Estimation of Silage Component Traits in Maize using Near-Infrared Reflectance Spectroscopy

Session 2: Silage Lab Analysis

ISC 2025
XX INTERNATIONAL SILAGE CONFERENCE

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Outline of Presentation

- Breeding program
- Phenotyping using NIRS
- NIRS model development

Broad Research Questions

- ❑Can we breed maize for silage quality traits?
- ❑Can we phenotype silage component traits?

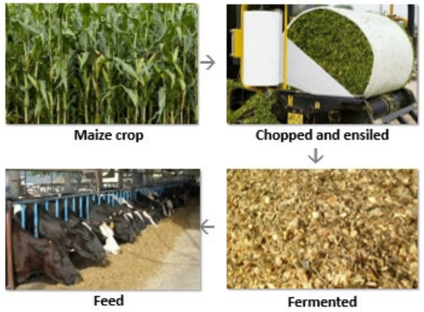
Can we breed maize for silage?

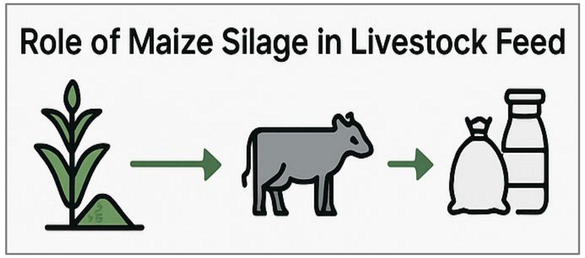
What is maize silage?

- Maize silage is an energy-rich high moisture fermented feed as nutritious as green fodder since it preserves the nutrients in their original form

Why is silage important?

- A key source of feed for ruminants
- Nutritional quality directly impacts livestock productivity





From breeding of view

- Based on protein and fiber content, some products are available in market
- Genetic dissection of silage component traits in maize using MHP is not studied
- Limited information is available on genomic regions and SNP markers associated with silage component traits in maize

Can we breed maize for silage?

Generation of multiple hybrid population (MHP)

- Development of MHP using sub-tropical and temperate inbred parents (vigour, biomass, grain, maturity)
- Large-scale analysis of combining ability and heterosis for silage component traits using MHP
- To perform MHP-based GWAS and Genomic prediction for silage component traits

Plant material

Inbred maize parents used to develop MHP – Spring 2023

Sub-Tropical Late

S. No.	PARENTS
1	CML - 313
2	CML - 579
3	CML - 581
4	PML- 22
5	PML- 24
6	PML- 48
7	PML- 368
8	PML- 387
9	PML-503
10	PML- 920
11	PML- 923-A
12	PML- 1118
13	PML- 1145
14	PML- 1179
15	PML-1230
16	PML- 1231
17	PML- 1233
18	PML- 1238
19	PML- 1247
20	PML- 1254
21	PML- 1275
22	RIL - 33
23	RIL - 64
24	VL - 1369 - 19
25	VL - 162590

Sub-Tropical Early

S. No.	PARENTS
1	EML - 101
2	EML - 123
3	EML - 124
4	EML - 145
5	EML - 159
6	EML - 168-I
7	EML - 201
8	EML - 220
9	EML - 227
10	EML - 236
11	EML - 258
12	EML - 261-I
13	E 222-1137
14	LM - 16
15	LM - 18

Sub-Tropical EARLY - Half Diallel - I: 58

Female↓ / Male→	EML - 101	EML - 123	EML - 124	EML - 145	EML - 159	EML - 168 - 1	EML - 201	EML - 220	EML - 227	EML - 236	EML - 258	EML - 261 - 1	E 222 1137	LM - 16
EML - 101														
EML - 123														
EML - 124														
EML - 145			x											
EML - 159														
EML - 168-I		x	x	x										
EML - 201			x			x								
EML - 220		x	x			x	x							
EML - 227		x	x			x		x						
EML - 236		x	x			x	x	x	x					
EML - 258		x	x	x					x					
EML - 261-I		x	x			x	x	x			x			
E 222-1137		x	x			x	x	x	x	x		x		
LM - 16		x	x			x	x		x	x	x	x	x	
LM - 18		x	x	x		x	x	x	x	x		x	x	x

Sub-Tropical EARLY - Half Diallel - II: 42

Male↓ / Female→	EML - 101	EML - 123	EML - 124	EML - 145	EML - 159	EML - 168 - 1	EML - 201	EML - 220	EML - 227	EML - 236	EML - 258	EML - 261 - 1	E 222 1137	LM - 16
EML - 101														
EML - 123														
EML - 124														
EML - 145			x											
EML - 159														
EML - 168-I														
EML - 201		x	x											
EML - 220						x								
EML - 227			x			x	x	x						
EML - 236						x	x	x	x					
EML - 258								x	x					
EML - 261-I							x	x	x	x	x			
E 222-1137						x	x	x	x	x	x	x		
LM - 16						x	x	x	x	x	x	x	x	
LM - 18						x	x	x	x	x		x	x	x

Sub-Tropical Late - Half Diallel - I: 204

Male↓																								
CML - 313																								
CML - 579																								
CML - 581		X																						
PML- 22			X																					
PML- 24		X	X																					
PML- 48		X	X		X																			
PML- 368		X	X	X	X																			
PML- 387		X	X		X		X																	
PML-503		X	X	X	X	X	X	X																
PML- 920		X	X		X		X	X	X															
PML- 923-A		X	X		X		X	X	X															
PML- 1118		X	X		X			X	X		X													
PML- 1145		X			X		X	X	X		X	X												
PML- 1179		X	X		X	X	X	X	X		X	X	X											
PML-1230		X	X		X			X	X		X	X	X	X										
PML- 1231		X	X		X			X			X	X	X	X	X									
PML- 1233		X			X	X	X		X		X	X	X	X	X	X								
PML- 1238		X	X	X	X		X	X	X		X	X	X	X	X	X								
PML- 1247		X			X			X	X		X	X	X	X	X		X							
PML- 1254		X	X		X		X		X		X	X	X	X		X	X		X					
PML- 1275		X		X				X	X		X		X	X	X		X	X	X	X				
RIL - 33		X	X		X	X		X	X	X	X		X	X	X	X	X		X	X	X			
RIL - 64		X	X		X	X	X	X	X		X	X	X	X	X	X			X	X	X	X		
VL - 1369 - 19		X	X		X		X	X	X		X	X	X	X	X	X	X		X	X	X	X		
VL - 162590		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X
Female➔	CML-313	CML-579	CML-581	PML-22	PML-24	PML-48	PML-368	PML-387	PML-503	PML-920	PML923A	PML-1118	PML-1145	PML-1179	PML-1230	PML-1231	PML-1233	PML-1238	PML-1247	PML-1254	PML-1275	RIL-33	RIL-64	VL-1369-19

Sub-Tropical Late - Half Diallel - II: 252

Female ↓ / Male →	CML-313	CML-579	CML-581	PML-22	PML-24	PML-48	PML-368	PML-387	PML-503	PML-920	PML923A	PML-1118	PML-1145	PML-1179	PML-1230	PML-1231	PML-1233	PML-1238	PML-1247	PML-1254	PML-1275	RIL-33	RIL-64	VL-1369-19
CML - 313																								
CML - 579	X																							
CML - 581	X	X																						
PML- 22	X																							
PML- 24	X	X	X	X																				
PML- 48	X	X	X	X	X																			
PML- 368	X	X	X	X	X	X																		
PML- 387	X	X	X	X	X		X																	
PML-503	X	X	X	X	X	X	X	X																
PML- 920	X	X		X																				
PML- 923-A	X	X	X	X	X	X	X	X	X	X														
PML- 1118	X	X	X	X	X	X	X	X	X	X	X													
PML- 1145	X	X	X	X	X	X	X	X	X	X	X	X												
PML- 1179	X	X	X	X	X	X	X			X	X	X	X											
PML-1230	X	X	X	X	X	X	X	X	X	X	X	X	X	X										
PML- 1231	X	X	X	X	X	X	X	X	X	X	X	X	X	X										
PML- 1233	X	X	X	X	X	X		X	X	X	X	X	X			X								
PML- 1238	X			X	X	X				X		X	X											
PML- 1247		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
PML- 1254	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
PML- 1275		X	X	X	X	X	X	X	X		X		X	X	X	X	X	X		X				
RIL - 33		X	X	X		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X			
RIL - 64	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
VL - 1369 - 19	X	X		X	X	X		X	X	X	X		X	X	X		X		X	X	X			
VL - 162590	X		X	X	X	X		X	X	X		X	X	X	X	X			X	X		X	X	

Parents used to develop MHP – Kharif (Jun-Oct) 2023

Sub-Tropical Late

S. No.	PARENTS
1	CML - 313
2	CML - 579
3	CML - 581
4	PML- 22
5	PML- 24
6	PML- 48
7	PML- 368
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24	VL - 1369 – 19
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Sub-Tropical Early

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8	EML - 220
9	EML - 227
10	EML - 236
11	EML - 258
12	EML - 261-I
13	E 222-1137
14	LM – 16
15	LM - 18

Temperate

S. No.	PARENTS
1	VL1010977
2	VL1010982
3	VL1010983
4	VL1010974
5	VL1010944
6	VL1010936
7	VL1010931
8	VL1010960
9	ZL132258
10	VL1010981
11	KL141701
12	KL141702

Mixed-type

S. No.	PARENTS
1	VL111354
2	VL18240
3	VL18242
4	VL181650
5	VL21952
6	VL21948
7	VL181668
8	VL181662

Sub-Tropical X Sub-Tropical NCD: 92
Mixed-type Diallel: 18 + 18
Temperate Diallel: 14 + 5

	TL1	TL2	TL3	TL4	TL5	TL6	TL7	TL8	TL9	TL10	TL11	TL12	TL13	TL14	TL15	TL16	TL17	TL18	TE1	TE2	TE3	TE4	TE5	TE6	TE7	M1	M2	M3	M4	M5	M6	M7	M8	Temp1	Temp2	Temp3	Temp4	Temp5	Temp6	Temp7	Temp8	Temp9	Temp10	Temp11	Temp12
TL1											X								X	X	X	X		X						X	X		X												
TL2				X		X	X	X		X							X		X	X	X	X		X	X					X	X	X	X						X						
TL3	X			X	X							X		X						X	X		X	X						X		X							X		X		X		
TL4					X	X		X											X	X		X		X							X	X	X						X						
TL5			X	X			X					X	X						X	X	X	X	X	X						X	X	X	X						X						
TL6							X							X	X				X											X	X	X							X		X				
TL7						X							X	X					X	X	X		X	X	X					X	X	X	X												
TL8		X			X		X												X	X	X	X	X	X							X	X	X						X						
TL9						X	X	X		X	X	X				X	X		X	X	X	X	X	X							X														
TL10		X				X	X		X		X				X		X	X	X	X	X	X	X	X	X																				
TL11						X	X			X					X				X	X	X	X	X																						
TL12						X	X	X		X	X				X		X	X	X	X	X	X		X	X																				
TL13																				X	X	X																							
TL14											X	X						X	X	X	X			X	X																				
TL15																	X	X	X	X	X	X		X	X																				
TL16		X					X		X									X	X	X	X			X																					
TL17					X				X				X					X	X	X	X		X	X	X																				
TL18					X			X						X					X	X	X	X		X	X																				
TE1	X			X										X				X																											
TE2	X																X																												
TE3	X																	X				X																							
TE4	X		X	X				X	X							X	X	X						X								X	X						X				X		
TE5	X	X		X										X			X							X	X					X									X	X			X		
TE6	X																						X							X		X							X				X		
TE7	X																			X			X								X						X		X						
M1																											X	X	X	X		X	X												
M2																										X				X		X													
M3							X																			X	X		X	X										X					
M4	X			X																						X	X	X			X	X	X							X					
M5		X	X	X	X																	X	X			X	X	X	X		X	X	X								X				
M6				X	X		X	X										X								X	X	X		X		X				X				X					
M7		X				X																X				X	X		X				X							X					
M8																																X													
Temp1																																													
Temp2																																													
Temp3																																													
Temp4																														X													X		
Temp5																																													
Temp6																														X				X	X	X	X	X		X			X		
Temp7																																	X			X			X		X				
Temp8																																						X					X		
Temp9																																													
Temp10																																		X	X			X	X	X	X				
Temp11																																													
Temp12																																													

Final set of hybrids used for evaluation

	Temp1	Temp2	Temp3	Temp4	Temp5	Temp6	Temp7	Temp8	Temp10	M1	M2	M3	M4	M5	M6	M7	M8	TE1	TE2	TE3	TE4	TE5	TE6	TE7	TL1	TL2	TL3	TL4	TL5	TL6	TL7	TL8	TL9	TL10	TL11	TL12	TL13	TL14	TL15	TL16	TL17	TL18
Temp1																																										
Temp2																																										
Temp3																																										
Temp4									x																																	
Temp5																																										
Temp6	x	x	x	x	x		x		x																																	
Temp7			x			x		x																																		
Temp8					x				x																																	
Temp10	x	x			x	x	x	x																																		
M1											x	x	x	x		x	x																									
M2										x				x		x																										
M3							x			x	x		x	x																												
M4							x			x	x	x			x	x	x																									
M5								x		x	x	x	x		x	x	x																									
M6			x				x			x	x	x		x		x																										
M7							x			x	x		x				x																									
M8																x																										
TE1																																										
TE2																		x																								
TE3																		x	x																							
TE4						x			x							x	x	x	x	x																						
TE5						x	x		x					x				x	x	x	x																					
TE6						x			x					x		x		x		x	x	x																				
TE7				x		x									x			x	x	x	x	x	x																			
TL1														x	x		x	x	x	x	x		x																			
TL2						x								x	x	x	x	x	x	x	x		x	x	x																	
TL3						x		x	x					x		x			x	x		x	x		x	x																
TL4						x									x	x	x	x	x		x		x		x	x	x															
TL5						x								x	x	x	x	x	x	x	x	x	x		x	x	x	x														
TL6						x		x						x	x	x		x							x	x	x	x	x													
TL7														x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x												
TL8						x									x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x											
TL9															x			x	x	x	x	x	x		x	x	x	x	x	x	x	x										
TL10																		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x									
TL11																		x	x	x	x	x			x	x	x	x	x	x	x	x	x	x								
TL12																		x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x							
TL13																			x	x	x				x	x	x	x	x	x	x	x	x	x	x							
TL14																		x	x	x			x	x	x	x	x	x		x	x	x	x	x	x	x	x					
TL15																		x	x	x	x		x	x	x		x	x	x	x	x	x	x	x	x	x	x	x				
TL16																		x	x	x			x			x	x	x	x	x	x	x	x	x	x	x	x	x	x			
TL17																		x	x	x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x		
TL18																		x	x	x	x		x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x			

MHP Evaluation at PAU, Ludhiana in Spring 2024

MHP Evaluation at PAU-RRS, Gurdaspur in Spring 2024






Material evaluated in Spring 2024

Total Hybrid Population (F1s)	354
Parents	42
Checks	4
Multiple hybrid population (MHP)	**400**
x 2 locations x 2 reps	**1600**
Agronomic Data	1600
NIRS-phenotyping	1600
Whole Genome Re-sequencing	42 parents

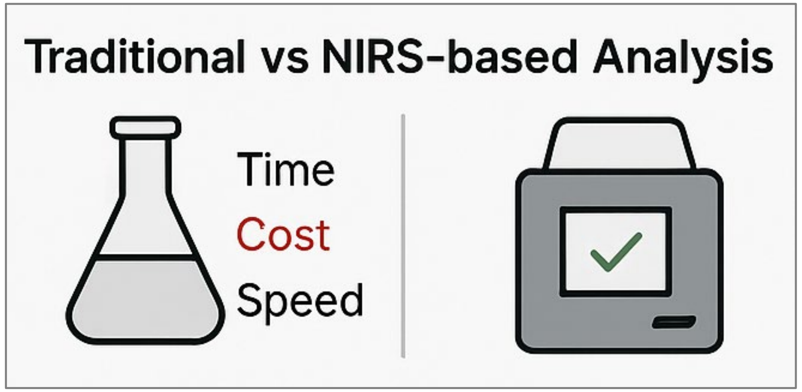
Can we phenotype silage component traits?

Wet chemistry!

NIRS

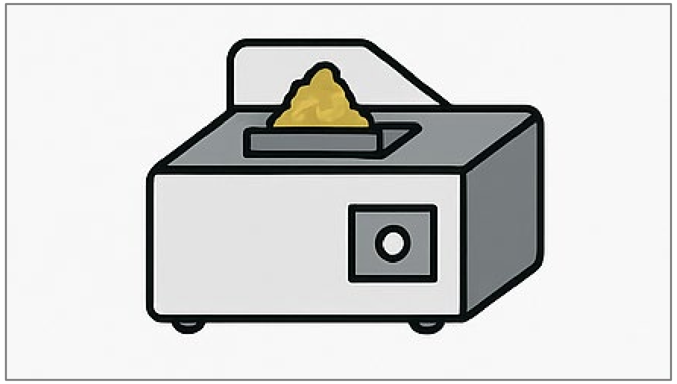
The challenge in conventional estimation of silage quality:

- Time consuming
- Expensive
- Low-throughput



Why NIRS:

- Non-destructive
- Rapid
- Cost effective



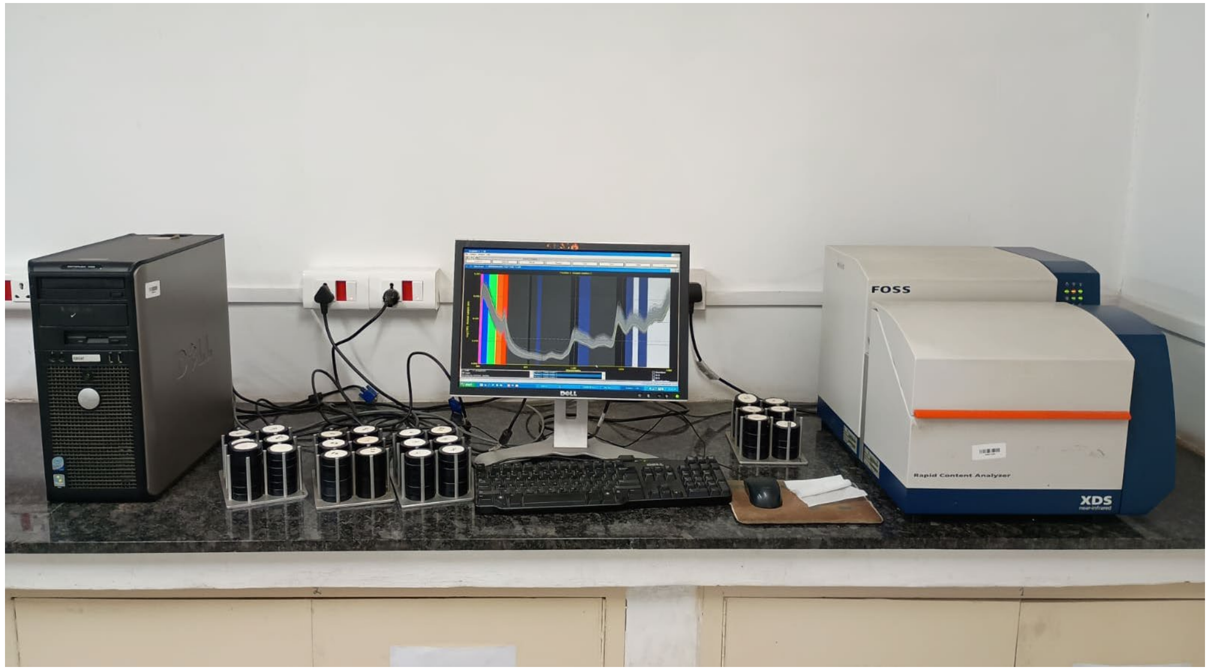
Research question

Can NIRS reliably predict the nutritional composition (e.g., crude protein, fiber) of silage maize hybrids?

Traits of interest:

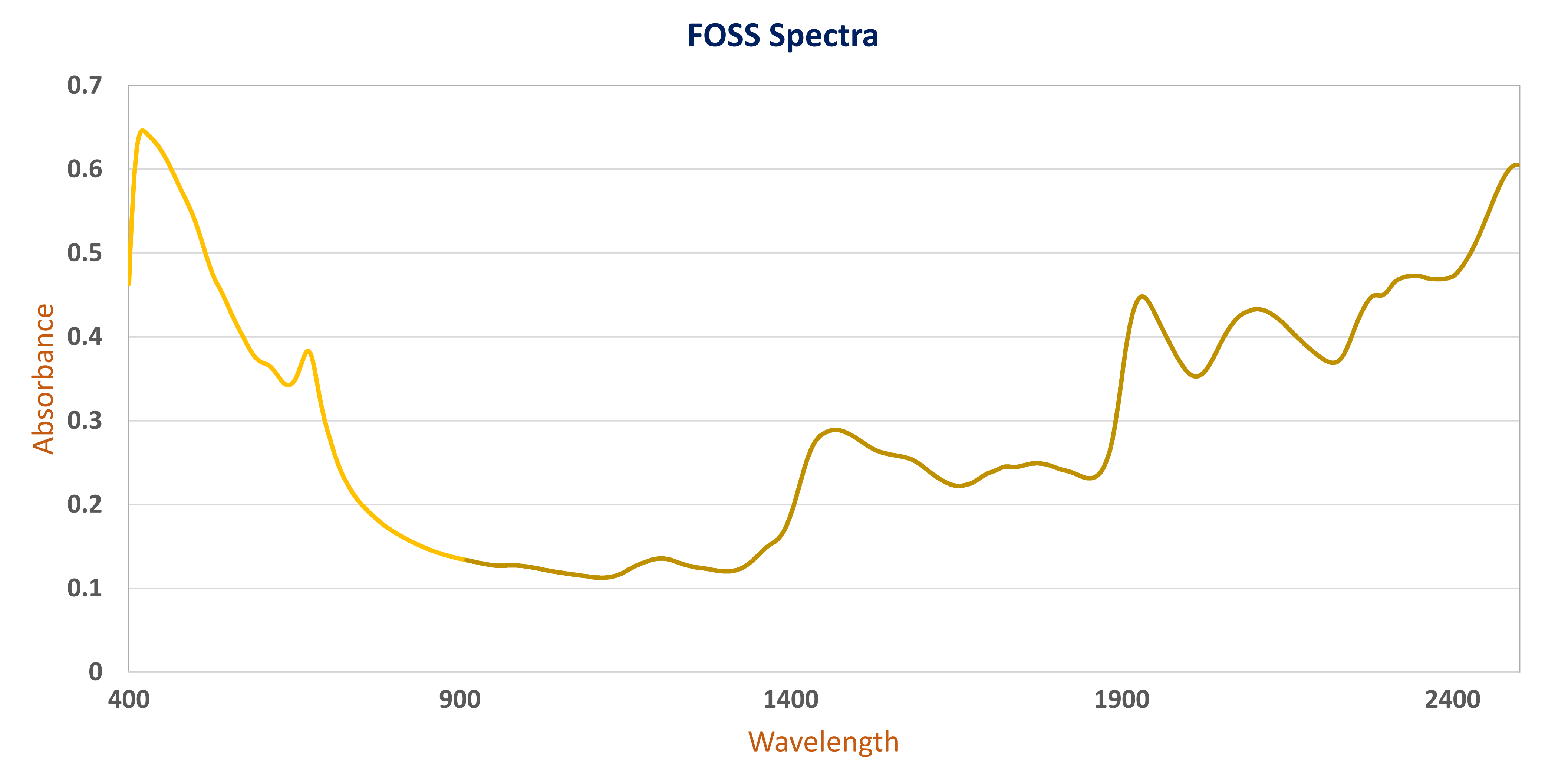
- Dry Matter (DM)
- Ash
- Crude Protein (CP)
- Acid Detergent Fiber (ADF)
- Neutral Detergent Fiber (NDF)
- Acid Detergent Lignin (ADL)
- Metabolizable Energy (ME)
- In-vitro Organic Matter Digestibility (IVOMD)

NIRS-based prediction models for crude protein



FOSS XDS

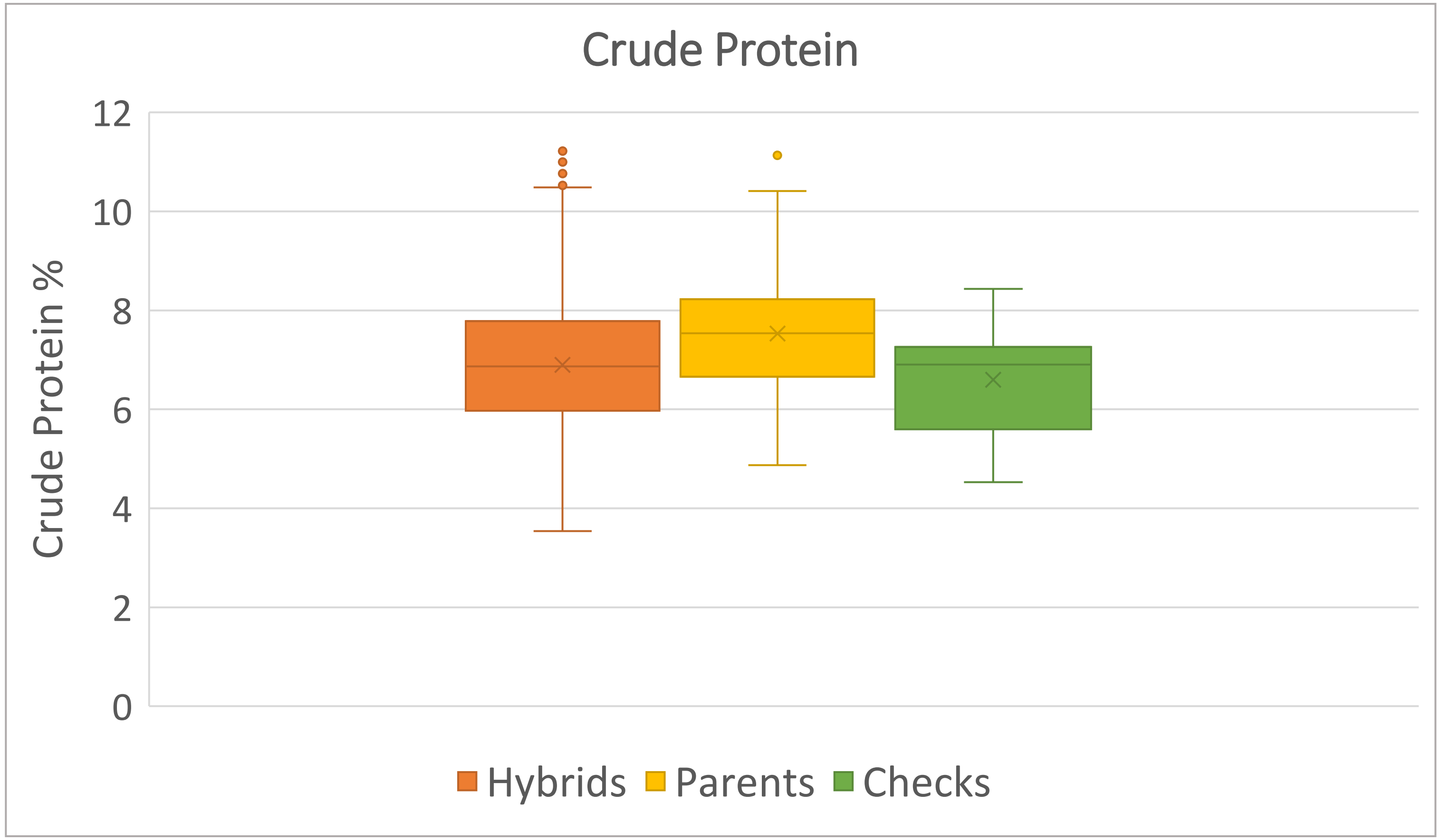
Spectral signature



ILRI's Global Fodder Maize Calibration Equation (GFMC)-665 model

Constituent	N	Mean	SD	Est. Min	Est. Max	SEC	RSQ	SECV	1-VR
DM	664	92.84	1.01	89.80	95.88	0.35	0.903	0.35	0.88
ASH	663	7.51	2.13	1.12	13.91	0.47	0.952	0.51	0.94
N	665	0.79	0.27	0.01	1.59	0.04	0.976	0.05	0.96
ME	654	8.36	0.89	5.68	11.03	0.23	0.933	0.93	0.92
IVOMD	659	56.17	5.58	39.42	72.92	1.47	0.931	0.93	0.91
NDF	663	69.27	6.43	49.98	88.57	1.14	0.969	1.23	0.96
ADF	667	38.45	5.58	21.71	55.19	0.92	0.973	1.01	0.97
ADL	666	3.31	0.94	0.48	6.14	0.34	0.870	0.87	0.86

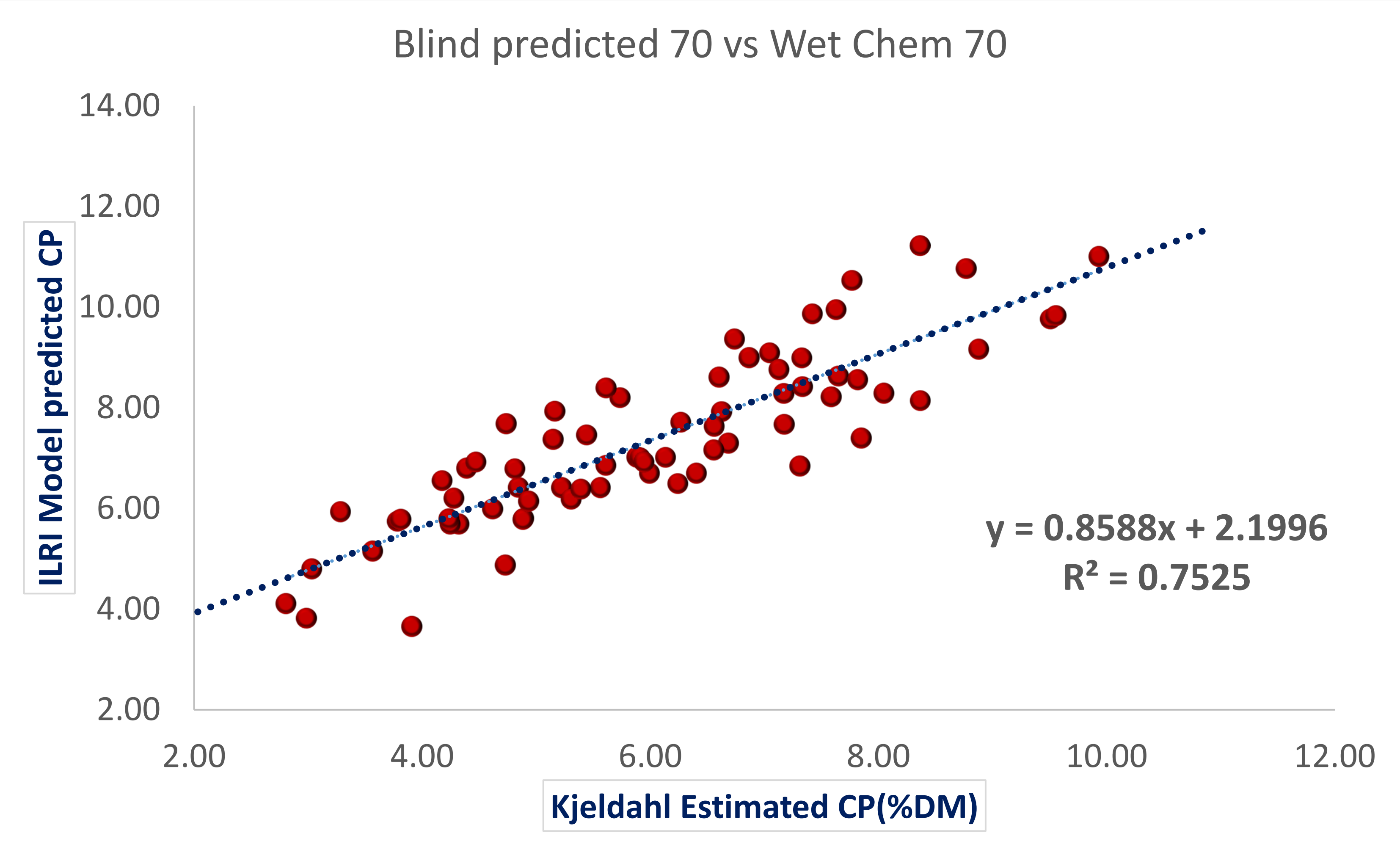
Prediction using ILRI's GFMC 665 model



For validation, 70 samples were analyzed initially

	DM%	ASH%	CPDM%
Max	90.66	9.57	9.93
Min	84.80	2.69	2.81
Average	88.88	5.88	6.01
Std D	*0.96*	*1.57*	*1.69*

Wet chemistry vs GFMC prediction



Further, additional samples were analyzed..

Initial 70 +

Inbred parents +

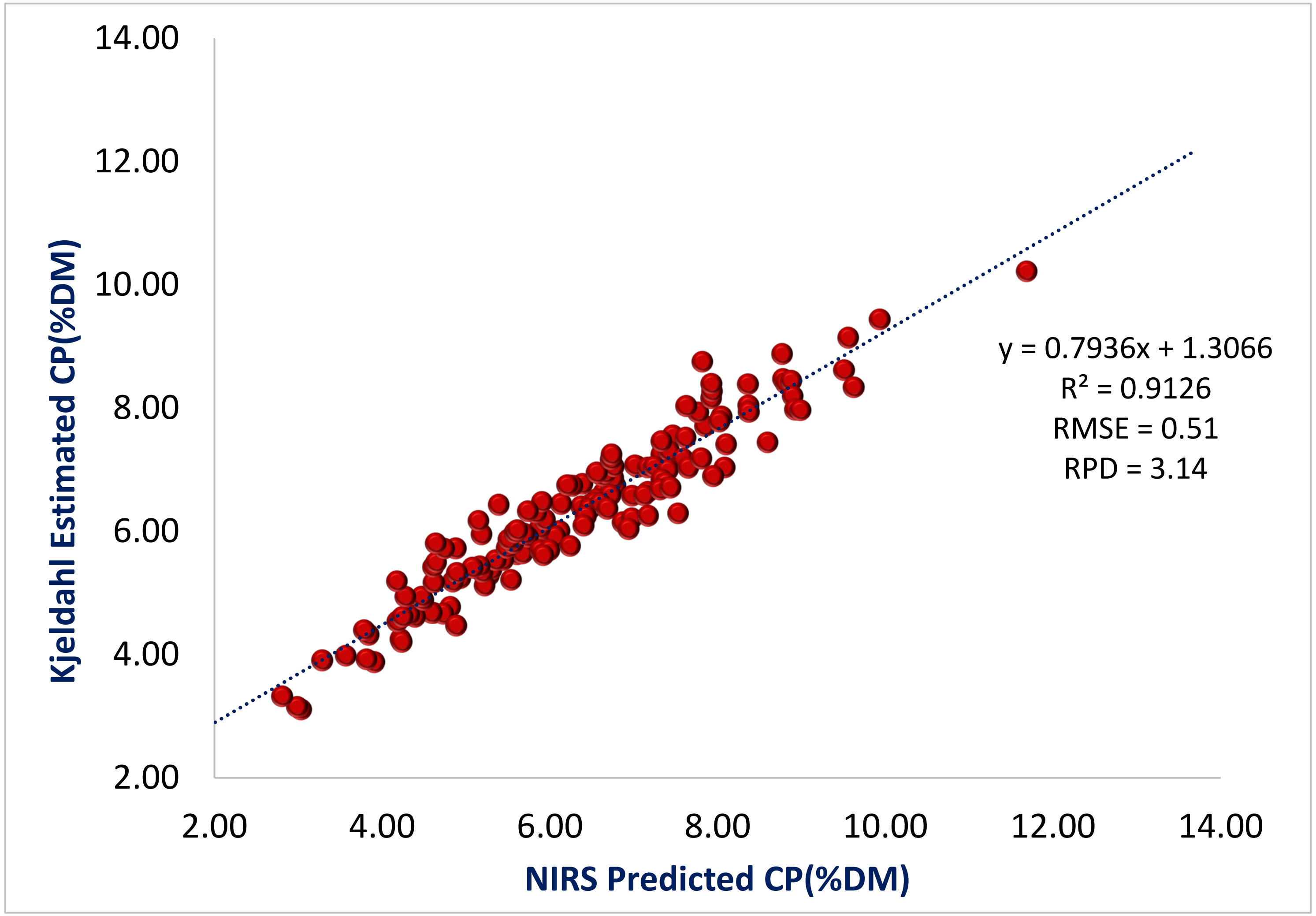
Range of hybrids (with high, medium and low CP)

Total: 148 samples

Based on 148 wetlab, silage maize equation was developed

Constituent	n	Mean	SD	Est. Min	Est. Max	SEC	RSQ	SEV	1-VR
DM	142	89.35	0.81	86.91	91.79	0.52	0.60	0.53	0.57
Ash	147	5.59	1.32	1.64	9.54	0.33	0.95	0.45	0.88
N	148	1.02	0.25	0.26	1.77	0.06	0.95	0.08	0.90

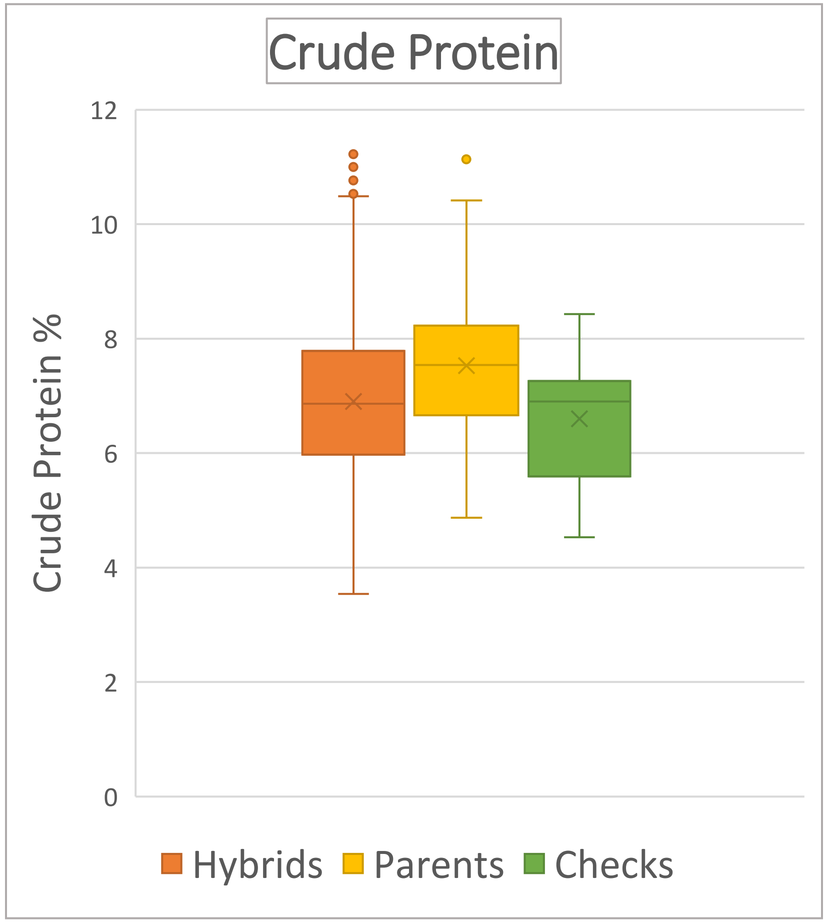
Wet Chemistry vs NIRS Predicted Crude Protein (%DM) for 148 samples



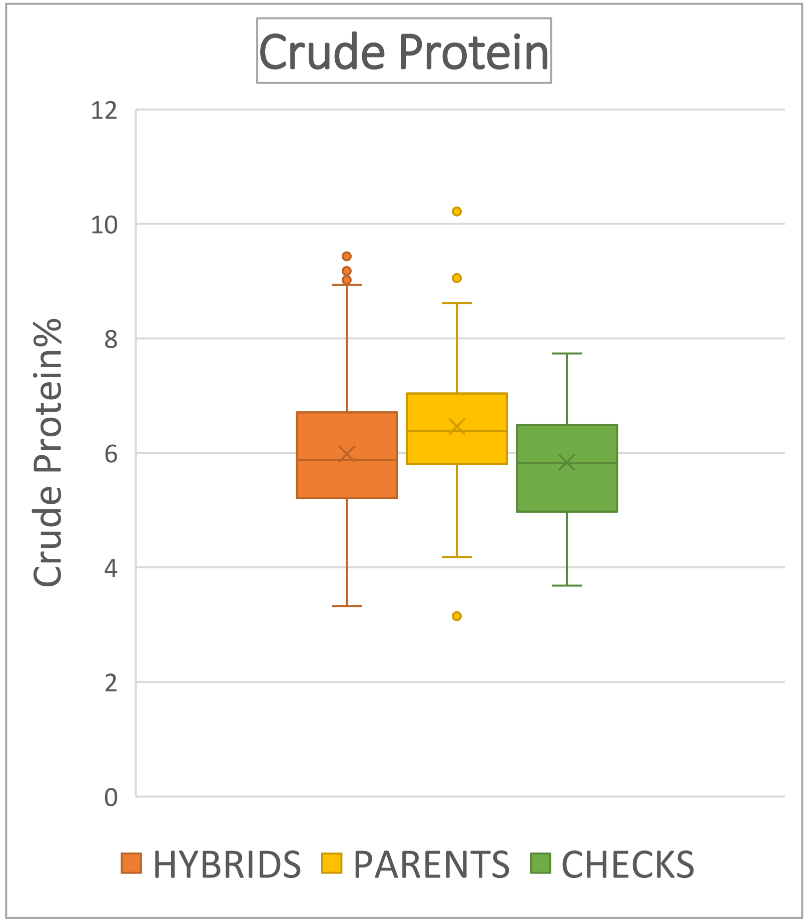
Expanded ILRI 813 model – model stats

Constituent	n	Mean	SD	Est. Min	Est. Max	SEC	RSQ	SEV	1-VR
DM	808	92.24	1.65	88.10	96.92	0.38	0.95	0.40	0.94
Ash	811	7.18	2.17	0.95	13.85	0.47	0.95	0.51	0.94
N	813	0.83	0.27	0.01	1.61	0.05	0.97	0.05	0.96
ME	802	8.30	0.88	5.68	10.93	0.23	0.93	0.25	0.92
IVOMD	807	55.88	5.45	39.52	72.24	1.44	0.93	1.62	0.91
NDF	663	69.23	6.43	49.94	88.51	1.09	0.97	1.21	0.96
ADF	667	38.46	5.61	21.63	55.29	0.91	0.974	1.02	0.97
ADL	666	3.31	0.95	0.47	6.15	0.34	0.87	0.36	0.86

Prediction using 'expanded GFMC' equation

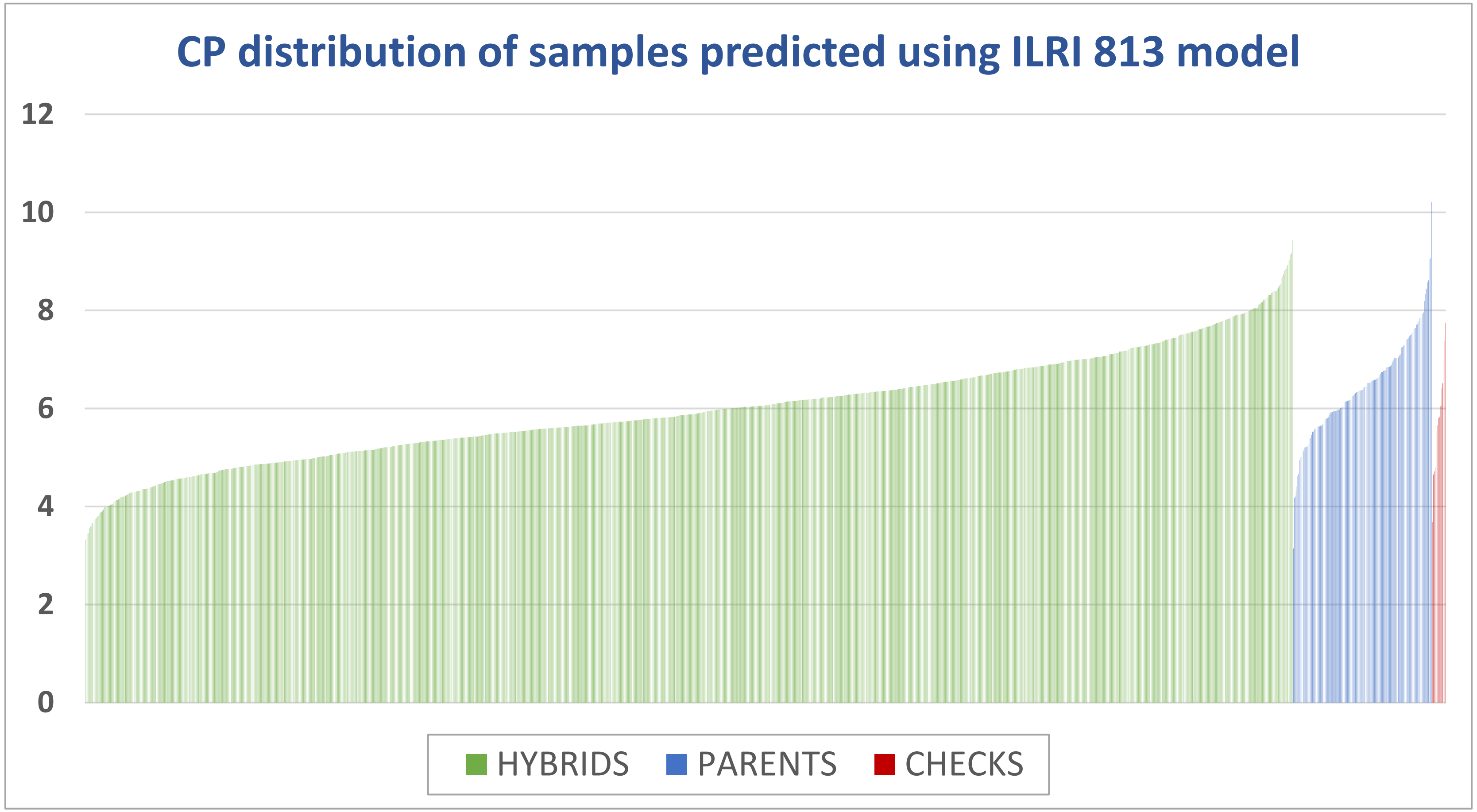


Existing GFMC (665 Model)

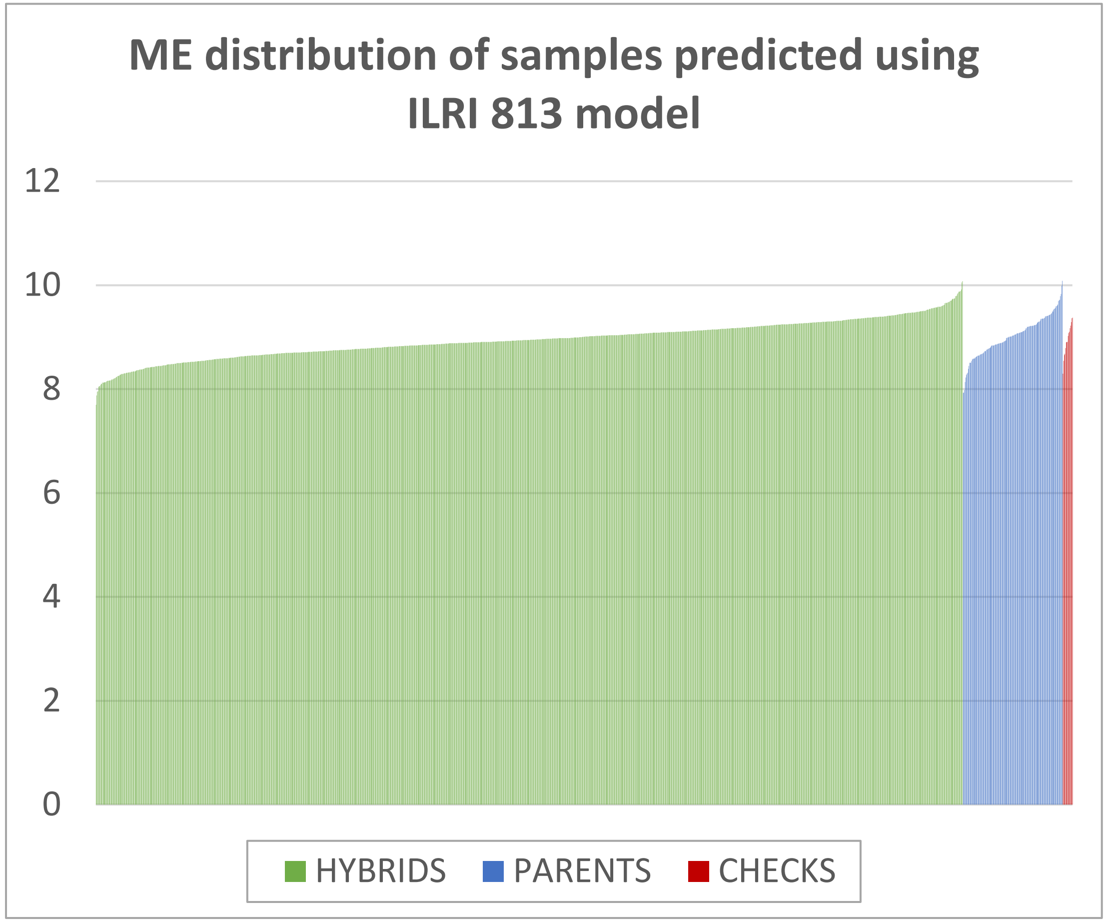


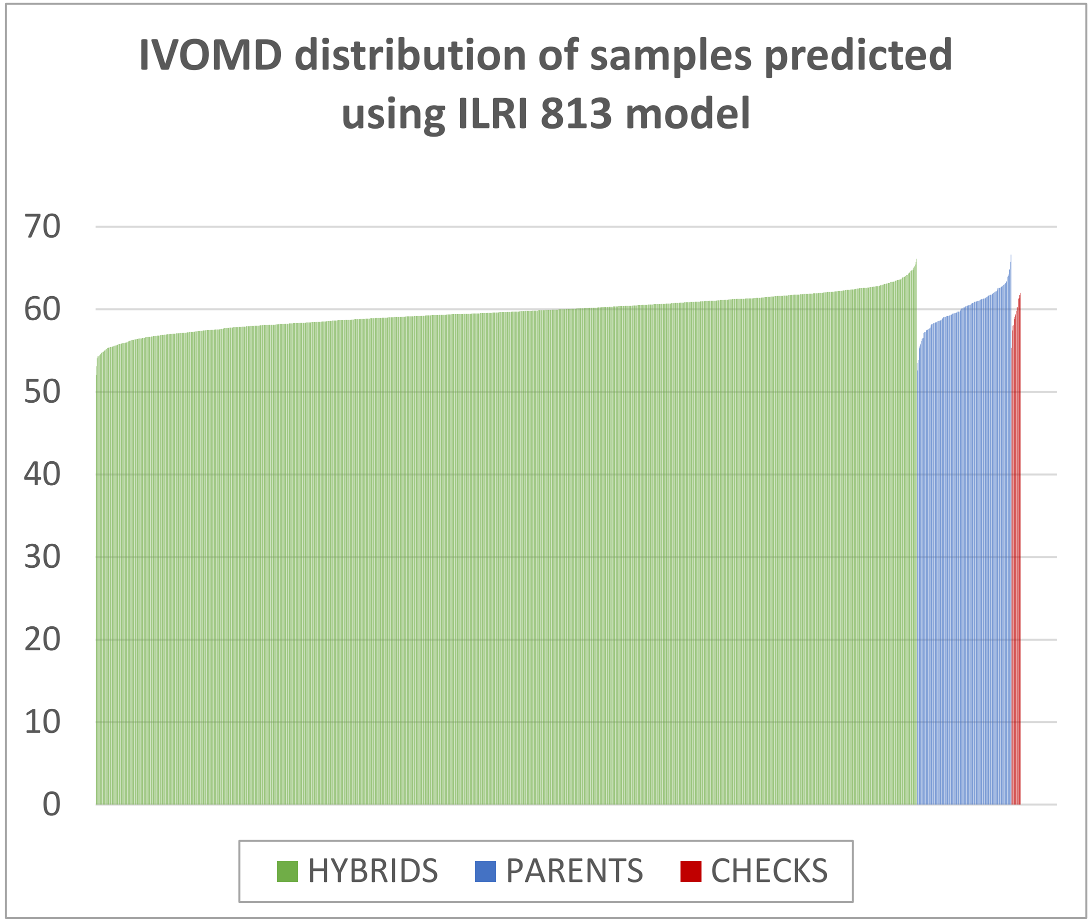
Expanded GFMC (813 Model)

Crude protein prediction



ME & IVOMD prediction





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For the study of plant species, maize occupies a position second to none....

-James A Birchler

Thank you