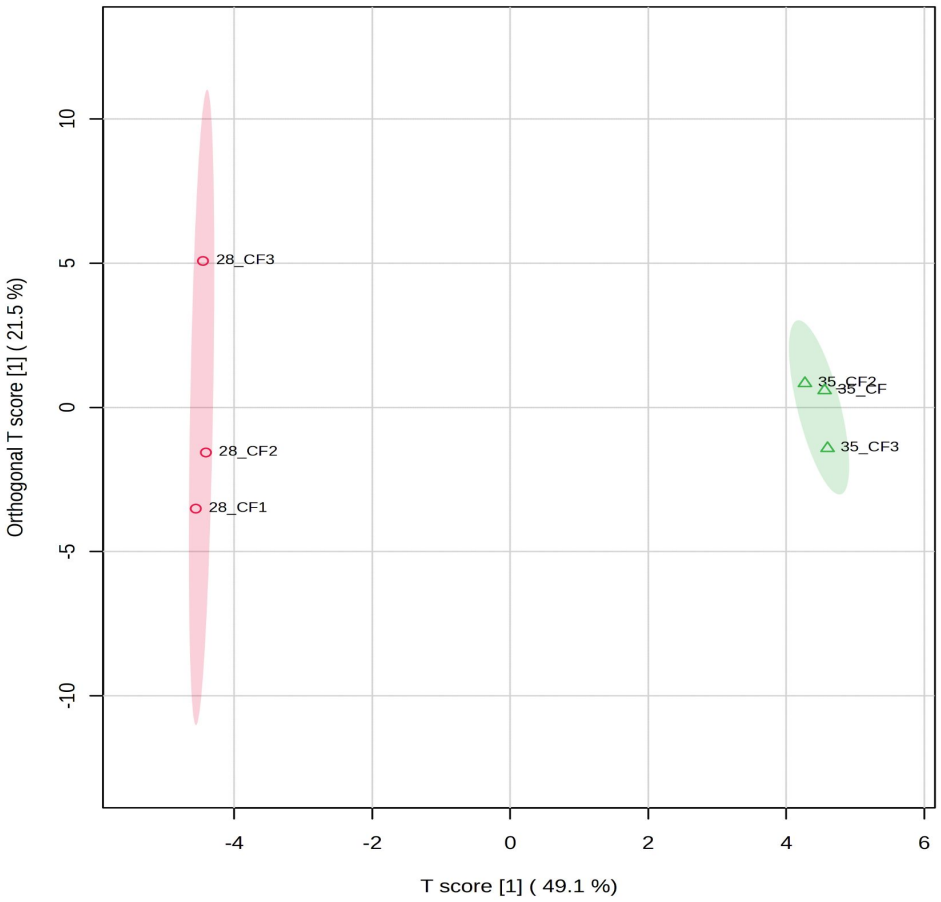


Enrichment Analysis

ECM Analysis



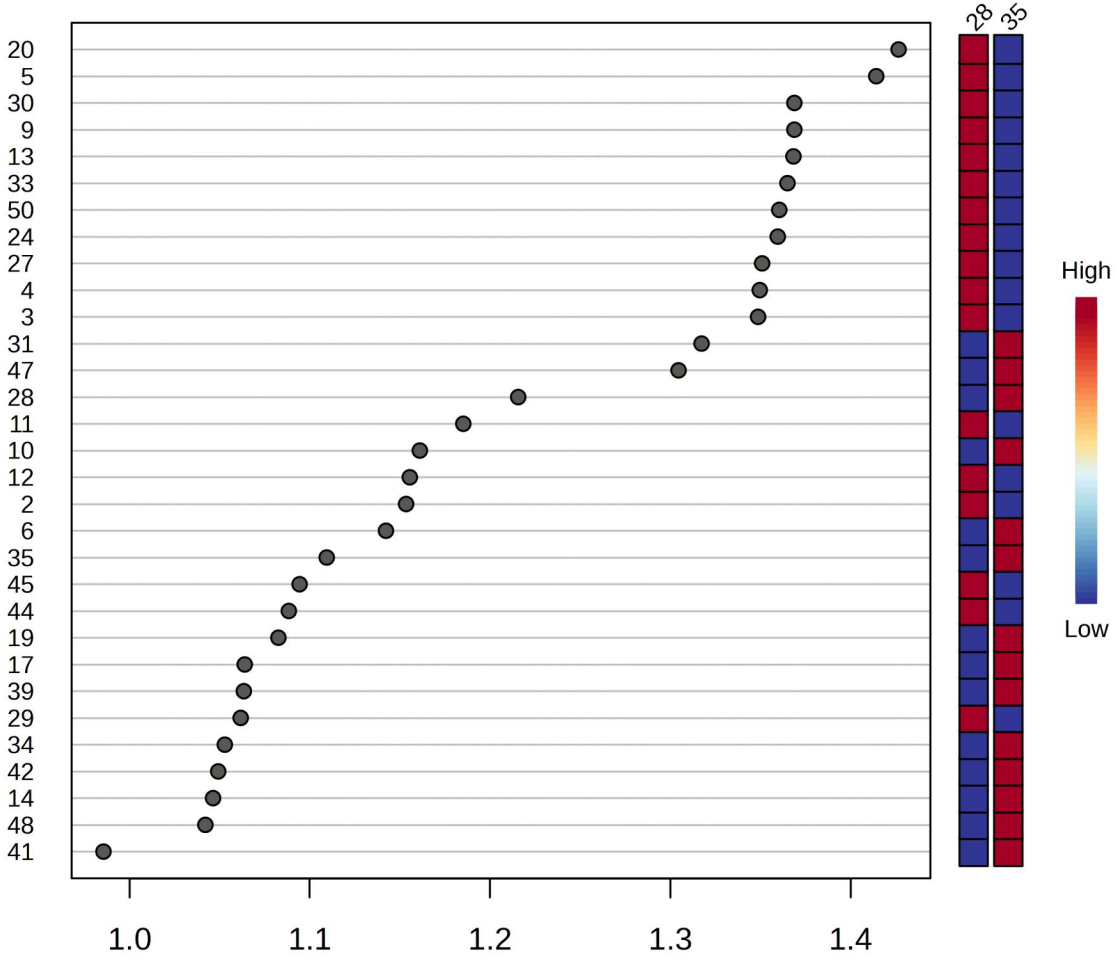




	p1	o1
R2X	0.491	0.215
R2Y	0.93	0.0692
Q2	0.838	0.0671

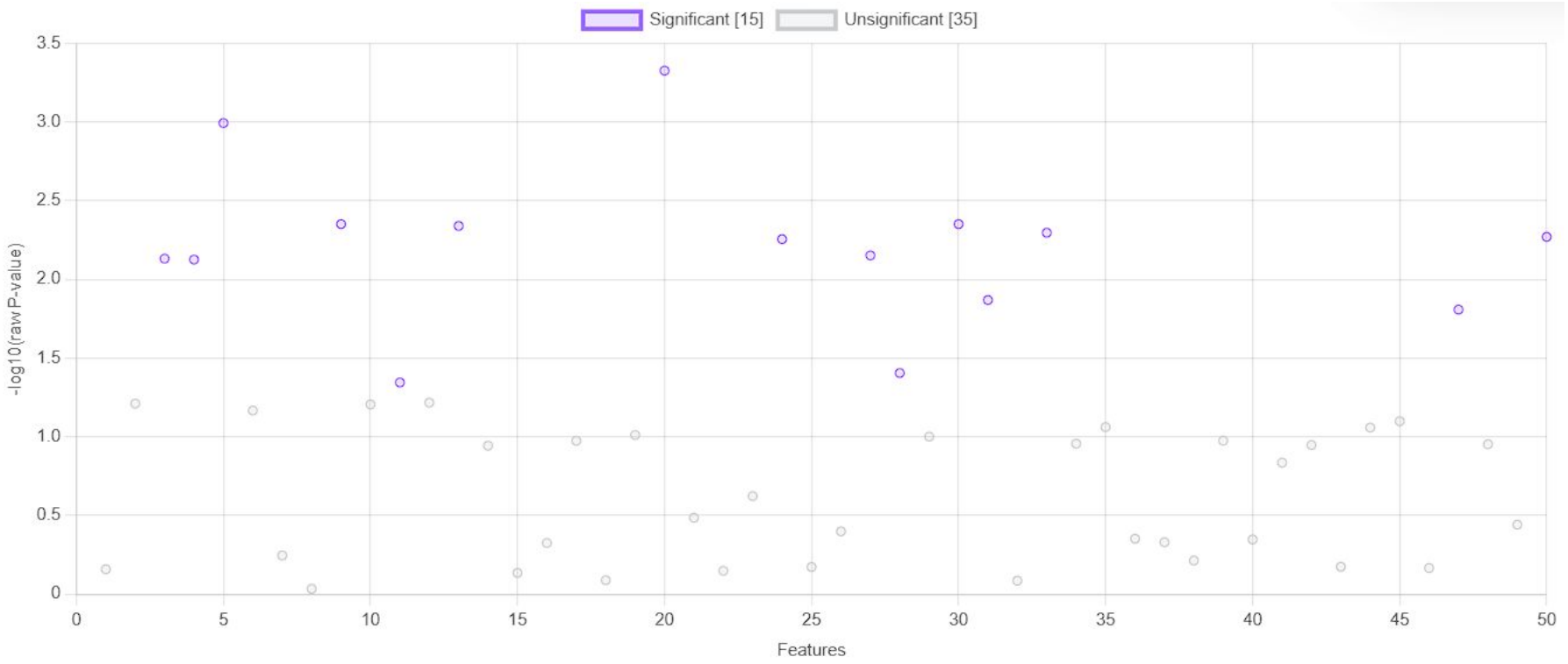
R^2X: 70.6% of total variation in X is explained by the model.
R^2Y: 99.92% of total variation in Y is explained by the model.
Predictive Variation: 49.1% of X correlates directly with 99.92% of Y.
Orthogonal Variation: 21.5% of X is orthogonal and irrelevant to Y.
Q^2: Predictive ability is 0.838.

OPLS-DA VIP



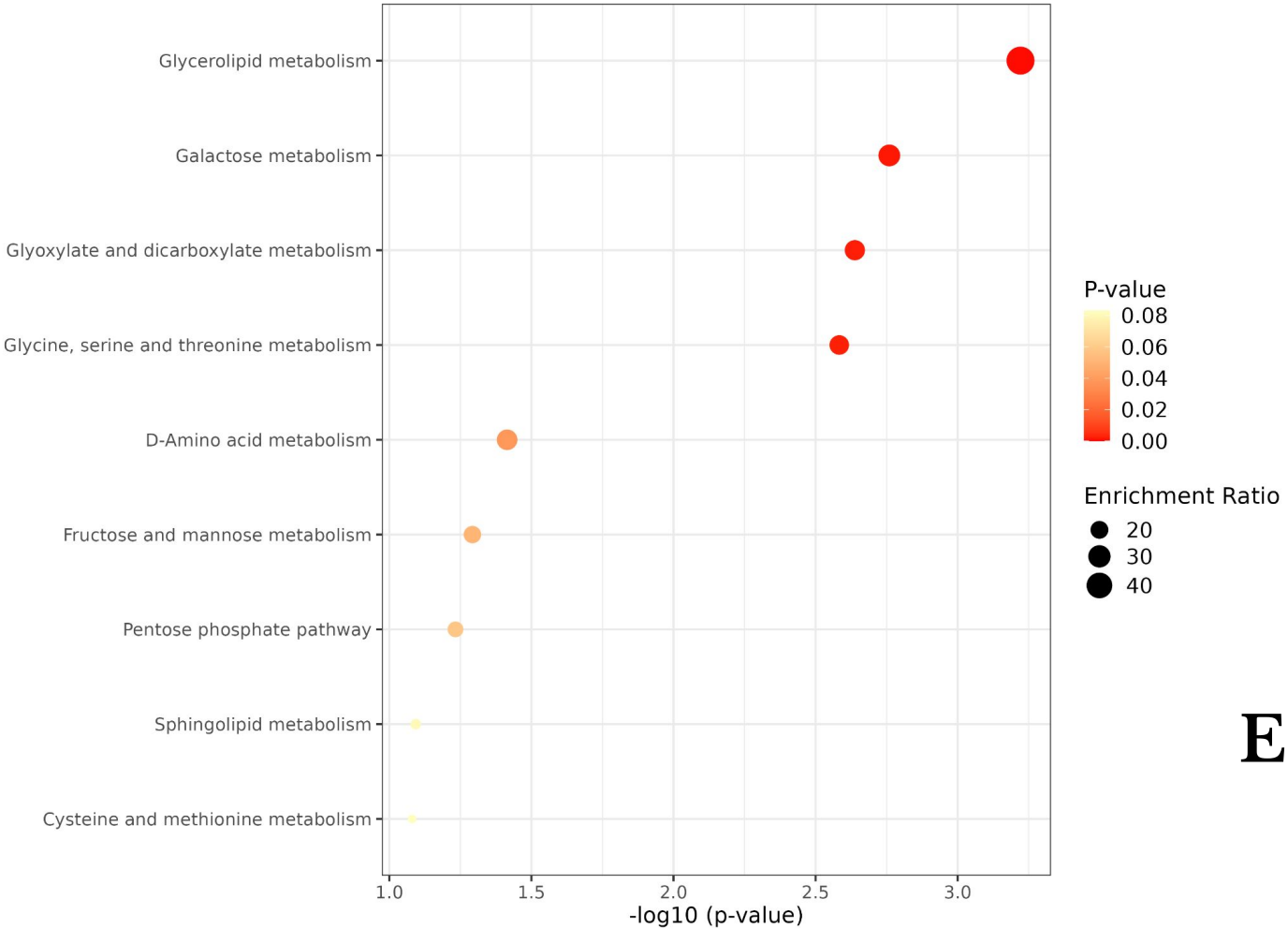
30 variables with VIP > 1

Student's t-test



	Compound Name	Type	P-value	VIP Score	Regulation
1	Glycerol	Sugar Alcohol	0.013532	1.317336695	Up
2	Serine	Amino Acid	0.015573	1.304557349	Up
3	DL-Phenylalanine	Amino Acid	0.039401	1.21563116	Up
4	D-Arabinose	Monosaccharide	0.00047159	1.426624576	Down
5	1-Phenyl-1,2-ethanediol	Aromatic Alcohol	0.0010163	1.414247467	Down
6	2-Isopropyl butyric acid	Fatty Acid	0.0044563	1.368812071	Down
7	Glyceric acid	Hydroxy Acid	0.0044563	1.368812152	Down
8	5-Hydroxymethyl-2-furoic acid	Aromatic Acid	0.0045759	1.368309928	Down
9	L-(+)-Threose	Monosaccharide	0.0050518	1.364990054	Down
10	Xylitol	Sugar Alcohol	0.0053726	1.360447342	Down
11	D-Glucitol	Sugar Alcohol	0.0055508	1.35959319	Down
12	Diethanolamine	Amine	0.007047	1.350925474	Down
13	beta-Gentiobiose	Disaccharide	0.0073855	1.348680227	Down
14	1-Deoxypentitol	Sugar Alcohol	0.0074892	1.349628766	Down
15	3,4,8-Trihydroxycoumarin	Aromatic	0.045221	1.185151802	Down

3 differentially upregulated, 12 differentially downregulated



Enrichment Analysis

Chapter 2: Cell Wall

Isolation of AIR

- Freeze-dried mycelium processed with ethanol, chloroform:methanol, and acetone.
- Pellet dried under nitrogen.

Preparation of AKI and AKS

- AIR treated with NaOH and $NaBH_4$ at 75°C.
- Centrifugation separated **AKS (supernatant)** and **AKI (pellet)**.

Processing AKS

- Acidified, precipitated with ethanol, washed, dialyzed, and lyophilized.

Processing AKI

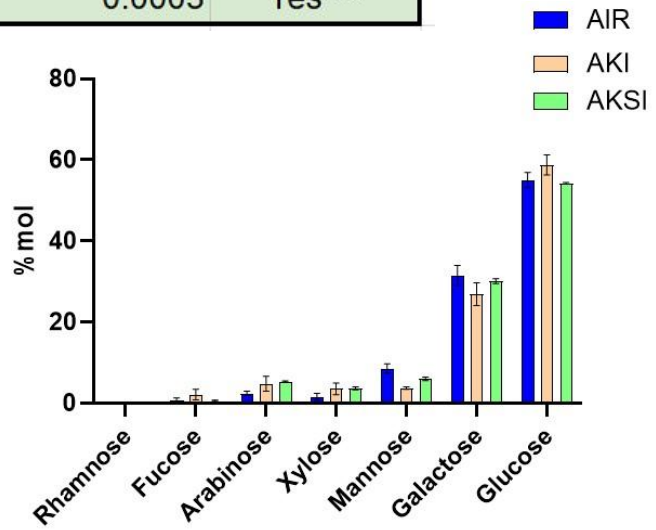
- Washed with ethanol, dialyzed, and lyophilized.

AIR	AKS/I	% Yield
5 g	300 mg	6%

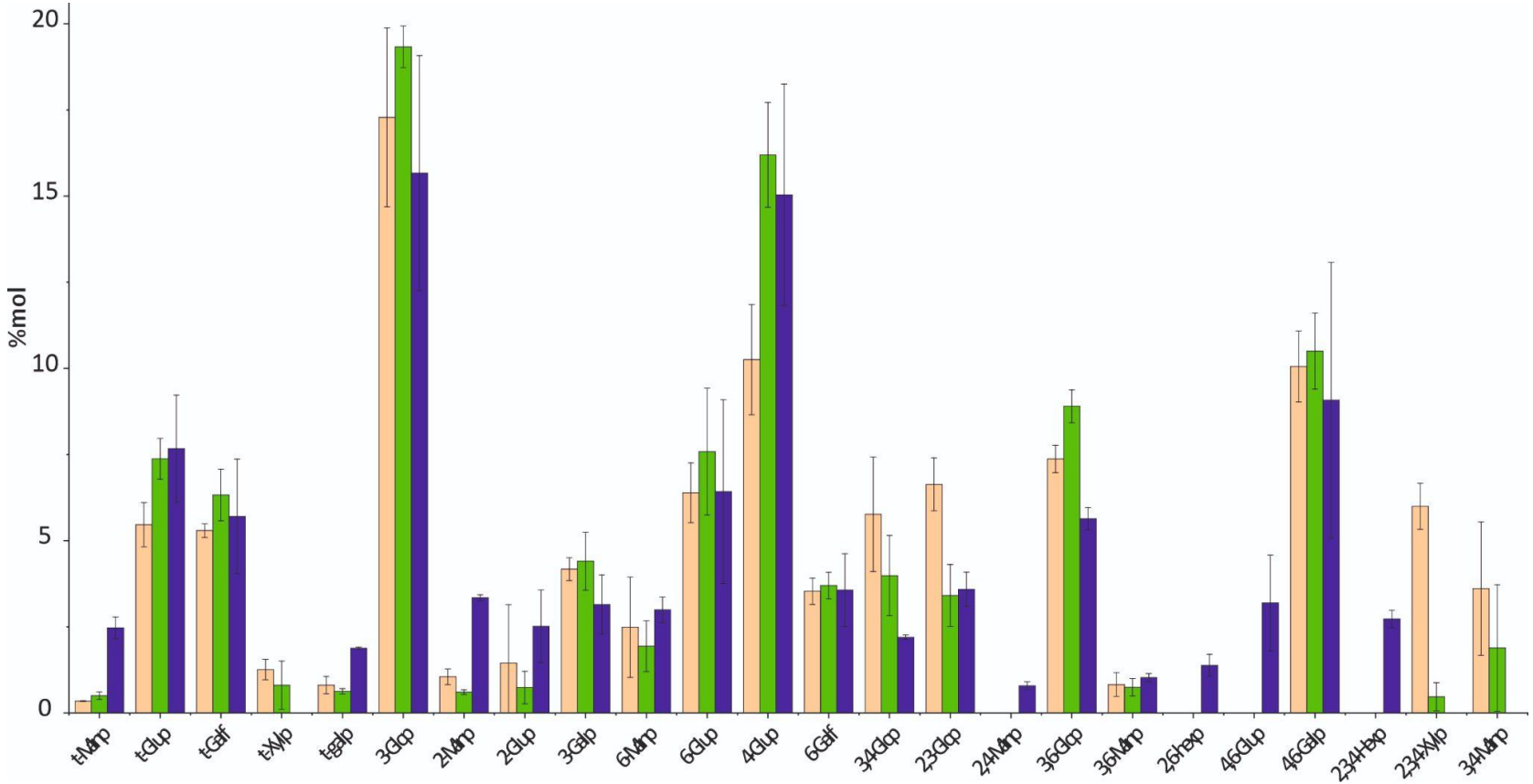
	AIR vs. AKI		AIR vs. AKSI		AKI vs. AKSI	
	P-value	Significant	P-value	Significant	P-value	Significant
Rhamnose	0.9998	No	0.9931	No	0.995	No
Fucose	0.3727	No	0.9891	No	0.3025	No
Arabinose	0.0756	No	0.022	Yes*	0.8574	No
Xylose	0.1614	No	0.1273	No	0.9915	No
Mannose	<0.0001	Yes****	0.045	Yes*	0.089	No
Galactose	0.0002	Yes***	0.4054	No	0.0095	Yes**
Glucose	0.0024	Yes**	0.7429	No	0.0003	Yes***

Monosaccharide Analysis

One star (*) = p-value less than 0.05
Two stars (**) = p-value less than 0.01
Three stars (***) = p-value less than 0.001
Four stars (****) = p-value is less than 0.0001



Glycosidic Linkage Analysis



		AIR vs. AKI		AIR vs. AKSI		AKI vs. AKSI	
No.	Linkage	P-value	Significant	P-value	Significant	P-value	Significant
1	t-Manp	0.0673	No	0.0989	No	0.984	No
2	t-Glup	0.055	No	0.9482	No	0.1115	No
3	t-Galf	0.9022	No	0.7886	No	0.5222	No
4	t-Xylp	0.3832	No	0.6749	No	0.8811	No
5	t-galp	0.4958	No	0.3858	No	0.9805	No
6	3-Glcp	0.2069	No	0.0005	Yes***	0.0817	No
7	2-Manp	0.0445	Yes*	0.0124	Yes*	0.8849	No
8	2-Glup	0.4967	No	0.1498	No	0.738	No
9	3-Galp	0.5263	No	0.3823	No	0.9675	No
10	6-Manp	0.8532	No	0.5057	No	0.8316	No
11	6-Glup	0.9992	No	0.4418	No	0.4201	No
12	4-Glup	<0.0001	Yes****	0.4435	No	<0.0001	Yes****
13	6-Galf	0.9993	No	0.9893	No	0.983	No
14	3,4-Glcp	0.0007	Yes***	0.1465	No	0.1486	No
15	2,3-Glcp	0.0046	Yes**	0.9802	Yes**	0.0025	No
16	2,4-Manp	0.6865	No	0.6865	No	>0.9999	No
17	3,6-Glcp	0.1642	No	0.0022	Yes**	0.2426	No
18	3,6-Manp	0.9751	No	0.9529	No	0.9963	No
19	2,6-hexp	0.3165	No	0.3165	No	>0.9999	No
20	4,6-Glup	0.0028	Yes**	0.0028	Yes**	>0.9999	No
21	4,6-Galp	0.5582	No	0.2925	No	0.8849	No
22	2,3,4-Hexp	0.0129	Yes*	0.0129	Yes*	>0.9999	No
23	2,3,4-Xylp	<0.0001	Yes****	0.8755	No	<0.0001	Yes****
24	3,4-Manp	0.0006	Yes***	0.1191	No	0.1675	No

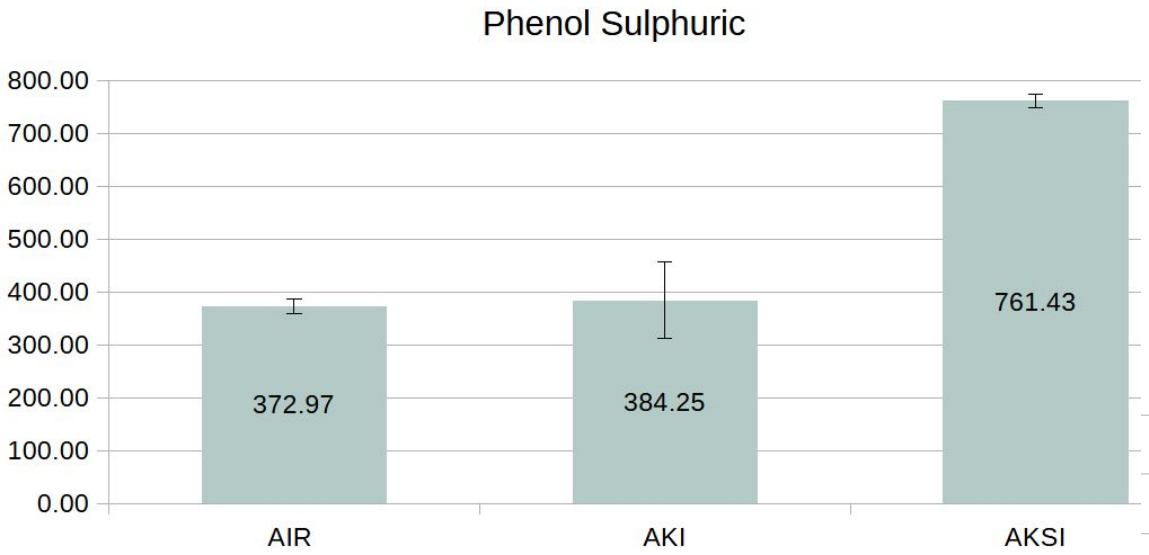
One star (*) = p-value less than 0.05

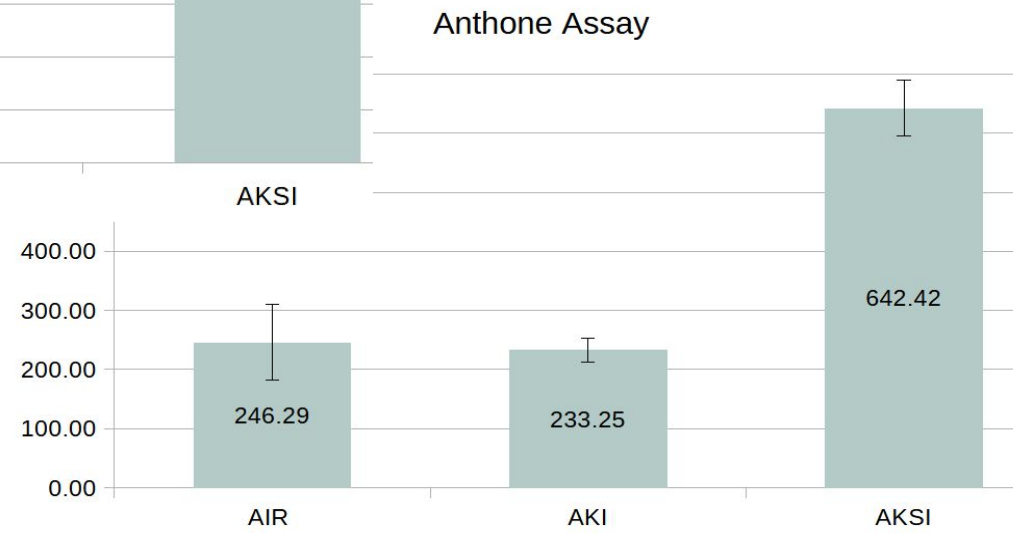
Two stars (**) = p-value less than 0.01

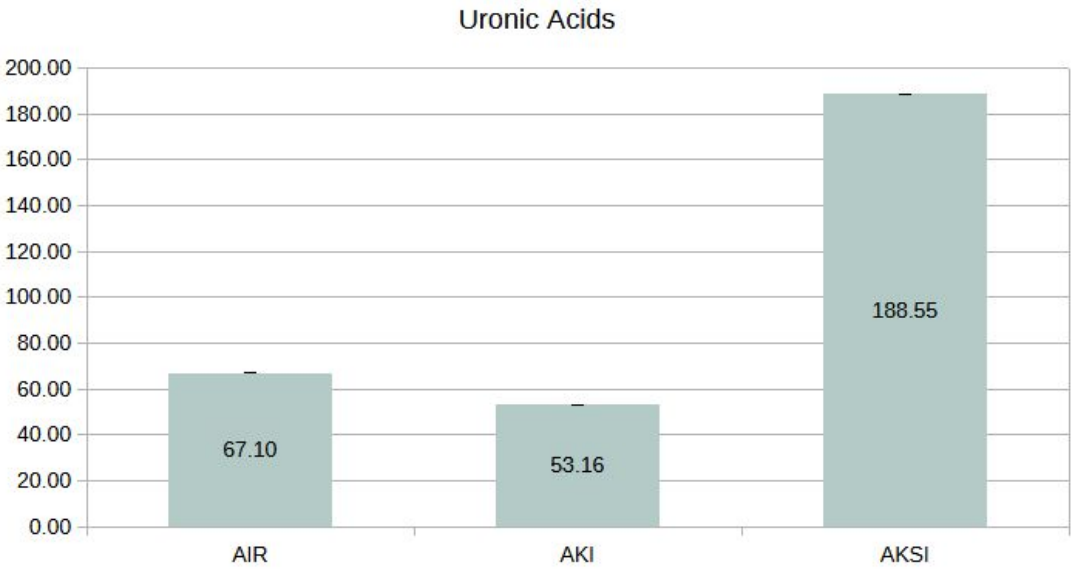
Three stars (***) = p-value less than 0.001

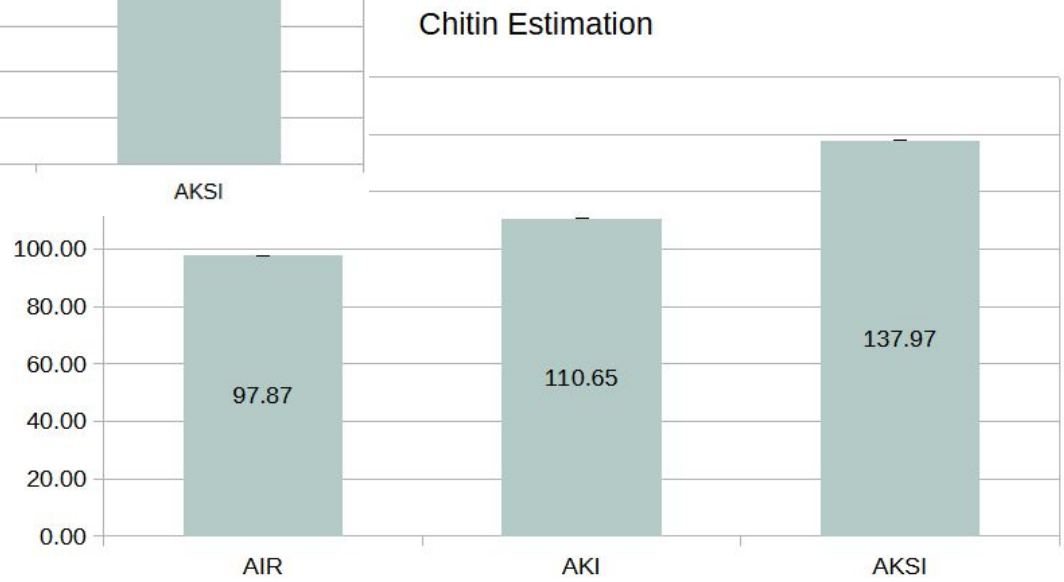
Four stars (****) = p-value is less than 0.0001

Quantification Assays

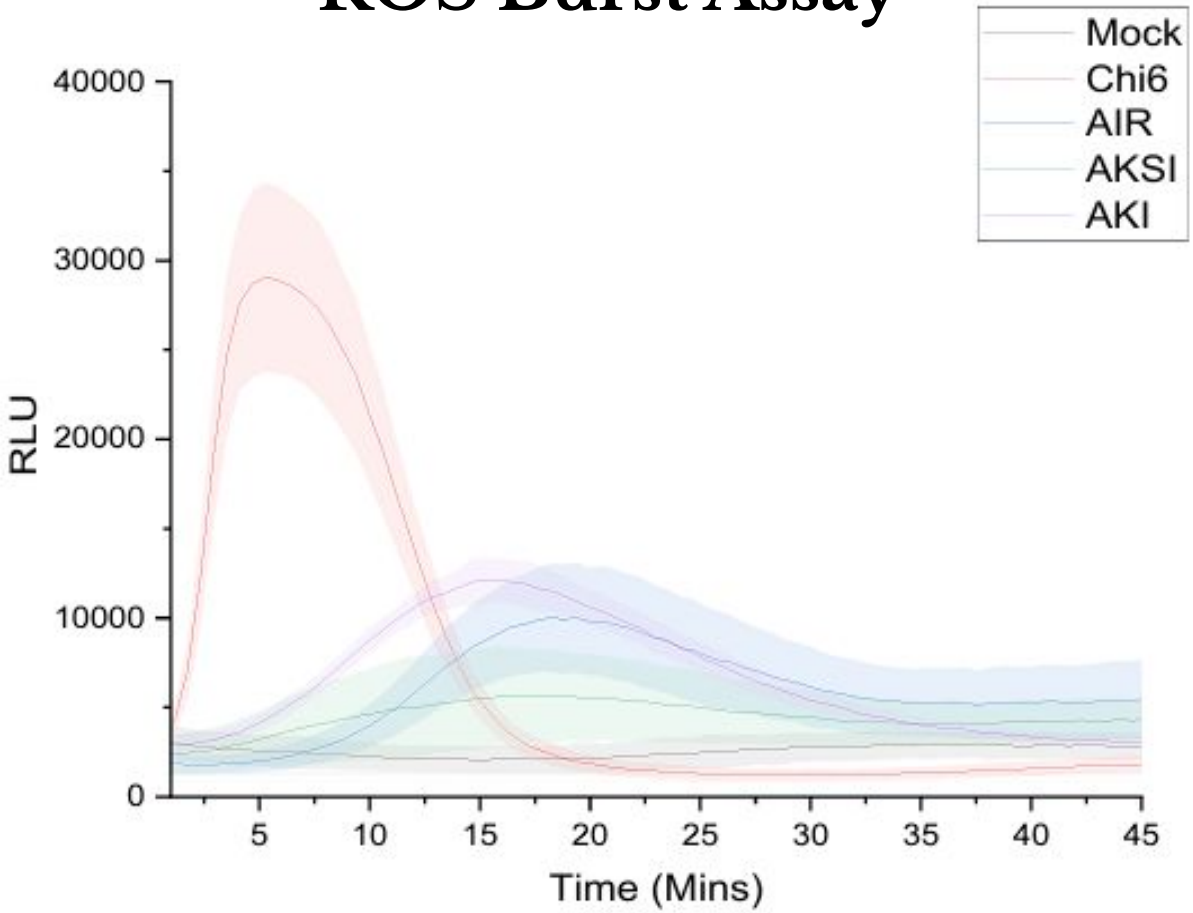





Uronic Acids
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
67.10
53.16
188.55
AIR
AKI
AKSI


Chitin Estimation
100.00
80.00
60.00
40.00
20.00
0.00
97.87
110.65
137.97
AIR
AKI
AKSI

ROS Burst Assay



CARBO-XXXVIII Poster

Characterization of *Macrophomina phaseolina* Cell Wall: Insights into Pathogenicity and Plant Immunity Interactions

42

Chemical Glycobiology Lab

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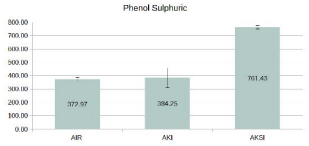
1. Introduction

Plants are constantly under attack from pathogens, which can devastate crops. Among these is *Macrophomina phaseolina*, a soil-borne fungus that kills plants from within and infects over 500 species, including staples like soybean, maize, and sorghum. Despite its massive impact, we know little about the tools this pathogen uses to infiltrate its hosts. One potential weapon lies in its cell wall, a dynamic structure that shields the fungus and interacts with the plant's immune system.

Our research delves into the fungal cell wall composition and how it may play a role in infection. By isolating and fractionating the cell wall at 28c, we identified its sugar building blocks and key linkages. The variations in these components suggest that different parts of the wall might trigger distinct immune responses in plants. To test this, we exposed plant roots and leaves to fungal cell wall fragments and measured early immune responses like the PTI response.

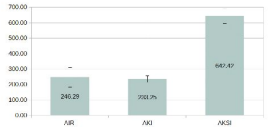
Our findings reveal that parts of the cell wall can act as "immunity alarms," priming plants to better resist diseases. This discovery not only sheds light on how *M. phaseolina* operates but also opens the door to eco-friendly strategies for protecting crops worldwide.

2. Quantification of General Sugars



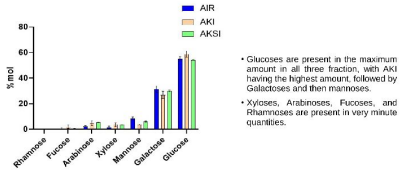
- AKSI fraction exhibits the highest sugar concentration at 761.43 µg per gram of fungal material, which is nearly double that of AIR (372.97 µg/g) and AKI (384.25 µg/g).
- The concentrations of AIR and AKI are relatively similar, with AKI being slightly higher by 11.28 µg/g.

3. Quantification of only Hexoses



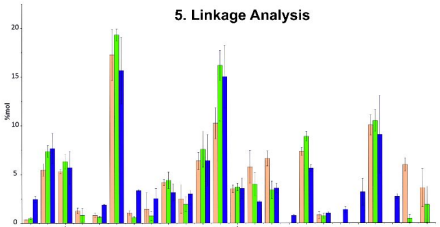
- Through anthrone assay, AKSI demonstrated a significantly higher concentration of hexoses (glucoses, fructoses etc.) as well, nearly triple that of AIR and AKI.
- AIR and AKI have comparable concentrations, with AIR being slightly higher by 13.04 µg/g.

4. Monosaccharide Analysis



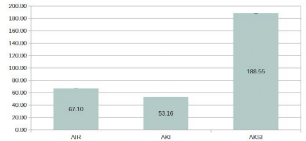
- Glucoses are present in the maximum amount in all three fraction, with AKI having the highest amount, followed by Galactoses and then mannoses.
- Xyloses, Arabinoses, Fucoses, and Rhamnoses are present in very minute quantities.

5. Linkage Analysis



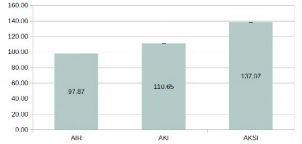
- Major linkages of AIR (blue color), AKI (orange) and AKSI (green) are shown above.
- Highest fraction of linkages in all the three fractions are 3-Glcp, 4-Glup, and 4,6-Galp.

6. Quantification of Uronic Acids



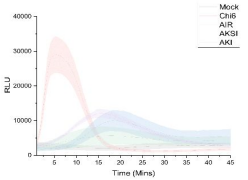
- AKSI demonstrated the highest concentration of uronic acids at 188.55 µg/g, almost triple of AIR and AKI.

7. Quantification of Chitin



- AKSI shows the highest quantity of Chitin, as measured by the glucosamine quantification assay, with 137.97 µg/g, followed by AKI and AIR.

8. ROS Burst Analysis



- AKI shows the highest ROS Burst out of all the three fractions, with the other two also showing bursts, which suggests that parts of the cell wall can act as "immunity alarms" for the plant.

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Thank you!