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Biofortification of pea and chickpea

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November 18, 2015

Health benefits of pulse crops

Whole grain/natural
Moderate protein content
Moderate starch/energy content
Moderate fiber content
Low fat content
Low glycemic index
Gluten free
Low alergencity

Can we improve them further?

Issues with gluten ….

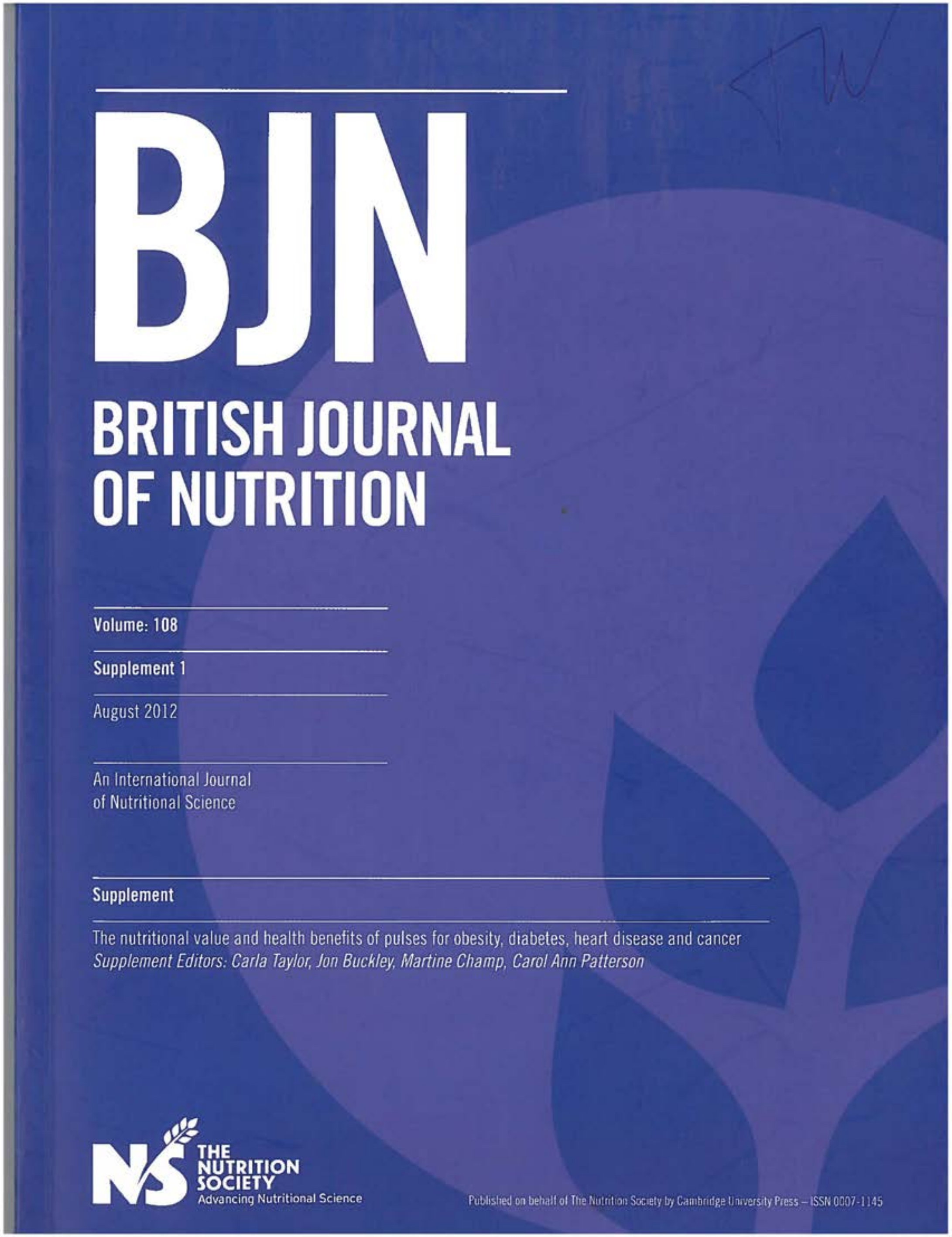
- 1-2% with celiac disease
- 2% with wheat allergy
- <u>5% with irritable bowel syndrome (gluten sensitive)</u>

  = 9% of the USA population

BUT,
40% of the USA population
avoided wheat in 2014

A good opportunity for pulses!

Source: Buhler (Switzerland)



The nutritional value and health benefits of pulses for obesity, diabetes, heart disease and cancer

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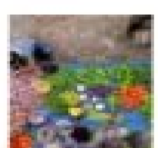
What's New

Dietary Diversity and Biofortification: Closer Than You Think
Oct 15, 2015



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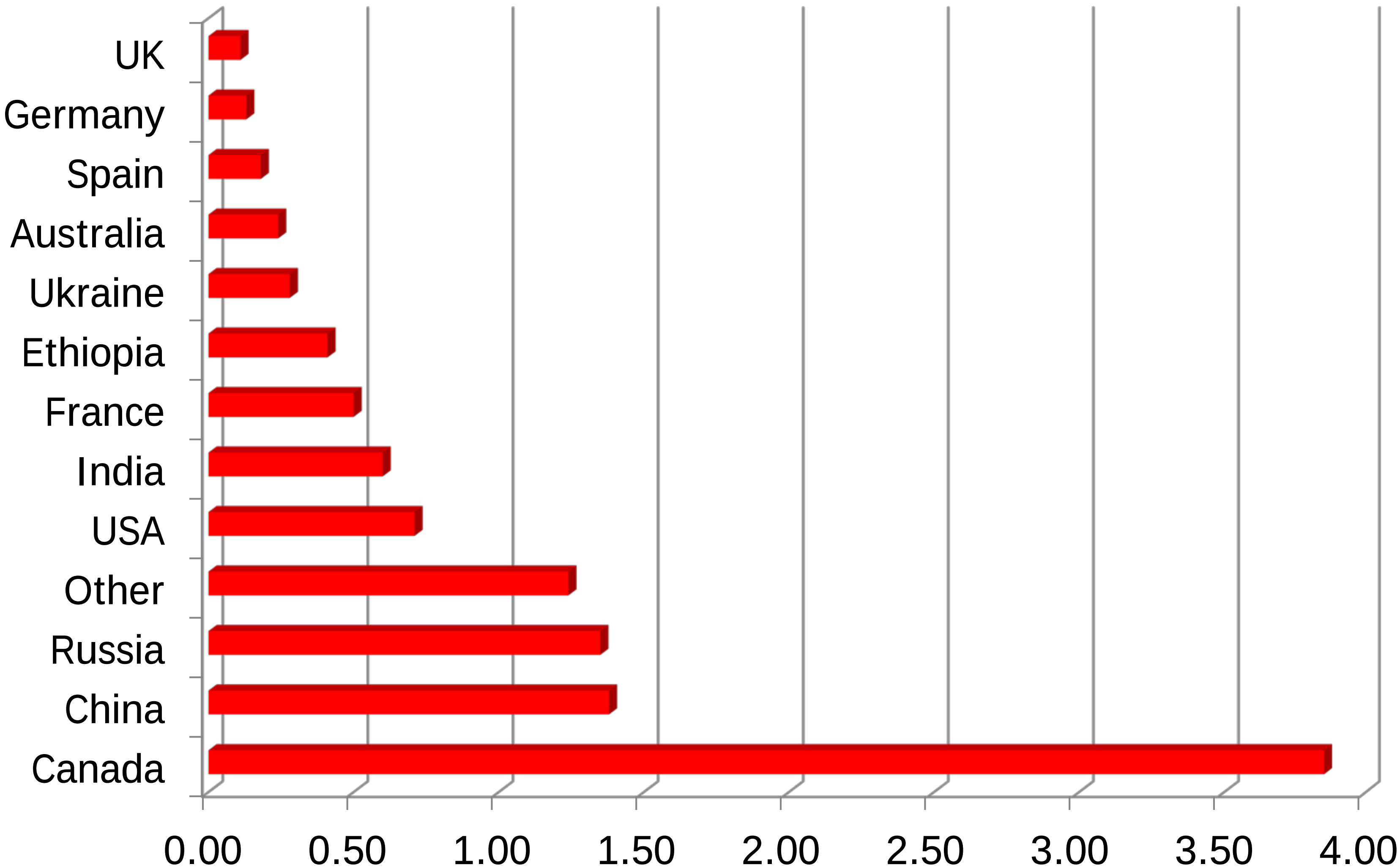
Pea production in 2013 (million tonnes)
Total 10.98



FAOSTAT

Top Canadian pea export destinations in 2013-14	
Country	tonnes
India	985,000
China	967,000
USA	193,000
Bangladesh	186,000
Others	449,000
Total	2,780,000

Statistics Canada, Saskatchewan Ministry of Agriculture, 2014

Vermicelli noodles
from Canadian yellow
pea starch (Shandong Prov. China)

Photos: Rachel Kehrig

Some emerging markets for pea ....

- **Protein fractions** from dry and wet milling
  in China (wet), integrated with starch noodle production
  in France and Belgium (wet) [Roquette, Cosucra]
  in USA (dry) [Alliance Grain Traders]

- **New food uses** as flours or fractions [Nestle, General Mills, others]

- Expanded **pet food** uses

- Flour incorporated into **Chinese wheat-based staple foods** (noodles, steamed bread, biscuits)

Key pea breeding/research goals

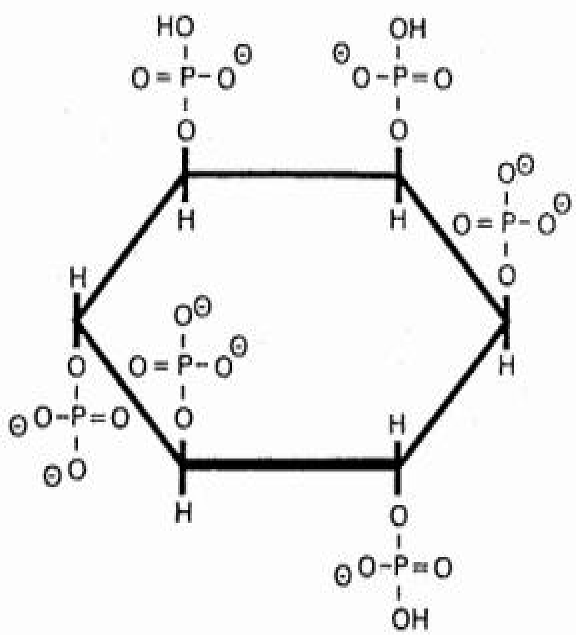
- 2% annual gain in yield
- Implement marker-assisted selection for key traits
  - mycosphaerella blight, lodging, Fe
- Genome sequence development and utilization
  - international consortium
- Biofortification
  - increased concentration
  - increased bioavailability (low phytate)
- Heat tolerance during flowering

Percentage of RDA provided by 100 g (dry wt.) pulses

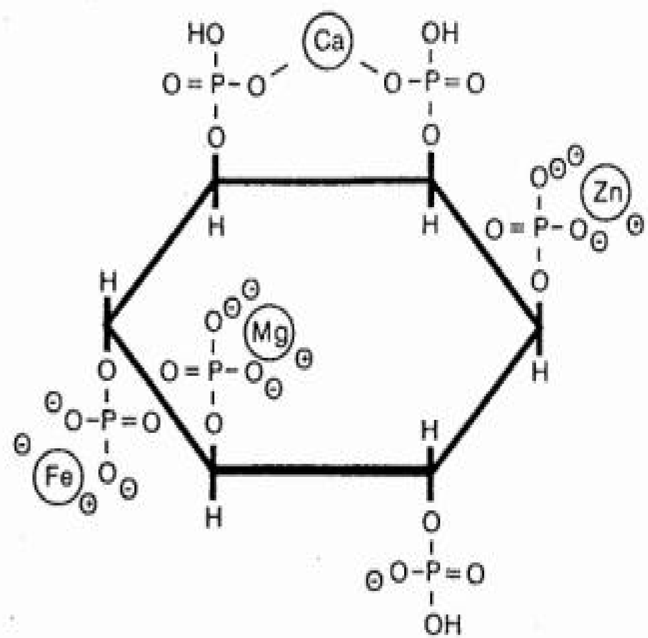
				% of RDA in 100 g dry wt							
Component	RDA			Bean		Chickpea		Field pea		Lentil	
	male	female	units	male	female	male	female	male	female	male	female
K	4700	4700	mg	44	44	n/a	n/a	22	22	20	20
Mg	420	320	mg	54	70	40	53	28	36	24	31
Ca	1000	1000	mg	n/a	n/a	5	5	n/a	n/a	3	3
Zn	11	8	mg	27	38	23	31	27	38	42	58
Fe	8	18	mg	87	39	65	29	67	30	109	48
Mn	2.3	1.8	mg	56	72	104	133	54	69	60	76
Cu	0.9	0.9	mg	113	113	78	78	64	64	90	90
Ni	1	1	mg	60	60	n/a	n/a	27	27	15	15
Se	55	55	μg	81	81	133	133	85	85	215	215

Ray et al. 2014 Crop Sci 54:1698-1708

- Phytic acid
    - Storage form of P in seeds
    - Mixed cationic salt
    - Not well digested
- Binds K^{+}, Mg^{++}, Ca^{++}, Mn^{++}, Zn^{++}, Fe^{+++}



Myo-inositol hexakisphosphoric acid



Phytate-Metal Complex

(Ref: http://www.seedbiology.de)

Performance of low-phytate pea lines 1-150-81 and 1-2347-144 in comparison to CDC Bronco in field trials in Saskatchewan in 2009 and 2010					
Variety	Grain yield (t/ha)	Seed weight (g/1000)	Total P (mg/g)	Phytate-P (mg/g)	Inorganic-P (mg/g)
1-150-81	5.15	203	3.37	1.12	1.08
1-2347-144	5.53	204	3.17	1.01	1.00
CDC Bronco	6.02	217	3.20	2.50	0.25
LSD (0.05)	0.42	6.6	ns	0.23	0.07
n	4	4	4	4	4

T.D. Warkentin, T. Delgerjav, G. Arganosa, A.U. Rehman, K.E. Bett, Y. Anbessa, B. Rossnagel, and V. Raboy, 2012. Development and Characterization of Low-Phytate Pea. Crop Science, 52:74-78.

F_2:$_4$ RILs of six populations from crosses of low and normal phytate field pea lines

Cross	Expected†			Observed			χ^{2}‡
	Normal	Segregating	Low	Normal	Segregating	Low	
1-150-81/CDC Bronco	59	119	59	68	125	44	5.57
CDC Bronco/1-150-81	42	83	42	41	91	34	2.13
1-150-81/CDC Meadow	51	101	51	50	108	44	1.33
1-2347-144/CDC Bronco	39	78	39	42	83	30	2.64
1-2347-144/CDC Meadow	44	88	44	37	105	34	6.67
1-150-81/1-2347-144	-	-	131	0	3	128	0.07§

† Based on single gene recessive (1:2:1 ratio for normal:segregating:low)

A. Rehman, A. Shunmugam, G. Arganosa, K.E. Bett, and T.D. Warkentin, 2012. Inheritance of the Low Phytate Trait in Pea. Crop Science, 52:1171-1175.

CDC Bronco (black bars) and low-phytate genotypes 1-150-81 (light grey bars) and 1-2347-144 (dark grey bars) assessed at Saskatoon and Rosthern in 2010 and 2011.

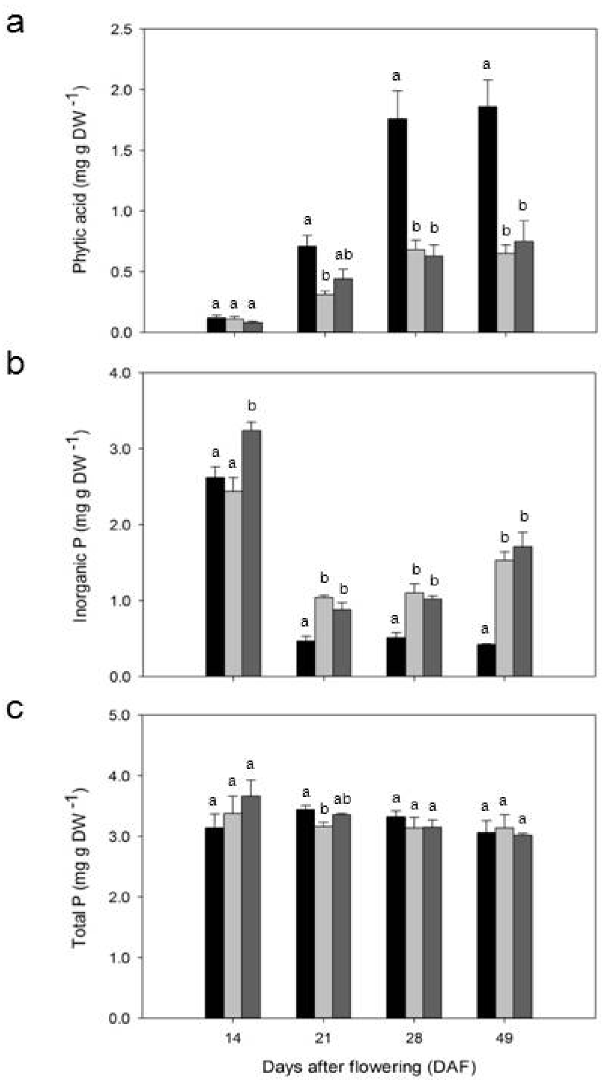
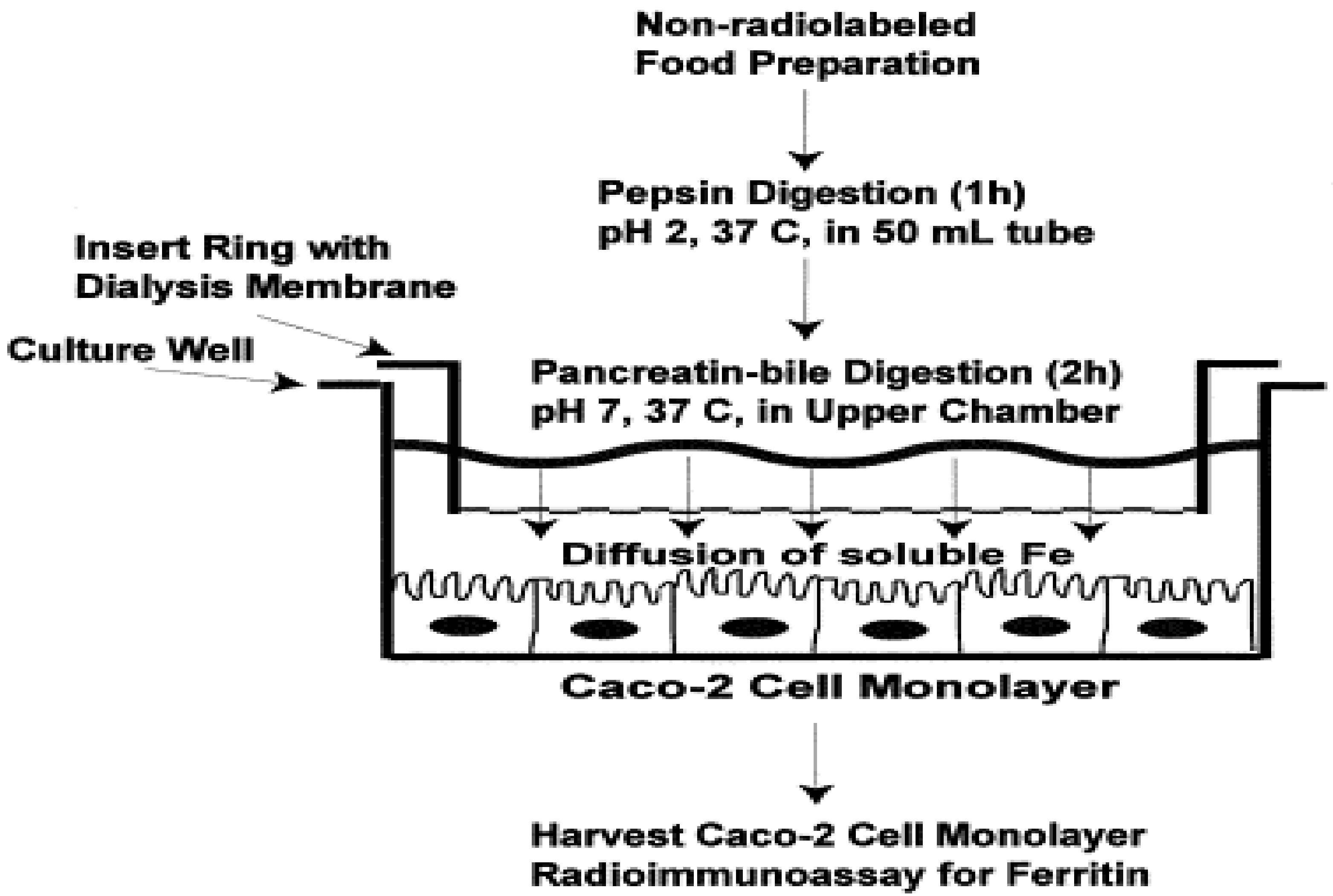


Fig. 2

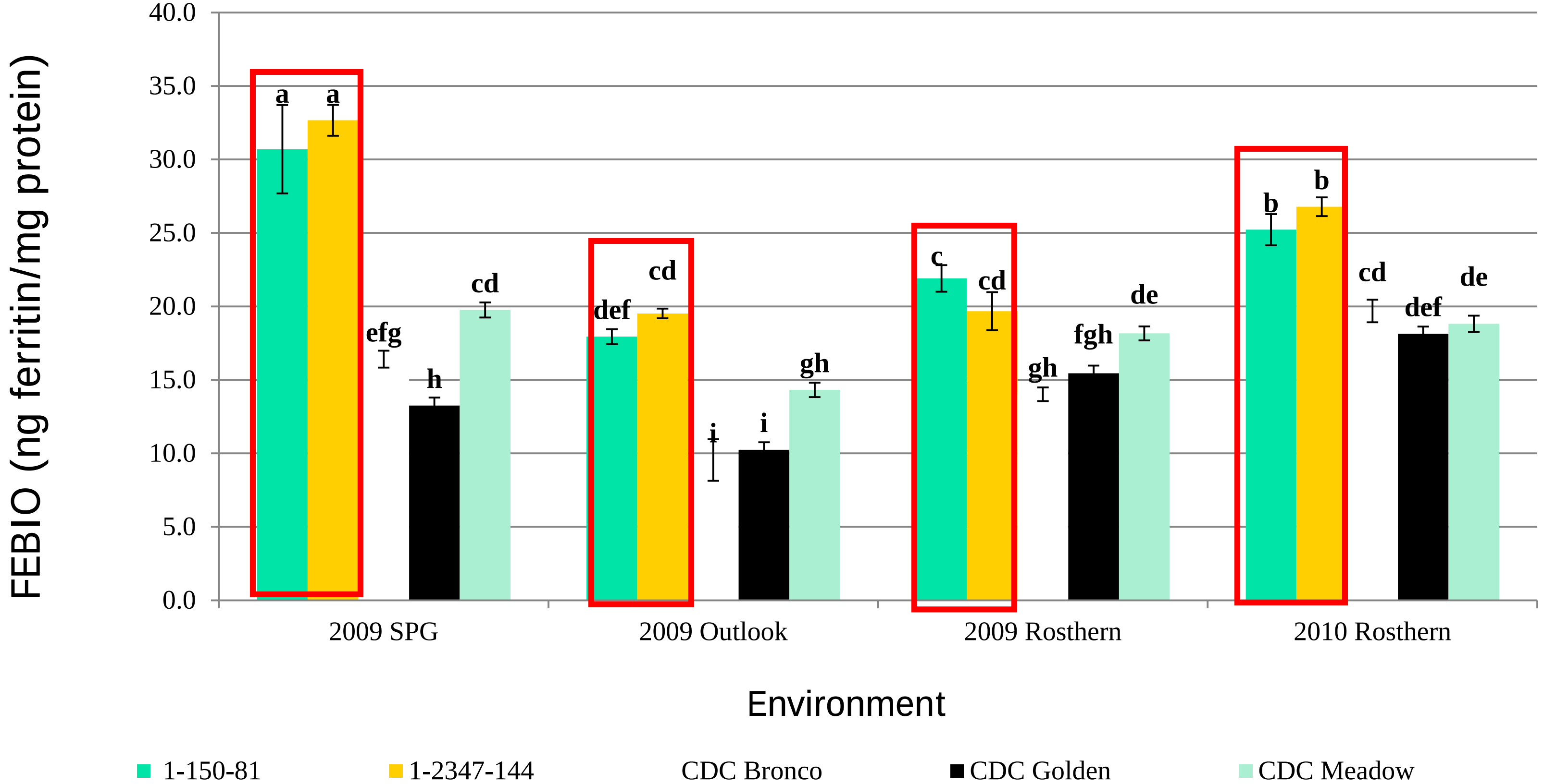
Shunmugam, A.S.K., Bock, C., Arganosa, G., Georges, F., Gray, G.R., and Warkentin, T.D. (2015) Accumulation of phosphorus-containing compounds in developing seeds of low-phytate pea (*Pisum sativum* L.) mutants. Plants 4:1-26.

- Iron Bioavailability (FeBIO) using Caco-2 cell culture assay



Source: http://www.atc-pharma.be/en/node/151

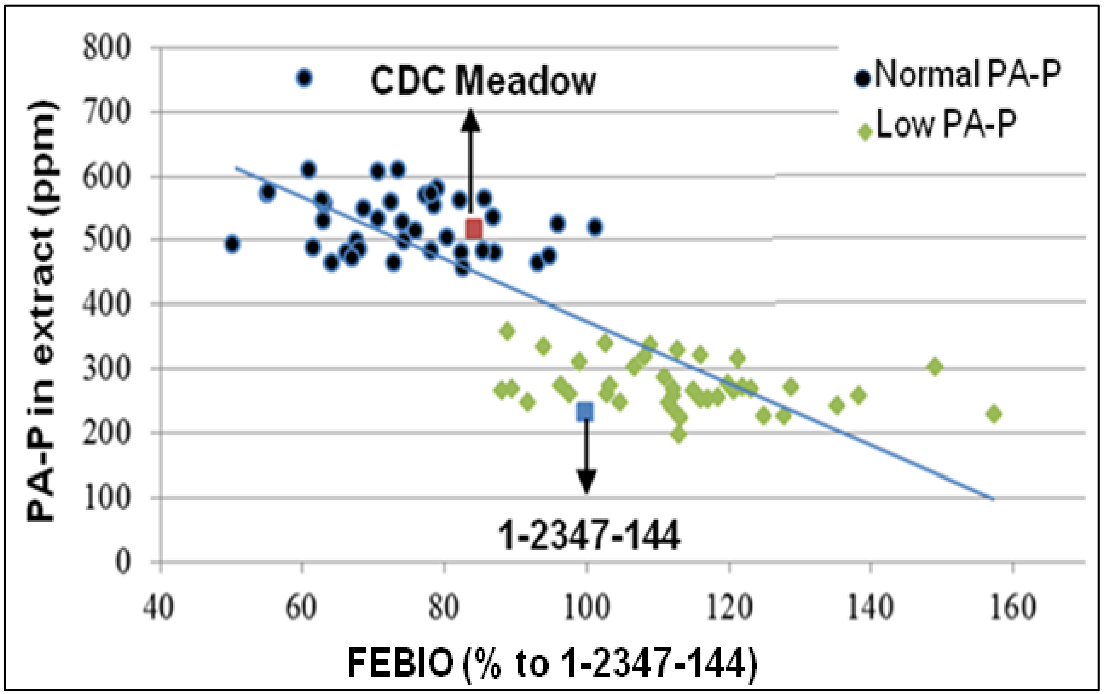
Glahn et al. (1998)



Iron bioavailability of two low phytate lines and three normal phytate varieties at 2009 SPG, 2009 Outlook, 2009 Rosthern and 2010 Rosthern environments.

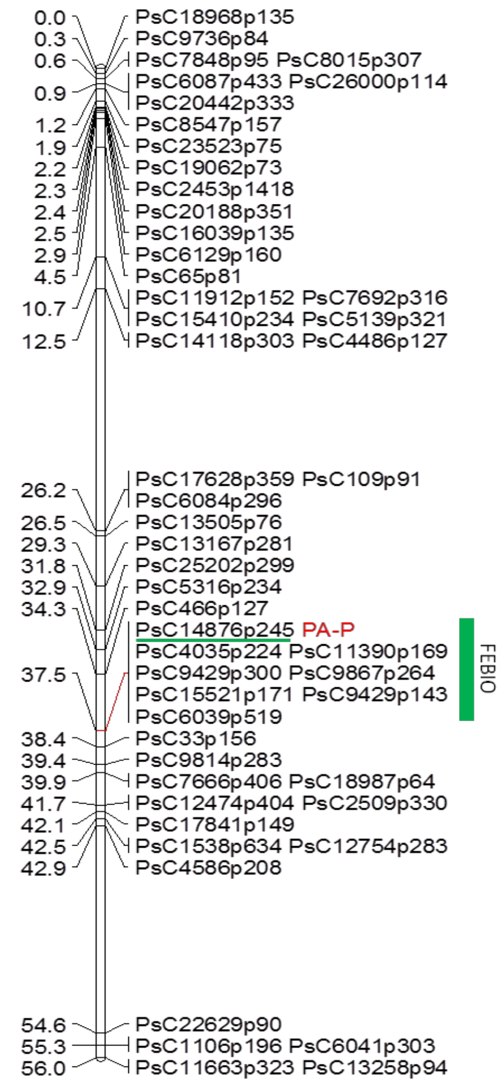
Liu, X., Glahn, R.P., Arganosa, G.C., and Warkentin, T.D. (2015) Iron bioavailability in low phytate pea. Crop Sci. 55:320-330.

QTL mapping of phytic acid-P and iron bioavailability



Correlation between phytic acid-P and iron bioavailability in lines from PR-15

Pea LG V



Shunmugam et al. 2015 Crop Sci 55: 828-836

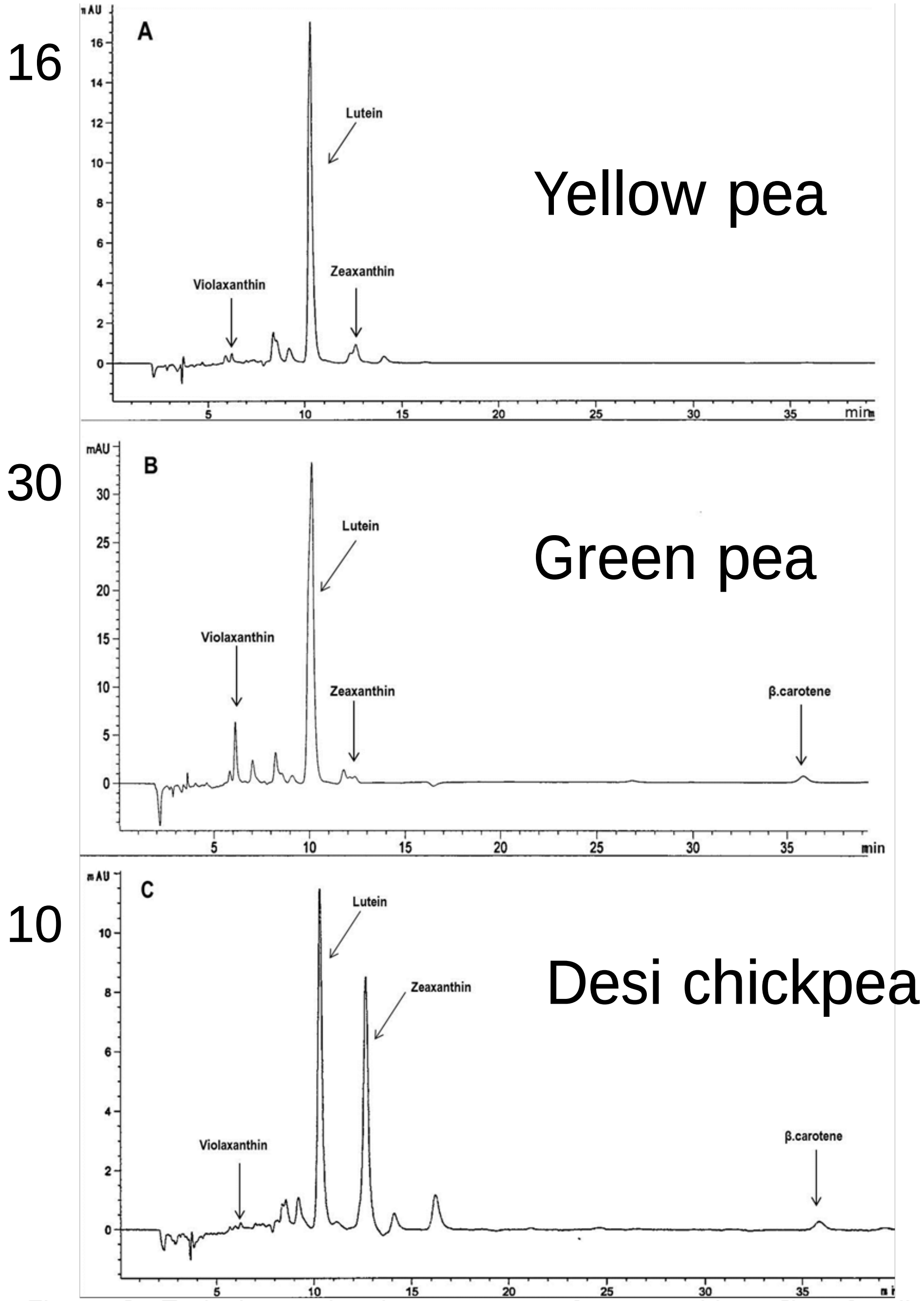
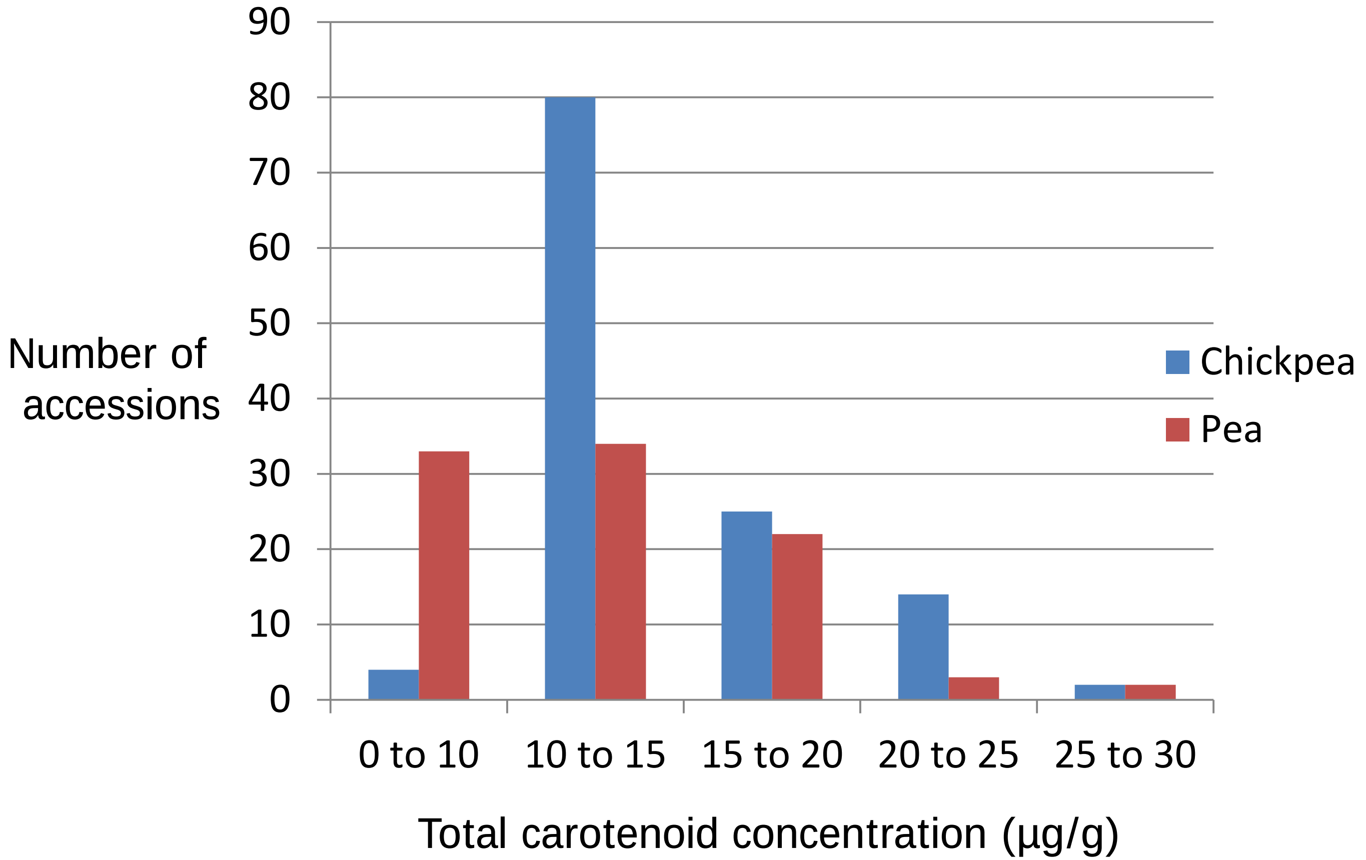


Figure 2. Typical sample chromatogram of carotenoid profile of yellow cotyledon pea cultivar CDC Meadow(A), green cotyledon pea cultivar CDC Patrick (B), and desi chickpea cultivar CDC Corinne (C).

Ashokkumar, K., Tar'an, B., Diapari, M., Arganosa, G. and Warkentin, T.D. (2014) Effect of genotype and environment on carotenoid profile in field pea and chickpea. Crop Sci. 54:2225-2235.

Total carotenoid concentration of genetically diverse accessions of pea and chickpea



Ashokkumar, K., Diapari, M., Jha, A., Tar'an, B., Arganosa, G., and Warkentin, T.D. (2015) Genetic diversity of nutritionally important carotenoids in diverse pea and chickpea accessions by HPLC analysis. J Food Composition and Analysis 43:49-60.

Correlation of ferritin with phytic acid and carotenoids in 4802-8 sublines

4802-8	Phytic Acid	Iron	Violaxanthin	Lutein	ß-carotene	Zeaxanthin
Ferritin	-0.34	-0.15	0.03	0.41	-0.17	-0.15
Pr>F value	0.02	0.34	0.82	0.01	0.25	0.32

Correlation of ferritin with phytic acid and carotenoids in 4803-4 sublines

4803-4	Phytic Acid	Iron	Violaxanthin	Lutein	ß- carotene	Zeaxanthin
Ferritin	-0.37	0.10	0.22	0.25	-0.03	0.04
Pr<F value	0.01	0.49	0.14	0.09	0.84	0.77

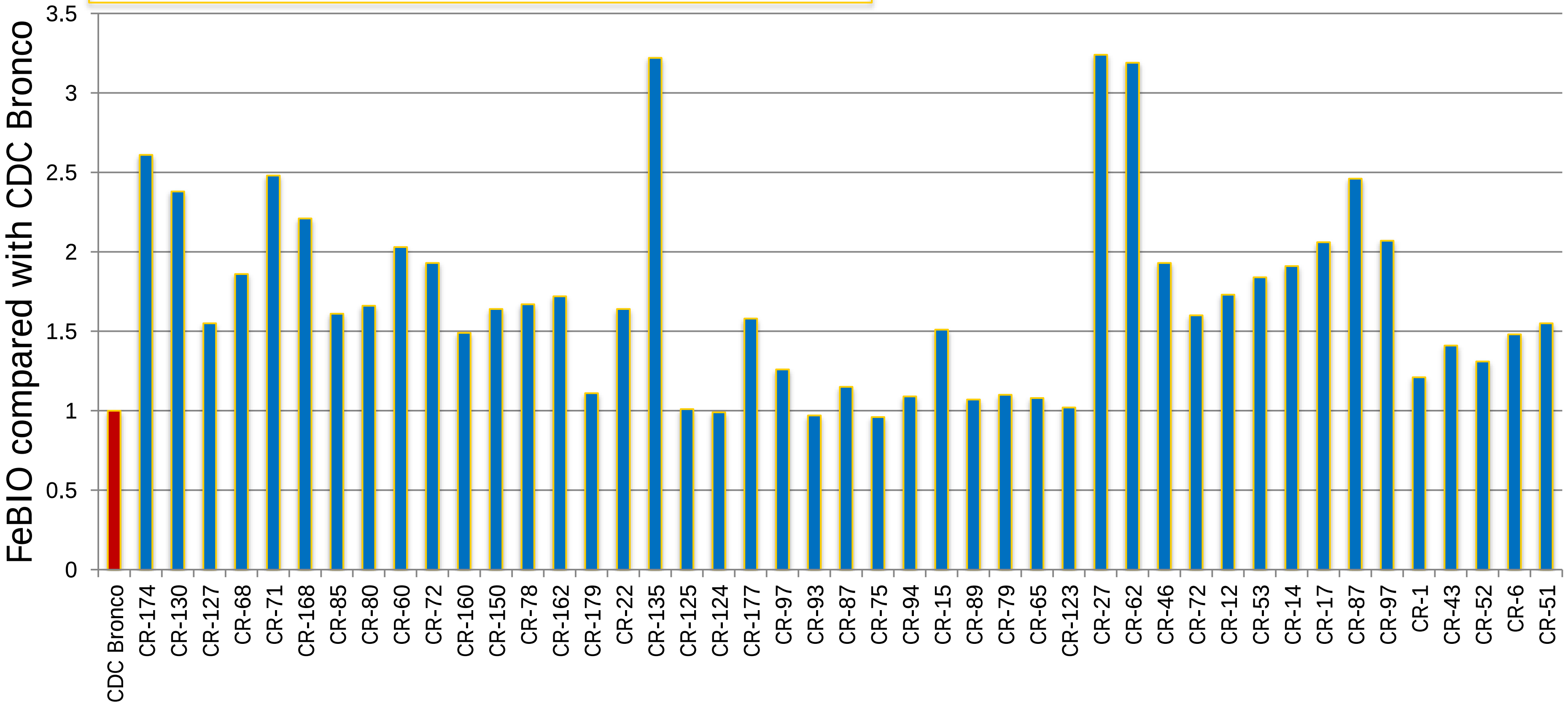
Parminderjit Bangar, MSc student

Correlation of ferritin with phytic acid and carotenoids in PR-07 RILs

	Phytic acid	Iron	Violaxanthin	Lutein	Zeaxanthin	ß-Carotene
FeBIO	-0.24	0.36	-0.09	-0.01	-0.24	-0.06
Pr<F Value	0.12	0.02	0.57	0.95	0.12	0.70

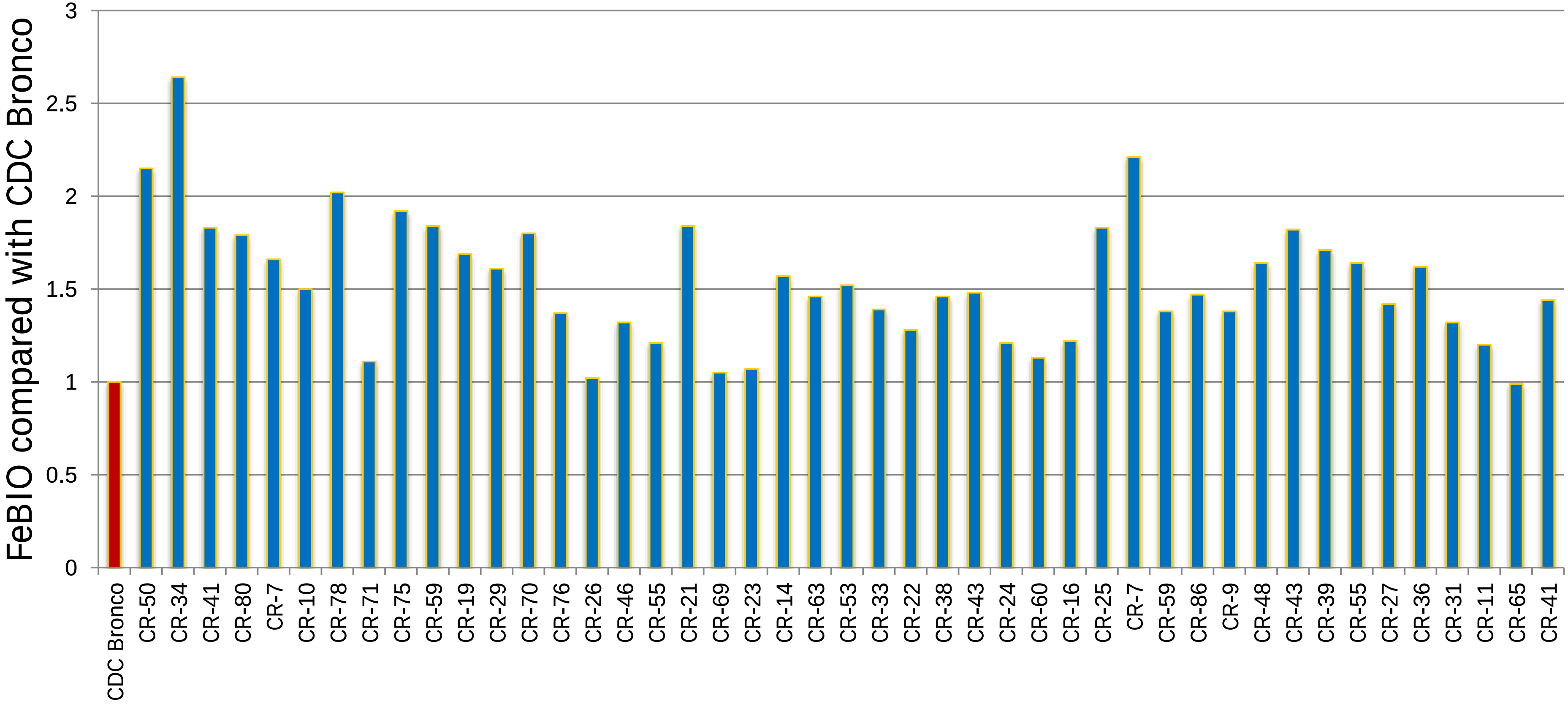
Parminderjit Bangar, MSc student

4802-8 Sublines



4802-8 Sublines showed up to 3.2 times higher iron bioavailability than CDC Bronco

4803-4 Sublines



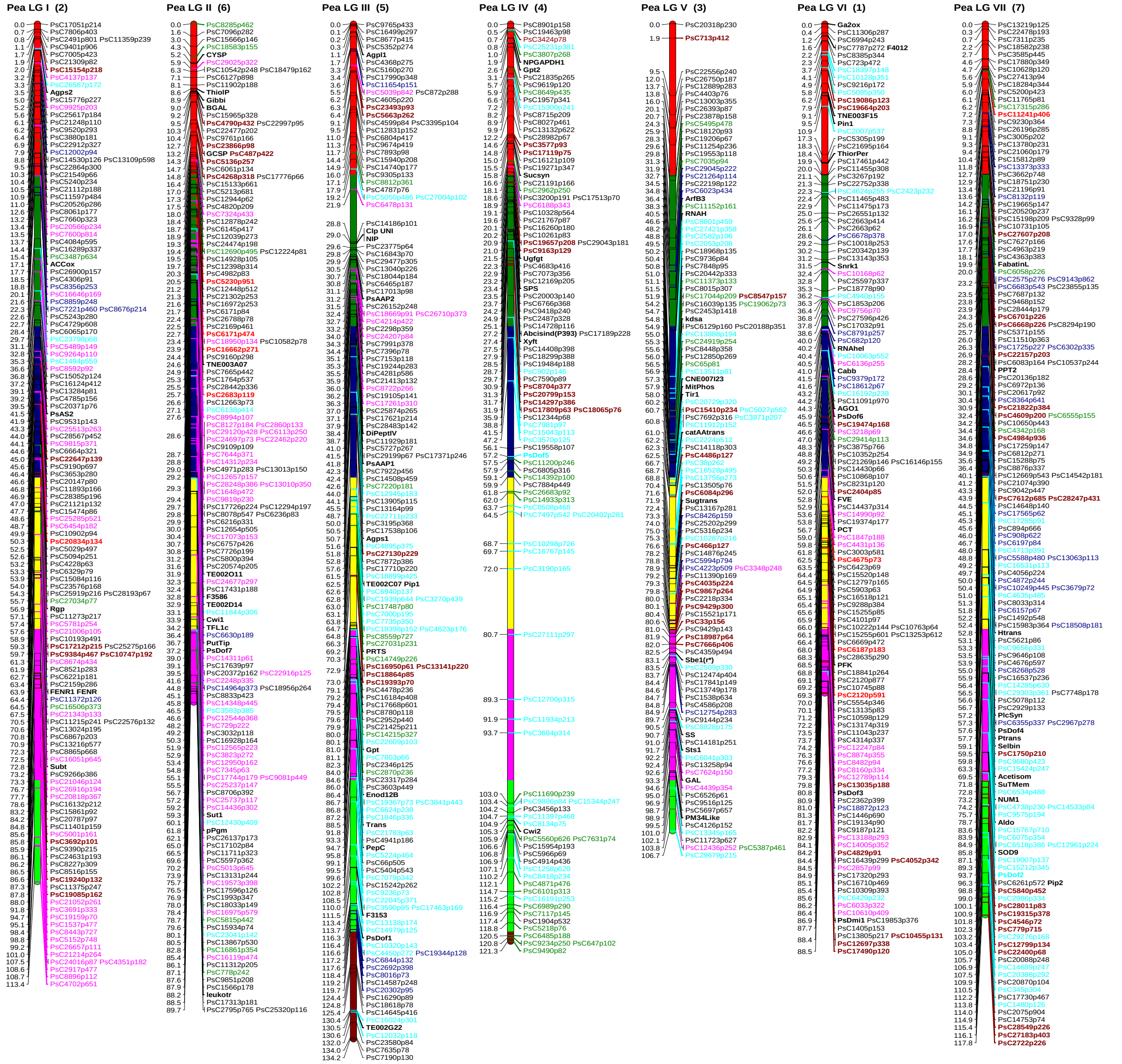
4803-4 Sublines showed up to 2.6 times higher iron bioavailability than CDC Bronco

Folates (µg/100 g) in common bean, lentil, chickpea and pea (mean of 4 cultivars X 3 reps X 2 locations)

Crop	Genotype	FA	10-FFA	THF	5-MTHF	5,10-MTHF	5-FTHF	Total folate
Bean[1]	Mean	10.8	3.1	27.7	75.5	11.3	63.1	191.6
	$LSD_{0.05}$	0.9	0.5	4.2	12.5	2.8	7.6	18.9
Lentil[2]	Mean	7.8	4.2	18.8	53.3	11.2	58.1	153.4
	$LSD_{0.05}$	0.7	0.9	2.7	12.5	1.5	3.5	21.7
Chickpea[3]	Mean	8.3	8.2	18.4	176.5	29.3	242	482.7
	$LSD_{0.05}$	3.0	3.7	4.9	26.4	8.7	78.5	90.3
Pea[4]	Mean	0.7	0.8	5.7	14.7	0.8	3.5	26.2
	$LSD_{0.05}$	0.1	0.2	0.9	1.7	0.3	1.1	3.1

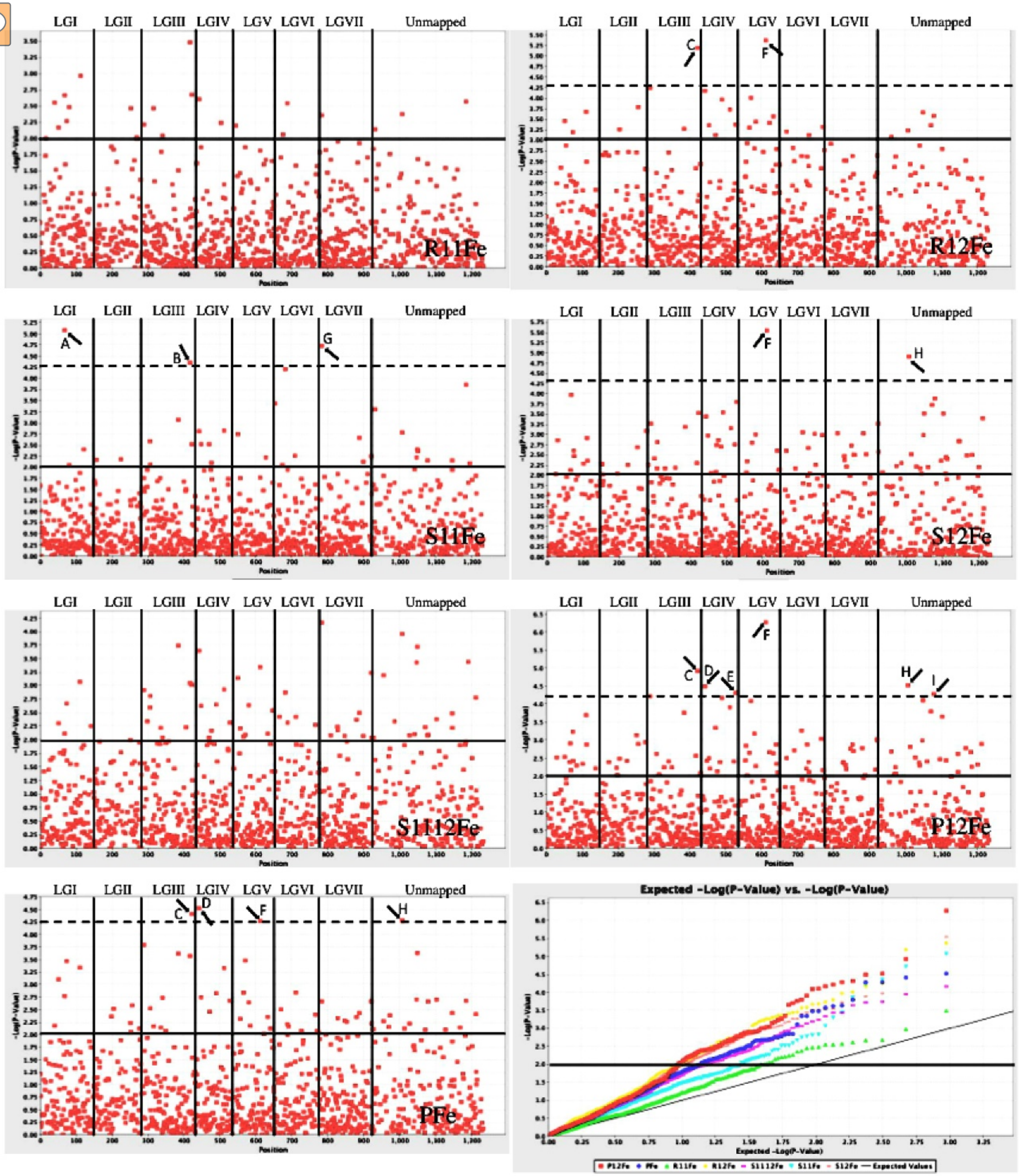
[1] Saskatoon & Rosthern; [2] Limerick & Saskatoon; [3] Elrose & Limerick; [4] Meath Park & Saskatoon;
FA, Folic acid; 10-FFA, 10 Formyl folic acid; THF, Tetrahydrofolate; 5-MTHF, 5-Methyl tetrahydrofolate; 5,10-MTHF, 5,10- Methenyl tetra hyrofolic acid; 5-FTHF, 5-Formyl tetrahydrofolic acid

Jha, A., Ashokkumar, K., Diapari, M., Ambrose, S. J., Zhang, H., Tar'an, B., Bett, K.E., Vandenberg, A., Warkentin, T.D., and Purves, R. (2015) Genetic diversity for folate profile in seeds of chickpea, lentil, common bean and pea. J Food Composition and Analysis 42:134-140.



Consensus SNP linkage map of *Pisum sativum* generated by using five RIL mapping populations.

Sindhu, A., Ramsay, L., Sanderson, L.A., Stonehouse, R., Li, R., Condie, J., Shunmugam, A.S.K., Liu, Y., Jha, A.B., Diapari, M., Burstin, J., Aubert, G., Tar'an, B., Bett, K.E., Warkentin, T.D., and Sharpe, A.G. (2014) Gene-based SNP discovery and genetic mapping in pea. Theoretical and Applied Genetics 127:2225-2241.



Diapari, M., Sindhu, A., Warkentin, T.D., Bett, K.E., Ramsay, L., Sharpe, A.G. and Tar'an, B. (2015) Population structure and marker-trait association studies of iron, zinc and selenium concentration in seed of field pea (*Pisum sativum* L.). Mol Breeding 35:30 doi 10.1007/s11032-015-0252-2.

Manhattans plot of $-\log_{10}$P-values of the marker-traits association study for the iron (Fe) concentration in seed in Pea Association Mapping (PAM) panel using mixed linear model (MLM). Threshold of $-\log_{10}(p\text{-value}) = 2.0$ indicated by the horizontal lines. Dashed lines indicate sequential Bonferroni correction at $-\log_{10}(p\text{-value}) = 4.27$. Letters indicated significant associated markers. A. PsC22912p327; B. PsC7893p98; C. PsC8677p415; D. PsC13009p652; E. PsC9886p84; F. PsC5316p234; G. PsC12961p224; H. PsC16473p224; I. PsC25762p728. Hypothetical quantile-quantile plots of the marker-traits association study for the (Fe) concentration in seed of 7 datasets.

International consortium for pea genome sequencing

- INRA, Dijon: J. Burstin, G. Aubert, J. Kreplak
- Genoscope, Paris: A. Madoui, P. Wincker
- University of Saskatchewan: T.D. Warkentin, B. Tar’an, K. Gali
- Curtin University, Australia: J. Lichtenzveig, D. Edwards, J. Batley
- USDA-ARS: C. Coyne
- Czech: J. Dolozel

<u>Approach</u>

Shot gun sequencing	– Genoscope
Whole Genome Profiling (Keygene)	– U of S
Genotype by Sequencing	– U of S, Curtin
PacBio sequencing	– USDA
Chromosome sorting	- Czech
Annotation	– INRA

Future directions

Chicken model

Phenolic effects

Markers for breeding

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Pulse crop breeding lab
Pulse crop molecular lab
Grains innovation lab
Pulse crop pathology lab
Pulse crop tissue culture lab
Pulse crop graduate students, post-docs
Breeder seed crew
Plant Sciences admin staff

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