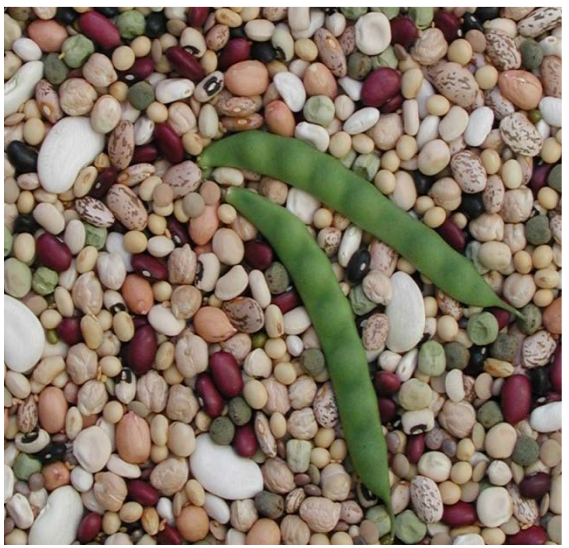
Nutritional Analyses



- Established Nutrients
  - Protein
  - Energy (Starch, Oil)
  - Fiber
  - Vitamins
  - Minerals
- Health-Promoting Phytochemicals

Dry Matter Yield

Nutrient Yield

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Nutritional Analyses

- Genetic diversity for many of these components is known to exist, but careful characterization of a range of cultivars for all traits is lacking.
- Eventual mapping of nutritional trait loci will enable breeders to develop more nutritious and health-beneficial cultivars in all market classes.

Dry and Snap Bean Evaluations

- **Minerals: ICP-OES (Grusak, USDA-TX)**
- **Iron absorption promoters: Caco-2 *in vitro* assay (Grusak, USDA-TX)**
- **Phytate: Colorimetric method (Cichy, USDA-MI)**
- **Protein, Oil, Crude Fiber: Near-infrared Diode Array Analyzer (Naggie, NDSU)**
- **Antioxidants and Sol/Insol Carbs: HPLC and colorimetric assays (Brick/Thompson, CSU)**
- **Carotenoids, Vitamin C, Fiber: HPLC (Myers, OSU)**

Dry Bean Samples Received/Processed

- 248 dry bean entries grown both in MI and WA in summer of 2010 (**496 total**) were received in Houston in the fall of 2010 (~150 grams each).
- Over **4,000 samples** grown at multiple sites in the summer of 2011 or winter of 2012 (~150 grams each) were received in Houston by spring 2012
- All samples are being ground (with stainless steel grinders), packaged, and sent to NDSU (60-100 gm) or MSU (15 gm) for analysis by cooperators; this was in addition to mineral analyses conducted in Houston.
- Early samples were ground with coffee grinders; this procedure was switched to an Udy Mill in mid 2012.

Current Status of 2011/2012 Dry Bean Samples

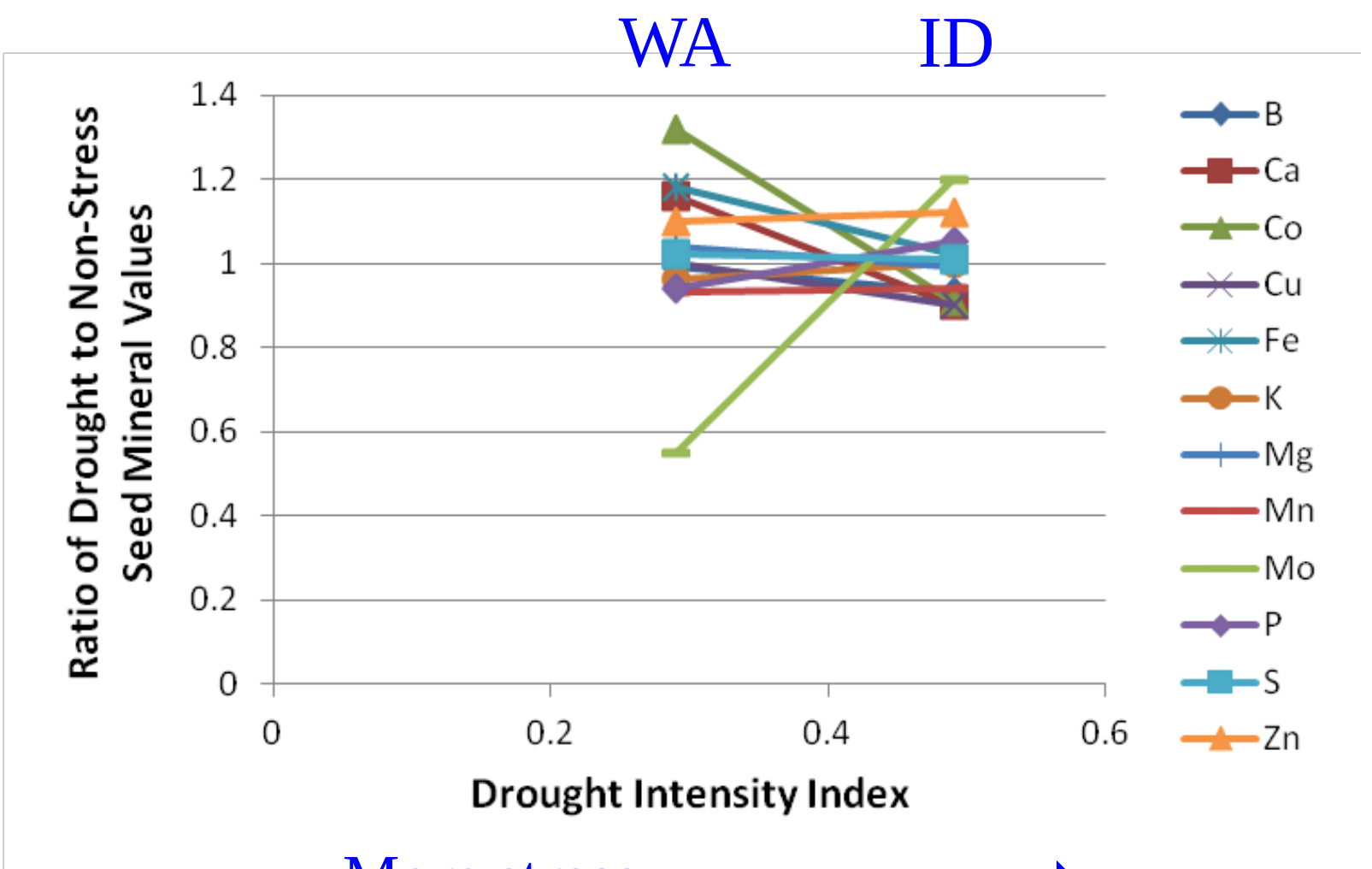
	Shipment Received	Grinding Completed	Ship to Cooperators	Digestion	Mineral Analyses	Protein, Fat, Crude Fiber	Phytate
300 Entries (2 reps)							
CO (~600 samples)	**X**	**80%**	**Apr 2013**				
MI (~600 samples)	**X**	**X**	**X**	**X**	**X**	**X**	**X**
ND (~600 samples)	**X**	**X**	**X**				
NE (714 samples)	**X**	**X**	**X**	**X**	**X**		
Drought/Irrigated (96 Entries x 2)							
ID (~384 samples)	**X**	**X**	**X**	**X**	**X**	**X**	
MI (~192 samples)	**X**	**X**	**X**			**X**	**X**
ND (0)	---	---	---	---	---	---	---
NE (~192 samples)	**X**	**X**	**X**	**X**	**80%**		
PR (~384 samples)	**X**	**Mar 2013**	**Apr 2013**				
WA (~384 samples)	**X**	**X**	**X**	**X**	**X**	**X**	

All analyses on 2010 samples have been completed.

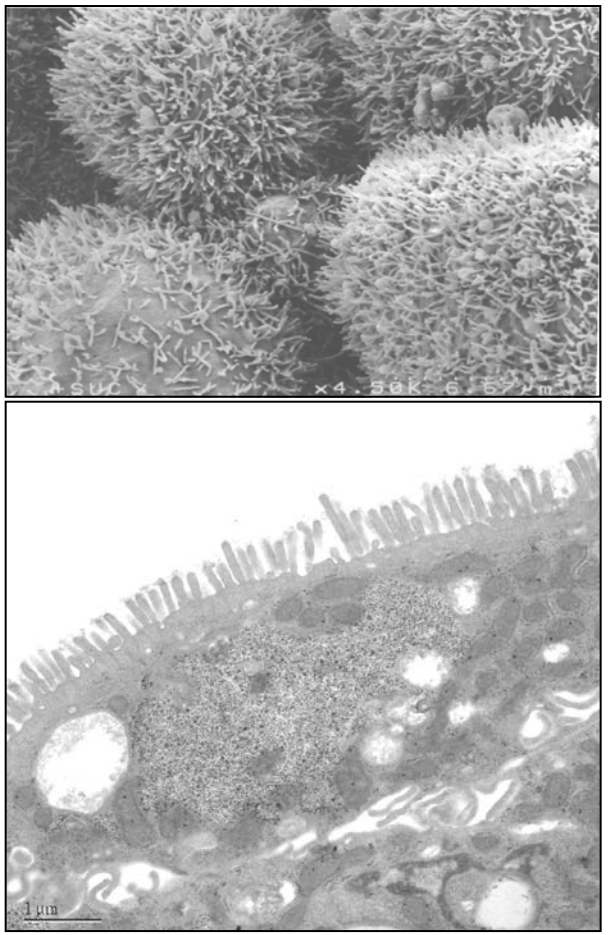
Elemental analysis of 96 Non-Stress Entries – Seeds

Elements	Prosser Range (2011)	Fold Range (Prosser)	Idaho Range (2011)	Fold Range (Idaho)	MSU 2010 Entries (248)
Ca (mg/g DW)	1.2 – 3.8	3.3 x	1.0 – 3.2	3.3 x	0.4 – 3.8
K (mg/g DW)	10.6 – 15.3	1.5 x	12.0 – 16.8	1.4 x	10.5 – 15.8
Mg (mg/g DW)	1.4 – 2.0	1.5 x	1.7 – 2.2	1.4 x	1.2 – 2.4
P (mg/g DW)	4.0 – 5.5	1.4 x	4.4 – 6.7	1.5 x	3.3 – 6.5
S (mg/g DW)	1.6 – 2.7	1.7 x	1.9 – 3.1	1.6 x	1.5 – 2.6
Cu (µg/g DW)	7.2 – 13.9	1.9 x	7.2 – 12.9	1.8 x	6.6 – 13.9
Fe (µg/g DW)	54.3 – 89.7	1.7 x	50.2 – 92.1	2.9 x	47.2 – 101.4
Mn (µg/g DW)	13.9 – 23.2	1.7 x	11.8 – 21.0	1.8 x	10.1 – 19.9
Mo (µg/g DW)	0.3 – 1.2	4.1 x	1.2 – 10.0	8.7 x	not detected
Ni (µg/g DW)	2.5 – 5.7	2.3 x	not detected		0.6 – 6.3
Se (µg/g DW)	not detected		not detected		0.3 – 1.0
Zn (µg/g DW)	23.1 – 43.0	1.9 x	35.5 – 62.6	1.8 x	31.0 – 68.5

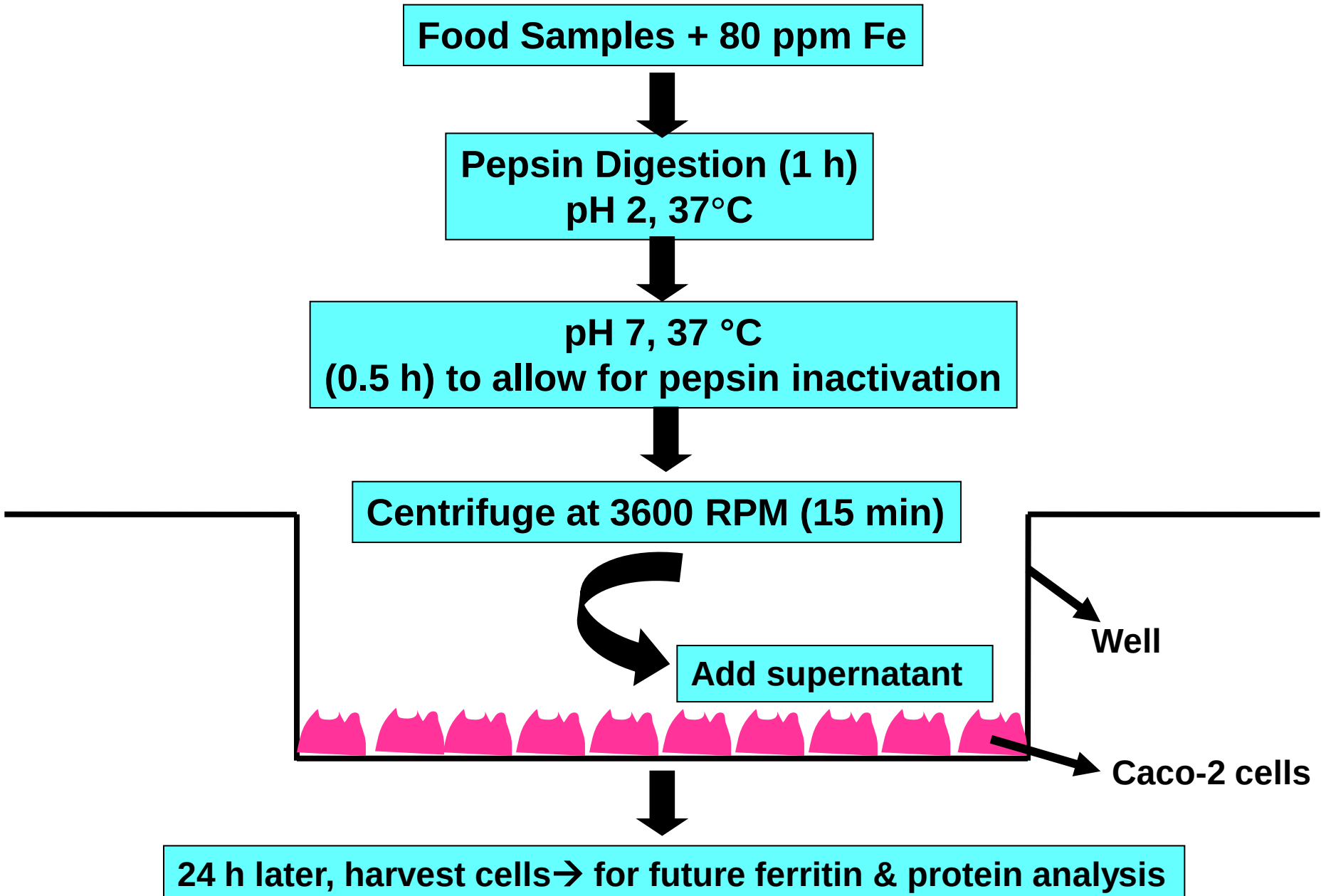
Drought Effect on Seed Mineral Concentrations



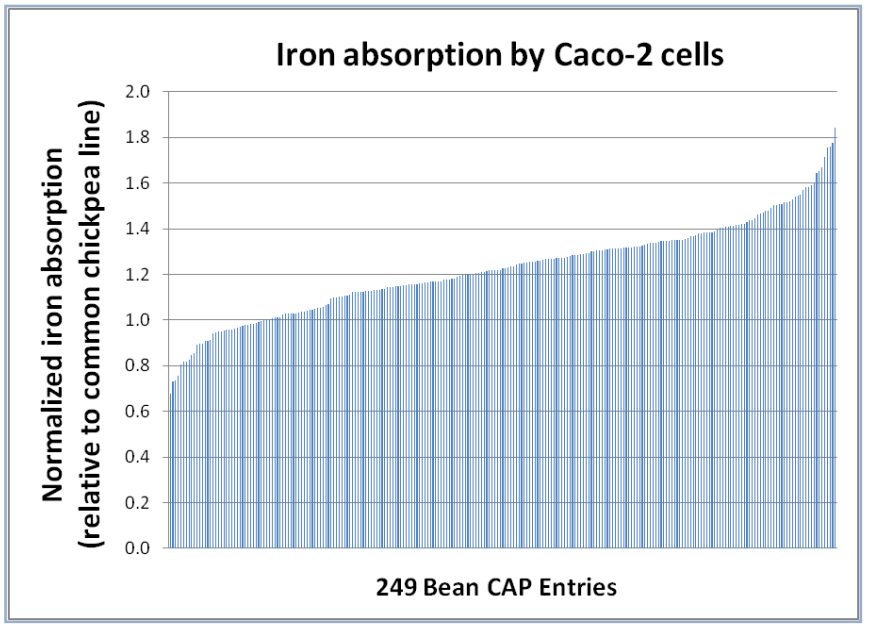
Caco-2 Cells

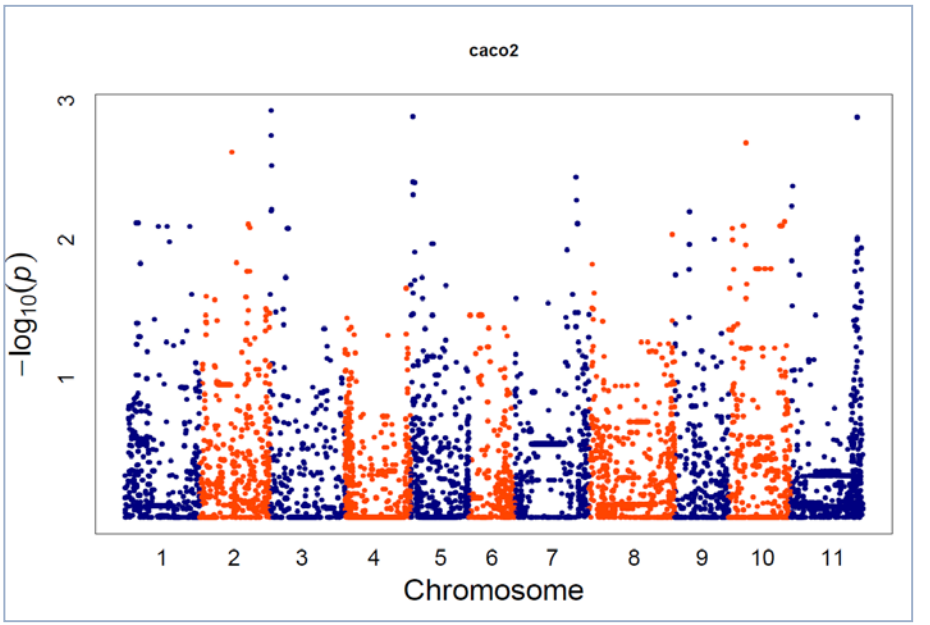


- Human colon adenocarcinoma cell line.
- Derived from a 72 year-old male Caucasian.
- In culture, the cells form a tight monolayer and exhibit features of small intestinal cells with brush border enzymes and transport proteins.
- Can be used as an *in vitro* model to study nutrient absorption.
- Well suited to screen for the effect of various inhibitors/enhancers on mineral bioavailability.


Food Samples + 80 ppm Fe
Pepsin Digestion (1 h)
pH 2, 37°C
pH 7, 37 °C
(0.5 h) to allow for pepsin inactivation
Centrifuge at 3600 RPM (15 min)
Add supernatant
Well
Caco-2 cells
24 h later, harvest cells→ for future ferritin & protein analysis

In vitro iron absorption using Caco-2 Cells





- Cooked bean samples were mixed with a standard amount of iron and fed to Caco-2 cells after *in vitro* digestion.
- Assay measured the potential of food components (in bean) to promote iron absorption.
- Normalized values demonstrated a 2.7-fold range.

Protein/Fiber/Fat Analyses (2010 samples)

	MI (MSU 2010)	WA (Prosser 2010)
% Protein (DW basis)	18.77 – 31.54	20.57 – 31.29
% Crude Fiber (DW basis)	3.43 – 8.35	3.18 – 8.33
% Fat (DW basis)	0.97 – 2.28	0.98 – 2.11

- Both NIR and chemical analyses were performed on 2010 samples in order to calibrate the NIR measurements.
- Had planned to just process all the 2011 samples by NIR, but new grinding procedure (finer grind) meant more calibrations were needed (NIR and chemical analysis comparisons).
- This is now completed and all further samples will be analyzed by NIR.

Update on Phytate Results

- Completed the analysis of 496 bean samples from 2010 and 950 bean samples from 2011 field seasons.
- An additional 300 lines (2011) have been prepared for analysis.
- Phytate levels are ranging from 2.5 – 7.2 mg/g (2.9-fold).
- A negative correlation was found between cooking time and phytic acid levels in a screening of 100 bean lines ($r = -0.45$, $p<0.0001$)

Plans for 2013

- Complete grinding of all 2011/2012 dry bean samples; distribute final subsamples to analytical cooperators.
- Complete mineral analyses on all samples.
- Work with Phil McClean's group on data analyses and Association Mapping.
- Plan and write manuscript(s), especially a characterization of 2010 field samples.