Evaluation of iron bioavailability in Pea (*Pisum sativum* L.) lines contrasting in nutritional traits

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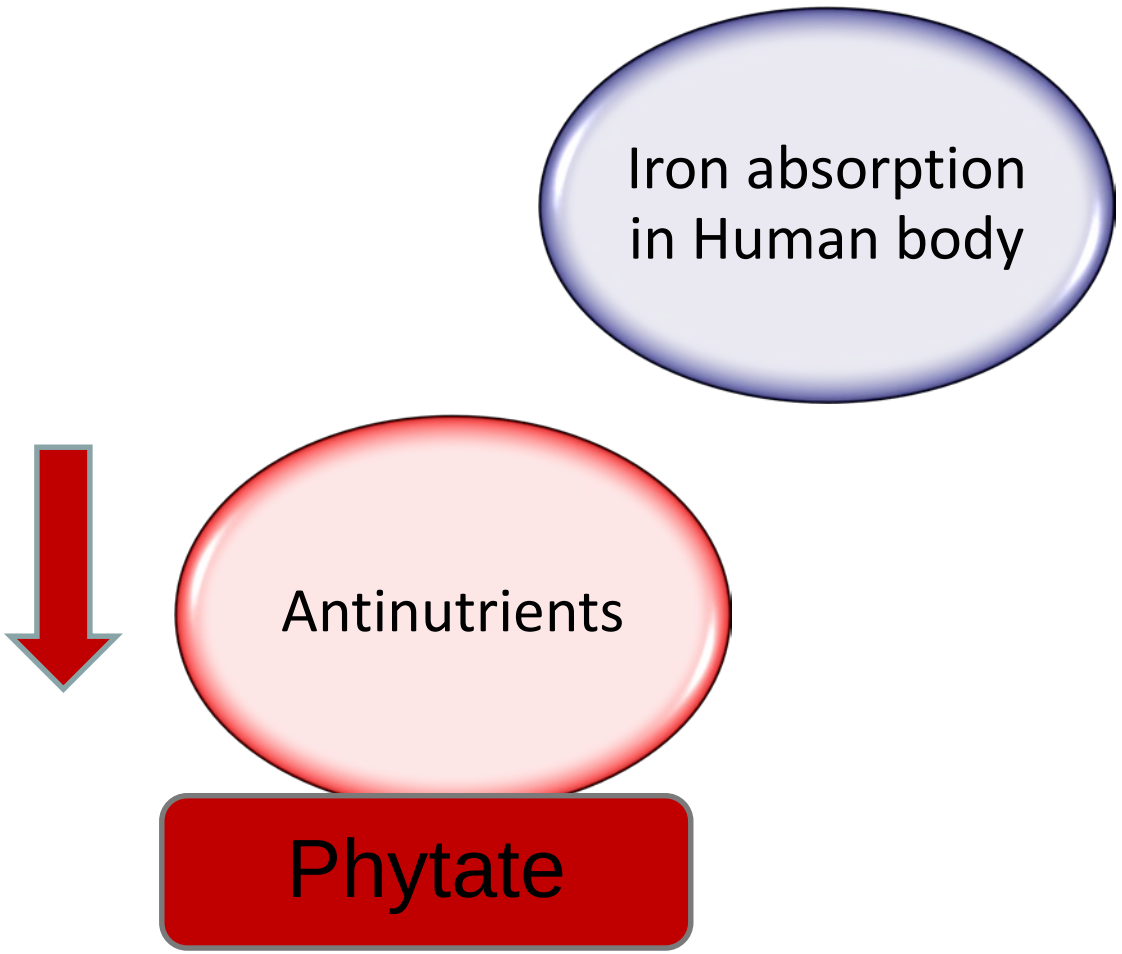
Field pea

- Source of proteins, carbohydrates, fiber, vitamins, essential amino acids and micronutrients.
- Canada is the largest global producer and exporter
- Annual production 3.1 million tonnes
- Saskatchewan (79% of the total production)

Iron

- Essential micronutrient
- Iron deficiency
  - ~2 billion people
  - Highly prevalent among infants and women
- Main cause: Low iron bioavailability

Iron Bioavailability



Low Phytate Pea

- Two *lpa* mutants (1-150-81 and 1-2347-144)
- Progenitor- CDC Bronco
- Method- Chemical mutagenesis.
- Phytate phosphorus reduced significantly:

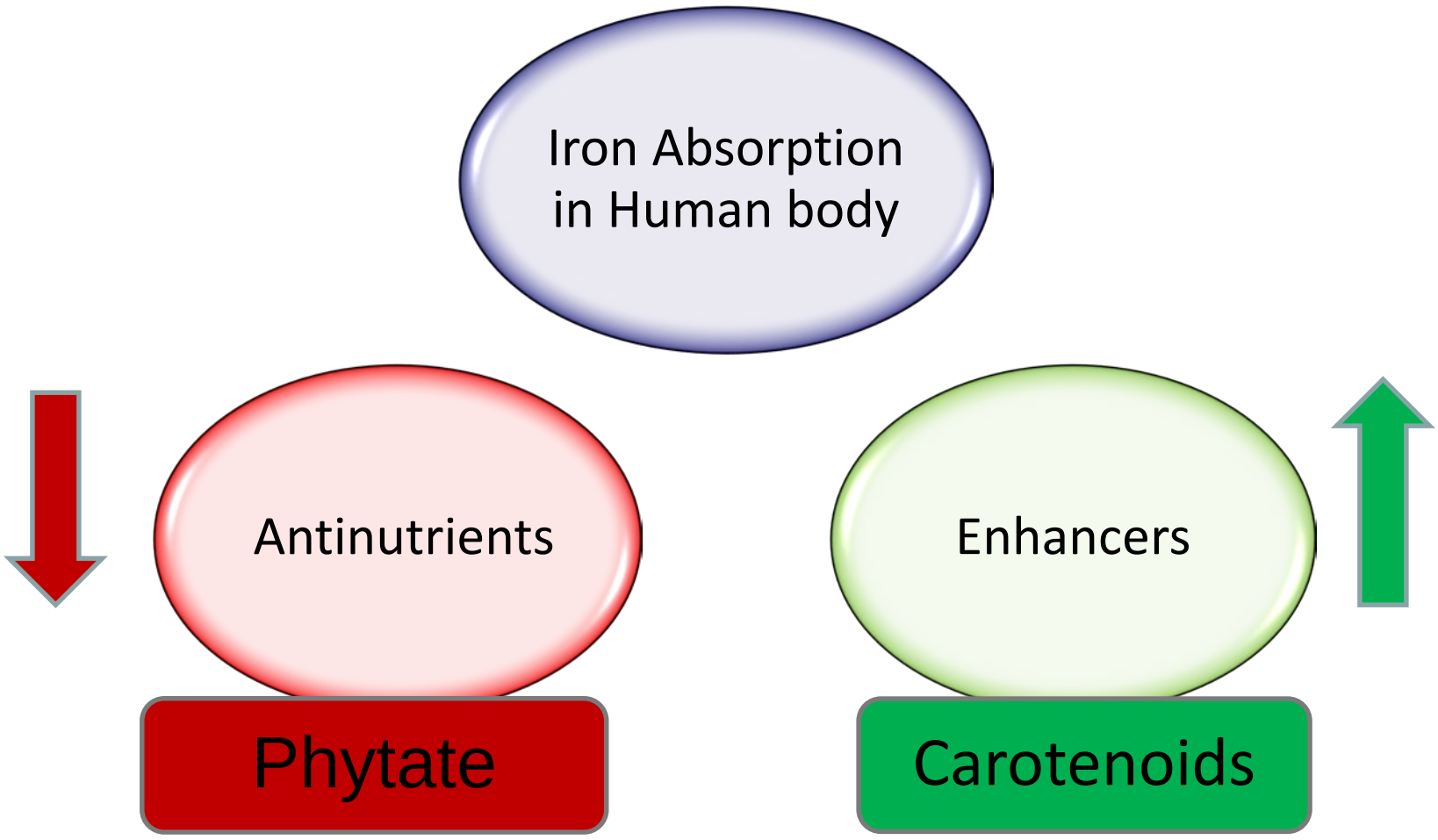
  1-2347-144 (1.1 mg/g)

  1-150-81 (1.2 mg/g)

  CDC Bronco (2.9 mg/g)
- Inorganic phosphorus - high
- Iron bioavailability higher (1.4-1.9 times) than CDC Bronco

Warkentin et al., 2012

Iron Bioavailability



Carotenoids

- Carotenoids increased iron bioavailability in cereal-based foods (Gracía-Casal, 2006)

- Green cotyledon pea varieties had greater concentration of carotenoids than yellow cotyledon varieties (Kaliyaperumal et al, 2013)

Hypotheses

Higher iron bioavailability in pea seeds with higher:

- Iron concentration
- Carotenoid concentration

Low phytate concentration with high carotenoid concentration in pea seeds may have additive benefits for iron bioavailability.

Objectives

To compare iron bioavailability in pea lines contrasting in

a. iron
b. Phytate and
c. carotenoid concentration

Techniques:

Atomic Absorption Spectroscopy

-Iron concentration

Modified colorimetric (Wade's reagent) method (Gao et al., 2007)

-phytate-phosphorus

Modified Chen's reagent method (Chen et al., 1956)

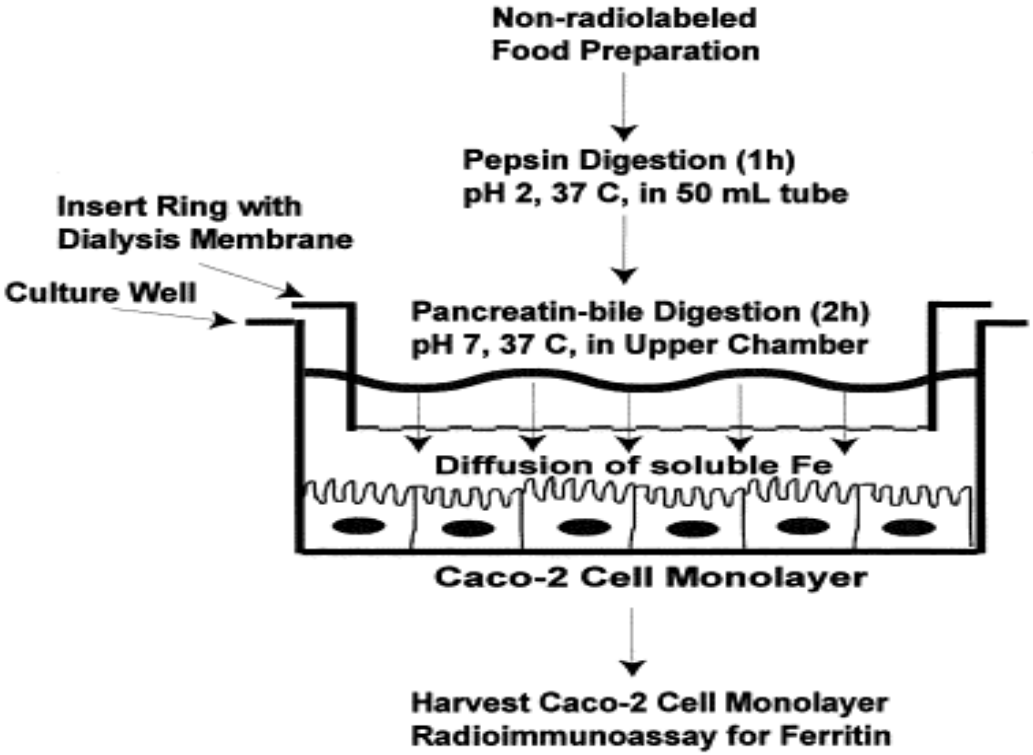
-Inorganic-phosphorus

High-performance Liquid Chromatography

-Carotenoid Concentration

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

Developed by
Glahn et al. (1998)



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

Objective # 1

To compare iron bioavailability in pea lines contrasting in

- Iron concentration
- Carotenoid concentration

Material- PR-07 RILs

Parents	Cotyledon color	Iron concentration Mean ± stdev (mg kg-1)
Carrera	Yellow	47.5 ± 5.11
CDC Striker	Green	41.6 ± 3.16

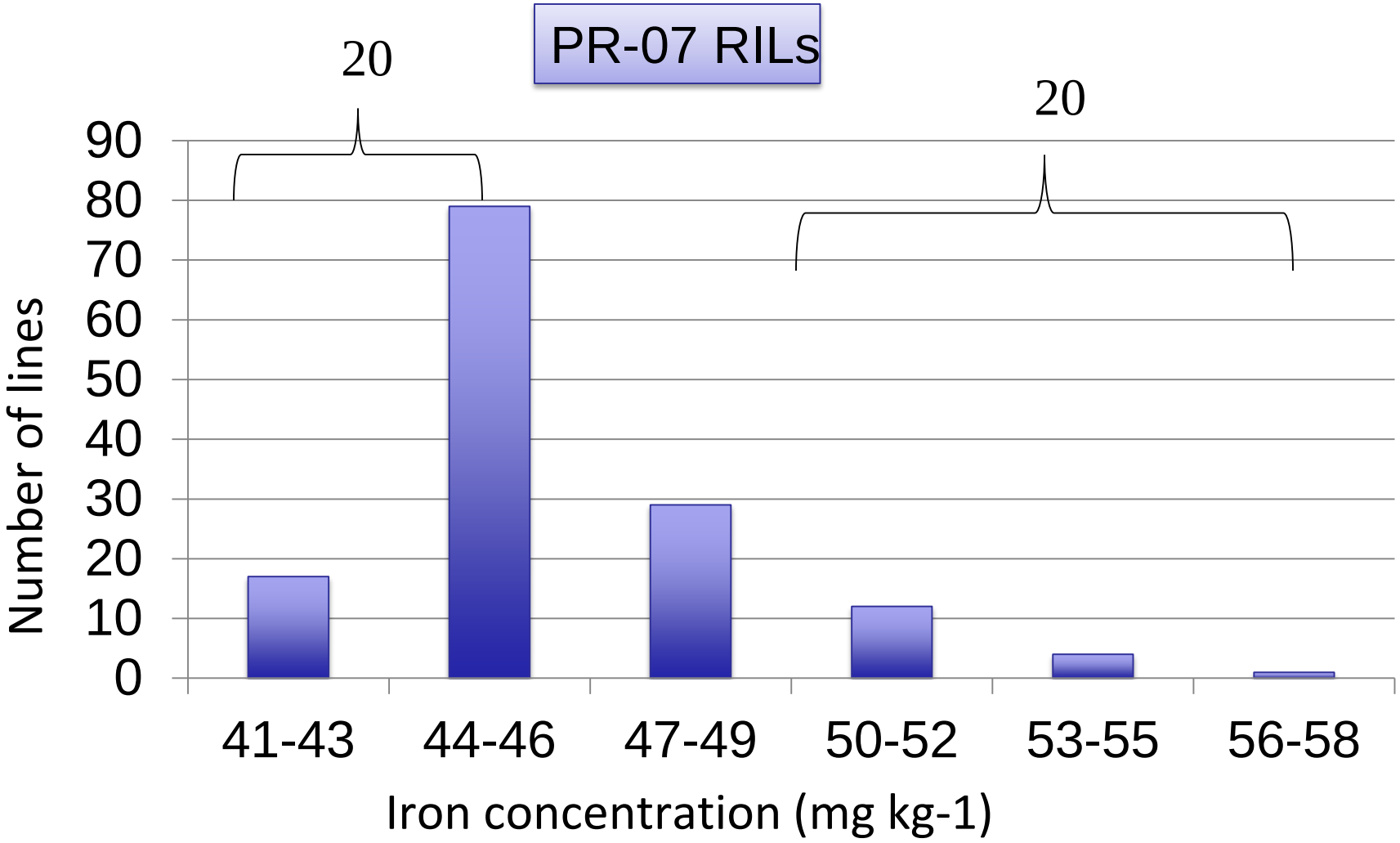


Fig: Frequency distribution of the average of least square means of iron concentration over three years (2010, 2011 and 2012) and two locations (Sutherland and Rosthern) for the Recombinant Inbred Lines of Carrera X CDC Striker populations.

Liu,Y 2012 MS thesis

Table: Mean squares of combined ANOVA, R-square, range and mean of different constituents in pea seeds of PR-07 RILs, parents (Carrera and CDC Striker) and check variety (CDC Bronco).

Source	DF	F Value	Range	Mean
Phytate (mg g^{-1})	6	14.5*	0.9-2.6	1.9
Iron (mg kg^{-1})	6	19.5*	37.2-53.8	46.9
Violaxanthin (mg kg^{-1})	6	7.2*	0.2-1.4	0.6
Lutein (mg kg^{-1})	6	6.8*	5.8-11.3	8.2
Zeaxanthin (mg kg^{-1})	6	5.9*	0.0-0.7	0.2
β-carotene (mg kg^{-1})	6	20.1*	0.2-0.3	0.2
Total Carotenoids (mg kg^{-1})	6	8.5*	6.2-13.6	9.2
FeBIO (ng ferritin mg^{-1} of protein)	6	1.0^{ns}	5.1-12.9	8.3

DF: degree of freedom; *: significant at <0.05 level; ns: not significant

Line/ Variety	Number of lines	CC	Phytate	Fe	TC*	FeBIO
			mg g^{-1}	mg kg^{-1}	mg kg^{-1}	ng ferritin mg^{-1} of protein
PR-07 GH	10	Green	1.4^{b}	50.8^{a}	11.1^{a}	8.4^{a}
PR-07 YH	10	Yellow	1.5^{b}	51.2^{a}	9.7^{a}	9.1^{a}
PR-07 GL	10	Green	2.0^{a}	42.4^{b}	9.8^{a}	7.3^{a}
PR-07 YL	10	Yellow	2.2^{a}	44.3^{b}	7.5^{b}	8.4^{a}
Carrera	2	Yellow	1.9^{ab}	47.3^{ab}	4.9^{b}	10.7^{a}
CDC Striker	2	Green	1.8^{ab}	44.5^{ab}	7.5^{ab}	8.8^{a}
CDC Bronco	2	Yellow	2.6^{a}	45.3^{ab}	8.7^{ab}	8.3^{a}

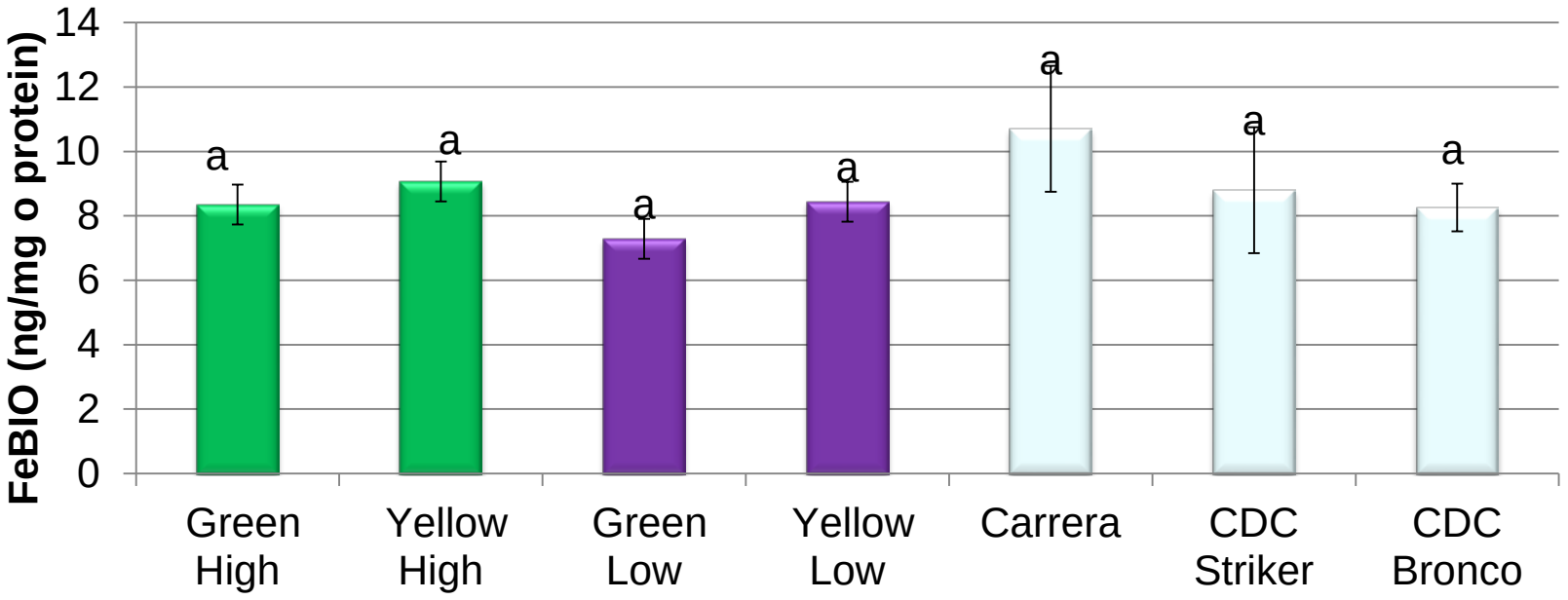


Table: Correlation of studied constituents (violaxanthin, lutein, zeaxanthin, β-carotene, total carotenoids, iron, phytate, molar ratio of PA:Fe and iron bioavailability) in selected PR-07 RILs

Variables	FeBIO
Violaxanthin	-0.04^{ns}
Lutein	0.08^{ns}
Zeaxanthin	-0.14^{ns}
β-Carotene	-0.02^{ns}
Total Carotenoids[a]	0.05^{ns}
Iron	0.38*
Phytate	-0.26^{ns}
Molar ratio PA:Fe	-0.29^{ns}

[a]: sum of four carotenoids (violaxanthin, lutein, zeaxanthin and β-carotene) measured; PA:Fe: molar ratio of phytic acid to iron; FeBIO: iron bioavailability; *: significant at 0.05 level; ns: not significant.

Conclusion

- Iron concentration had the greatest association with iron bioavailability

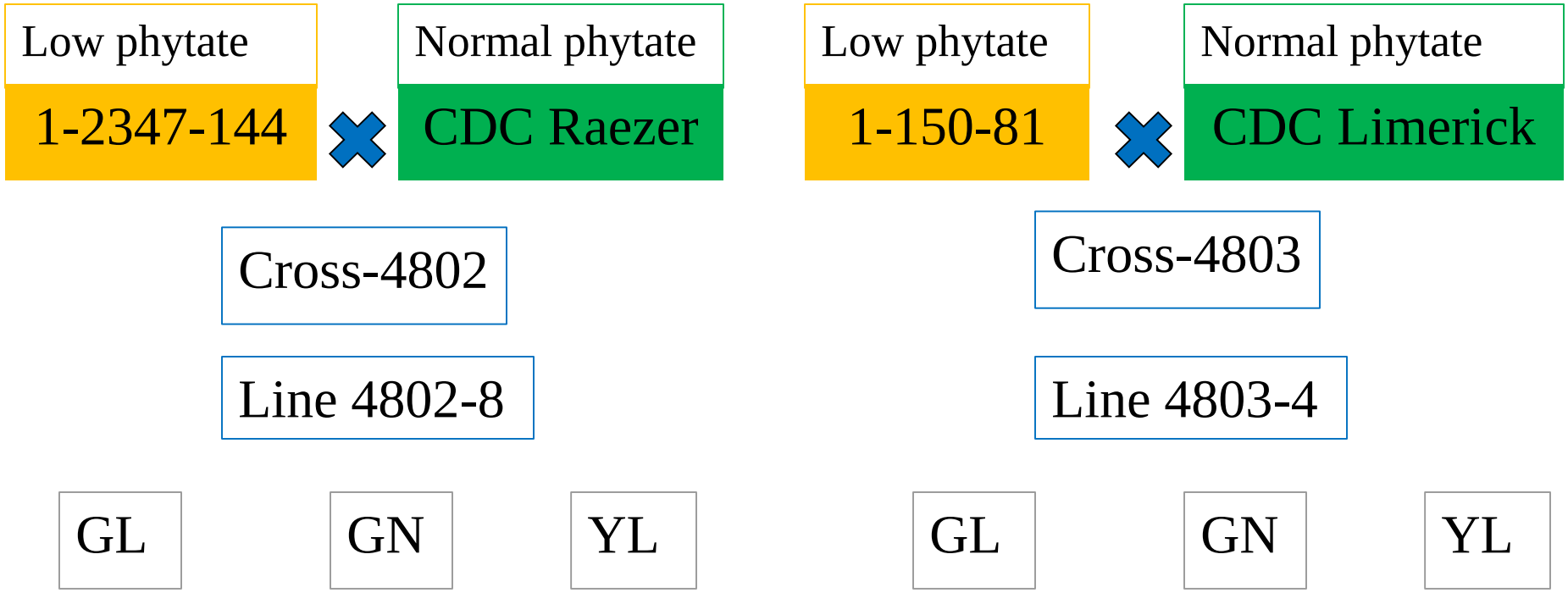
- Selecting pea lines with greater iron concentration may result in increased iron bioavailability for humans and monogastric animals.

Objective # 2

To compare iron bioavailability in pea lines contrasting in

- phytate concentration
- carotenoid concentration

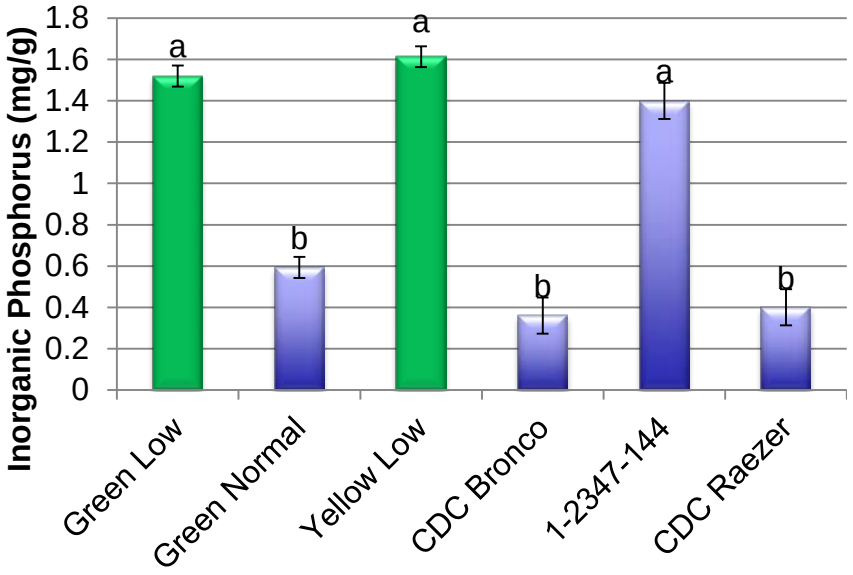
Materials



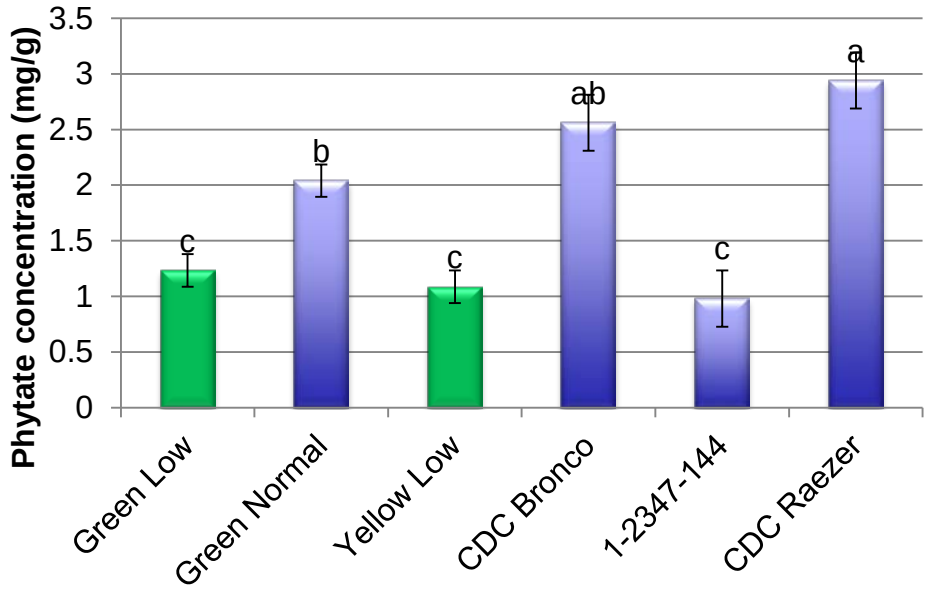
Analyzed for:

- Inorganic phosphorus
- Phytate
- Iron and
- Carotenoid concentration
- Iron bioavailability

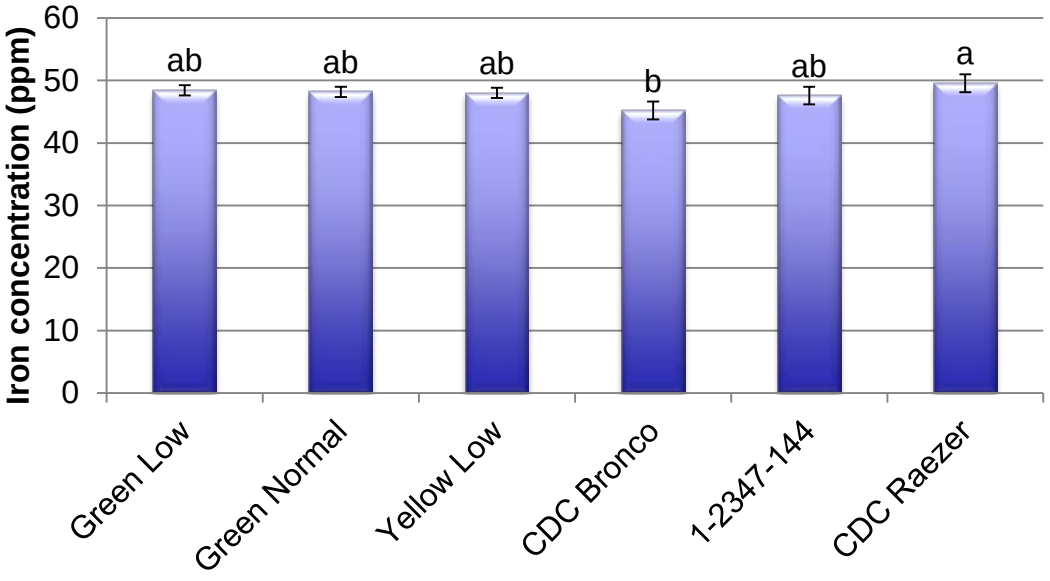
Results for 4802-8 categorized sub-lines



F-Value
127.5*

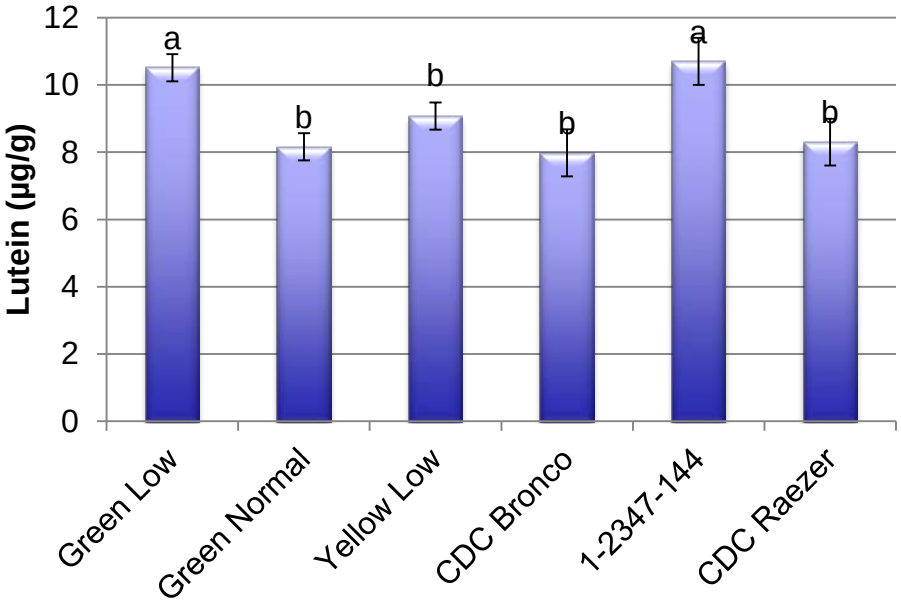


F-Value
26.2*

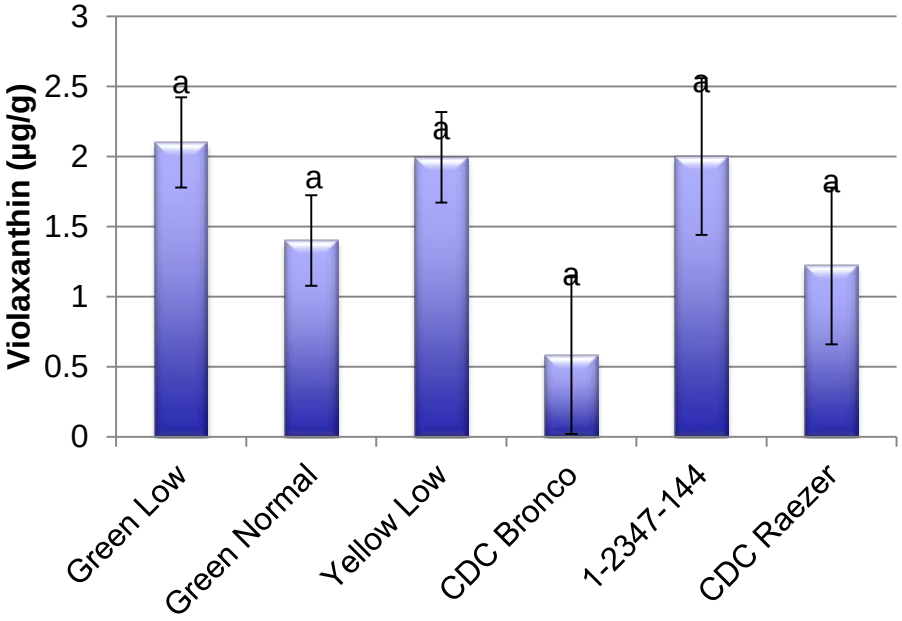


F-Value
2.5*

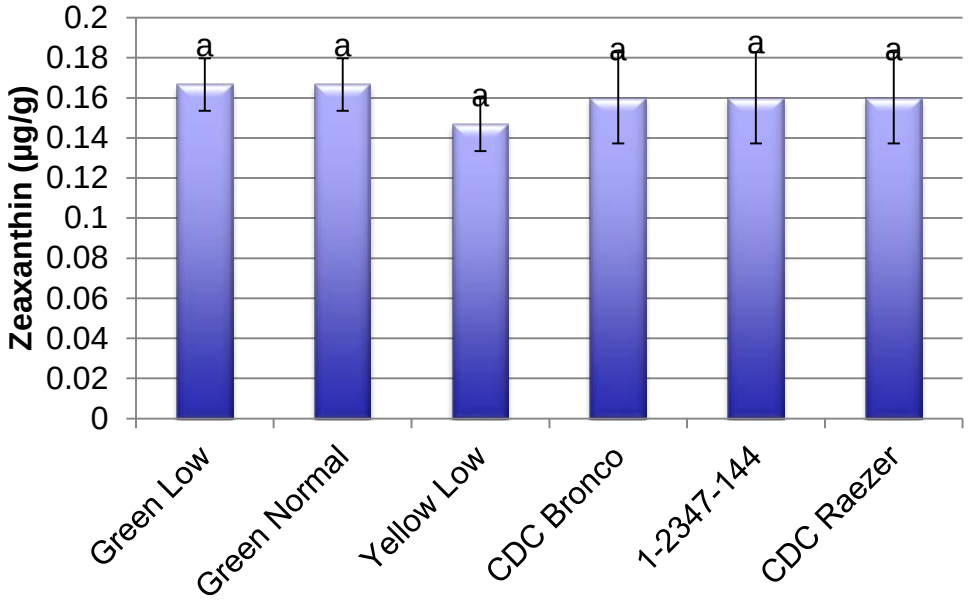
* Significance p<0.05



F-Value
11.0*



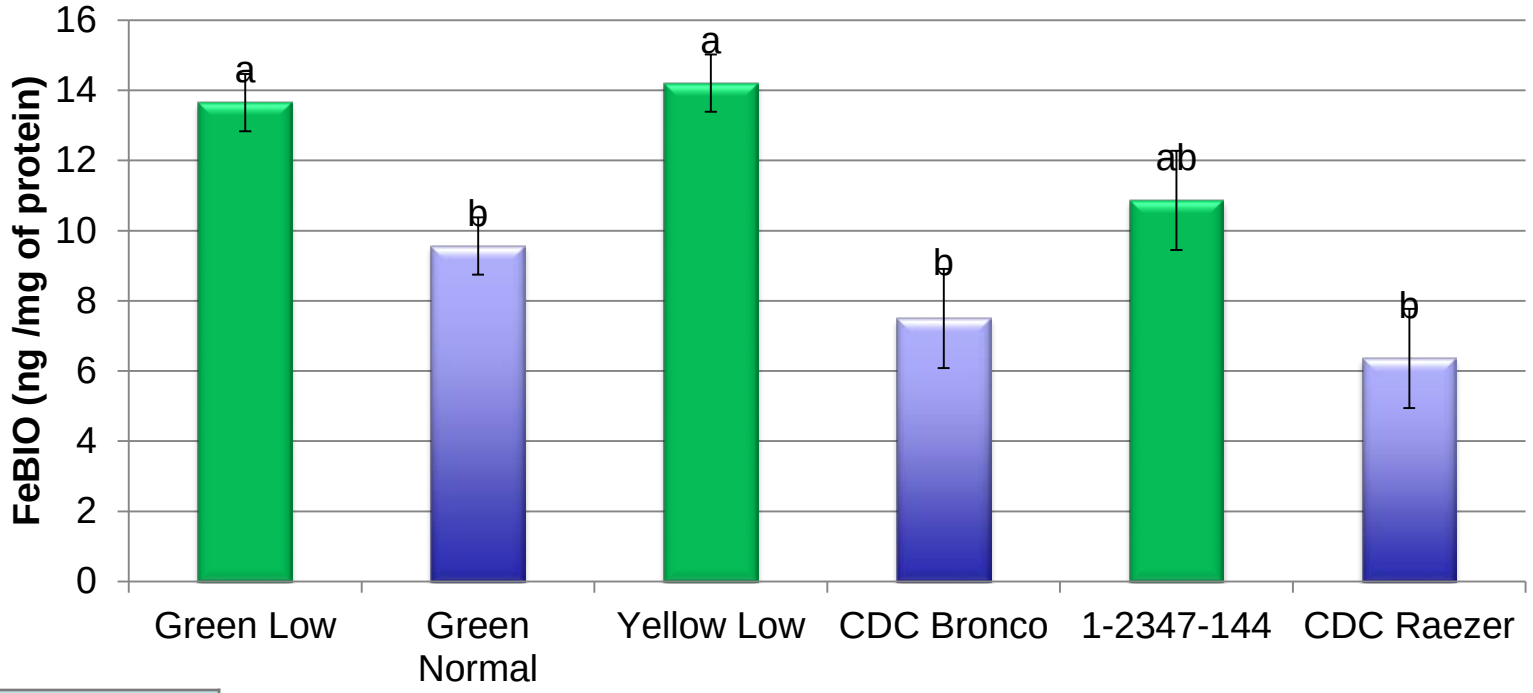
F-Value
3.0*



F-Value
0.31^{ns}

* Significance p<0.05 ; ^{ns} Not significant

Table: Iron bioavailability (FeBIO) from 4802-8 sub-lines categorized on the basis of cotyledon color and phytate concentration , parents of 4802 cross (1-2347-144 and CDC Raezer) and check variety (CDC Bronco).



F-Value
8.9*

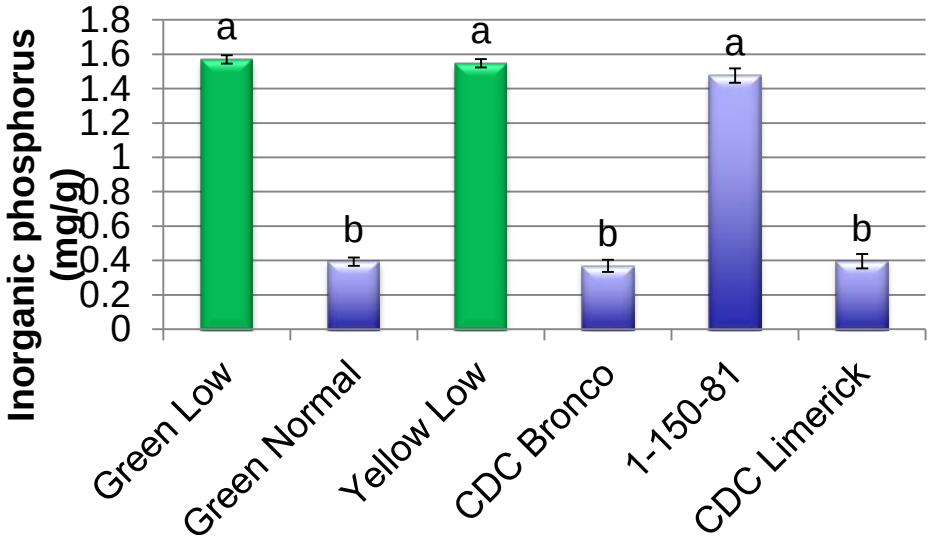
* Significance p<0.05

Table: Correlation studied constituents (violaxanthin, lutein, zeaxanthin, β-carotene, total carotenoids, inorganic phosphorus, phytate, iron, molar ratio of PA:Fe and iron bioavailability) in 4802-8 categorized sub-lines

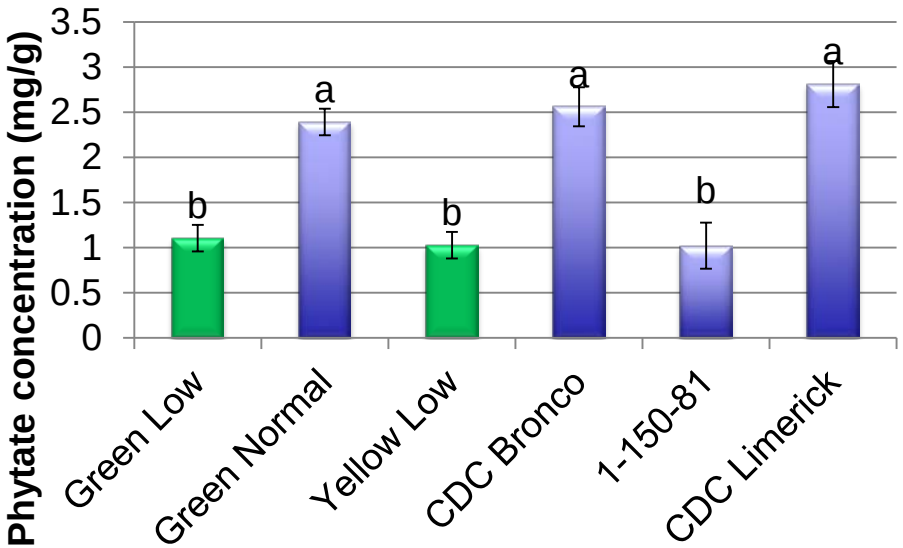
Variables	FeBIO
Violaxanthin	0.03^{ns}
Lutein	0.41*
Zeaxanthin	-0.05^{ns}
β-Carotene	-0.18^{ns}
Total Carotenoids[a]	0.26^{ns}
Phytate	-0.34*
Iron	-0.15^{ns}
PA:Fe	-0.18^{ns}

[a]: sum of four carotenoids (violaxanthin, lutein, zeaxanthin and β-carotene) measured; PA:Fe: molar ratio of phytic acid to iron; FeBIO: iron bioavailability; *: significance at 0.05 level; ns: not significant.

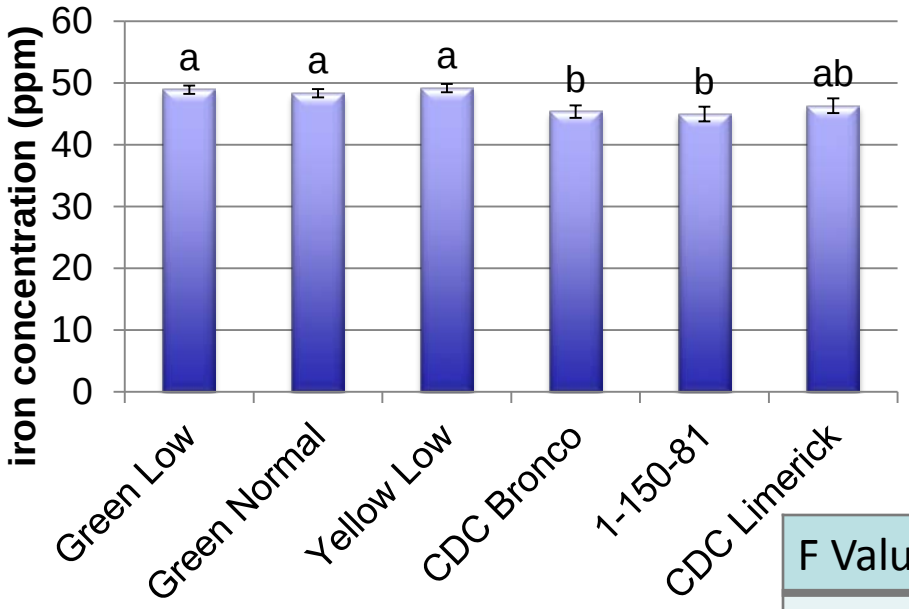
Results for 4803-4 categorized sub-lines



F Value
856.7*

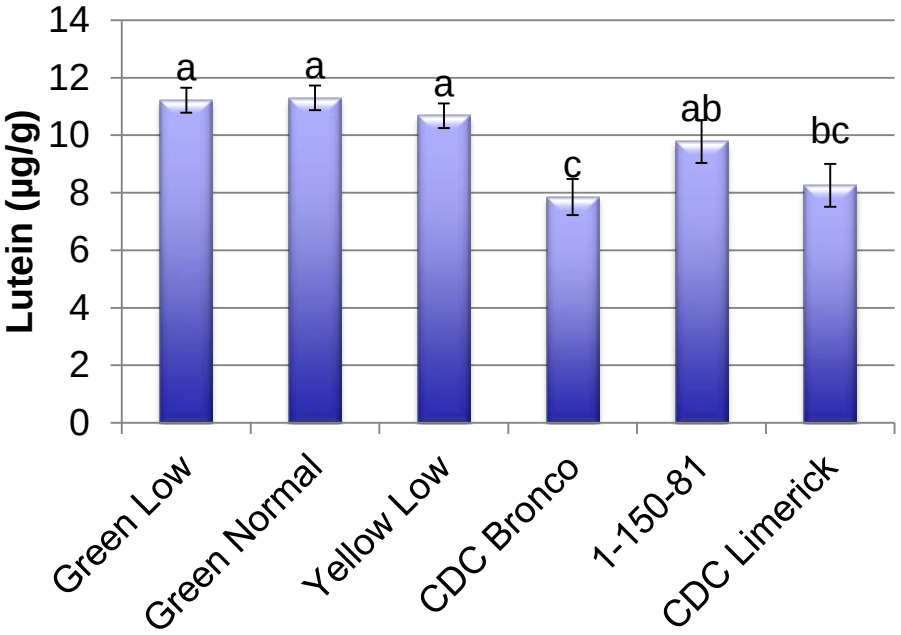


F Value
40.0*

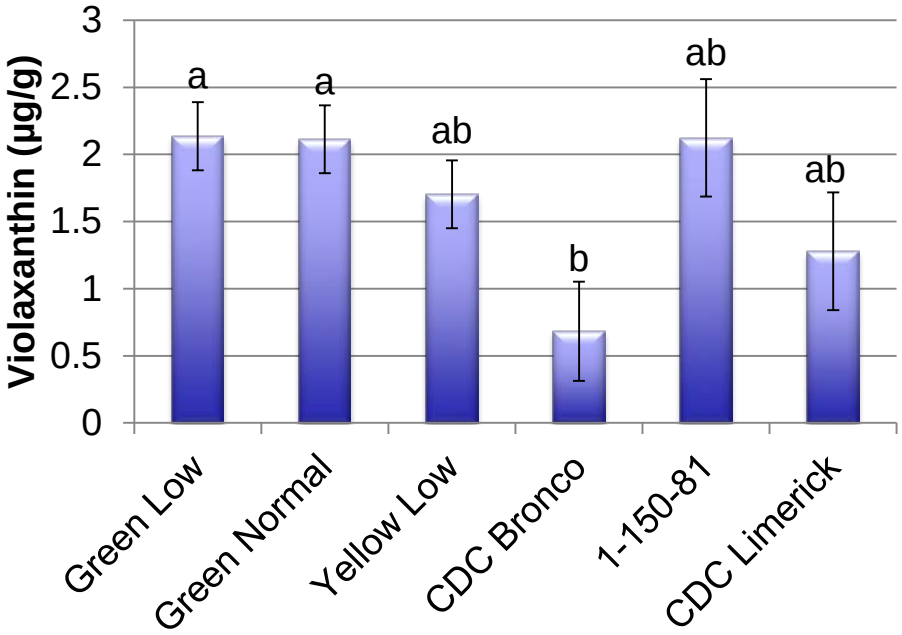


F Value
7.1*

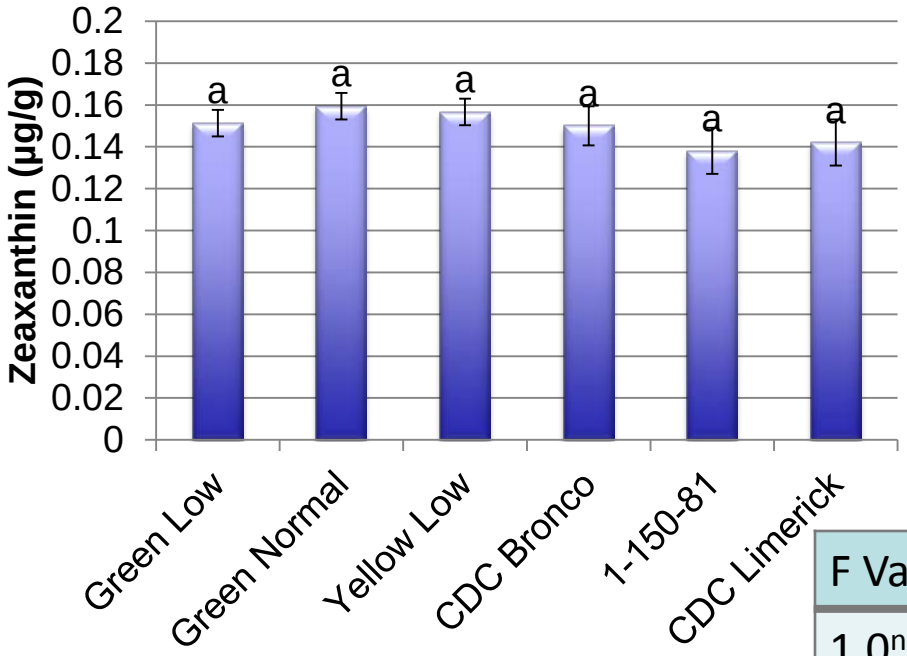
* Significance $p<0.05$



F Value
11.9*



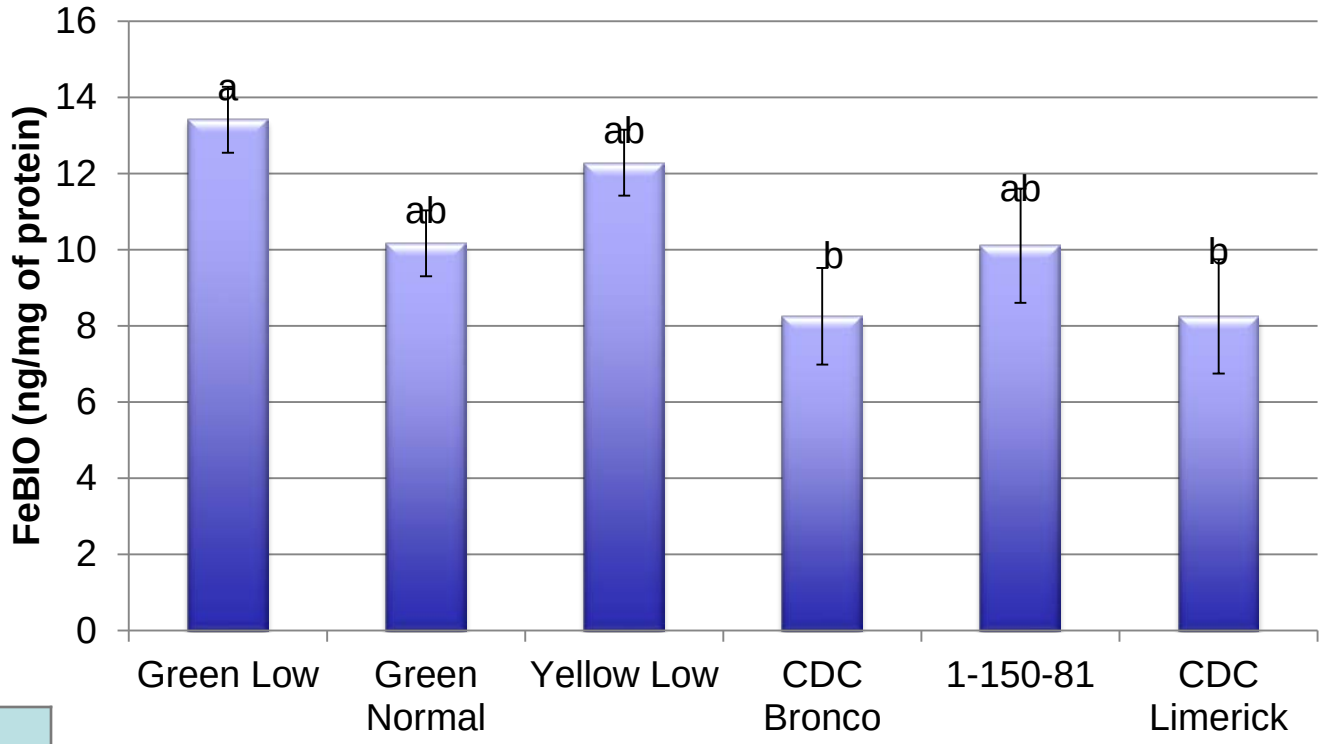
F Value
4.4*



F Value
1.0^{ns}

* Significance p<0.05 ; ^{ns} Not significant

Table: Iron bioavailability (FeBIO) from 4803-4 sub-lines categorized on the basis of cotyledon color and phytate concentration, parents of 4803 cross (1-150-81 and CDC Limerick) and check variety (CDC Bronco).



F Value
3.85*

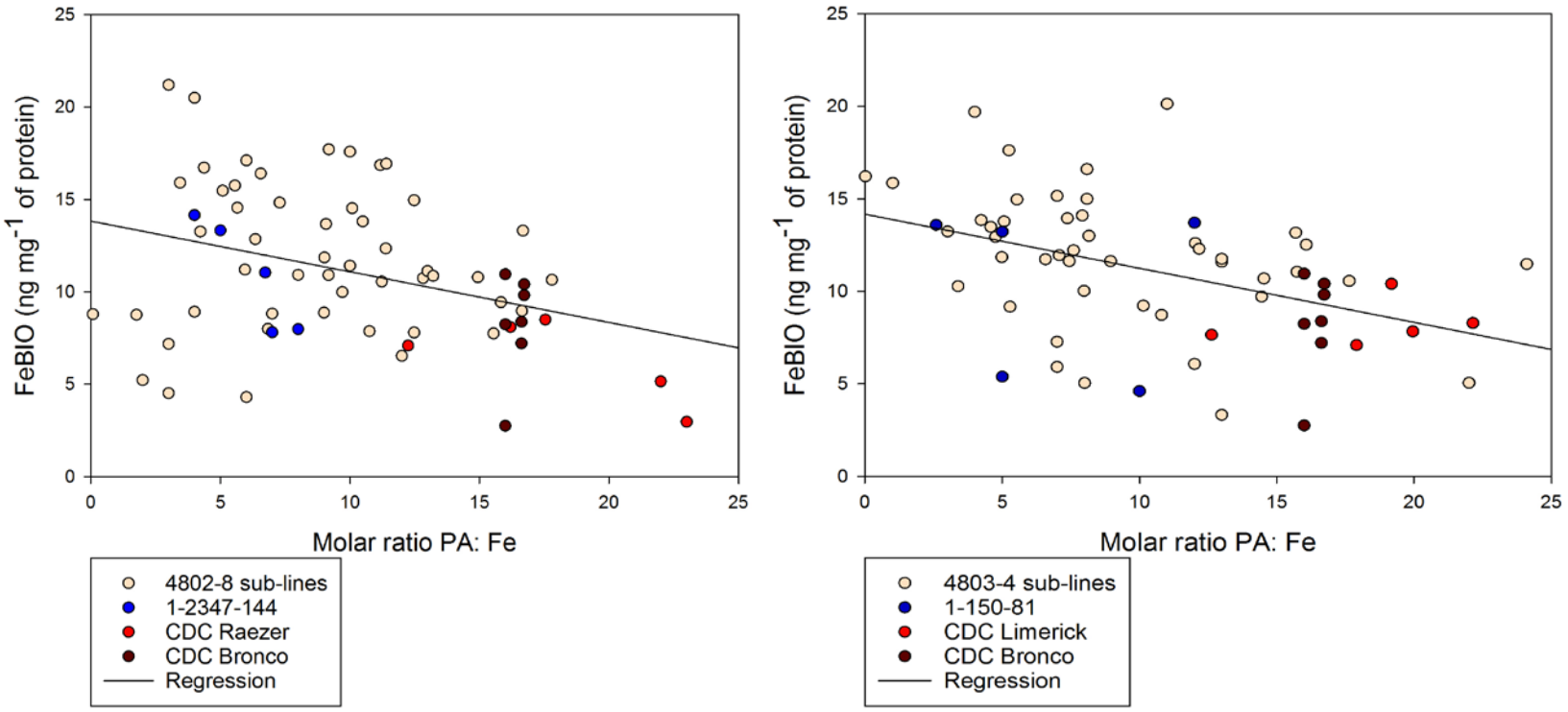
* Significance $p<0.05$

Table: Correlation matrix of studied constituents (violaxanthin, lutein, zeaxanthin, β-carotene, total carotenoids, inorganic phosphorus, phytate, iron, molar ratio of PA:Fe and iron bioavailability) in 4803-4 categorized sub-lines.

Variables	FeBIO
Violaxanthin	0.19^{ns}
Lutein	0.24^{ns}
Zeaxanthin	0.04^{ns}
β-Carotene	0.02^{ns}
Total Carotenoids[a]	0.26^{ns}
Phytate	-0.37*
Iron	0.06^{ns}
PA:Fe	-0.38*

[a]: sum of four carotenoids (violaxanthin, lutein, zeaxanthin and β-carotene) measured; PA:Fe: molar ratio of phytic acid to iron; FeBIO: iron bioavailability; *: significance at 0.05 level; ns: not significant

Figure: Changes in FeBIO (iron bioavailability) with the change on molar ratio of phytic acid (PA) to iron (Fe) in 4802-8 and 4803-4 sub-lines



A non-significant regression (0.03) was observed in 4802-8 sub-lines. Whereas, in the 4803-4 sub-lines, a significant, but weak, regression (0.14) of molar ratio (PA:Fe) with iron bioavailability was observed

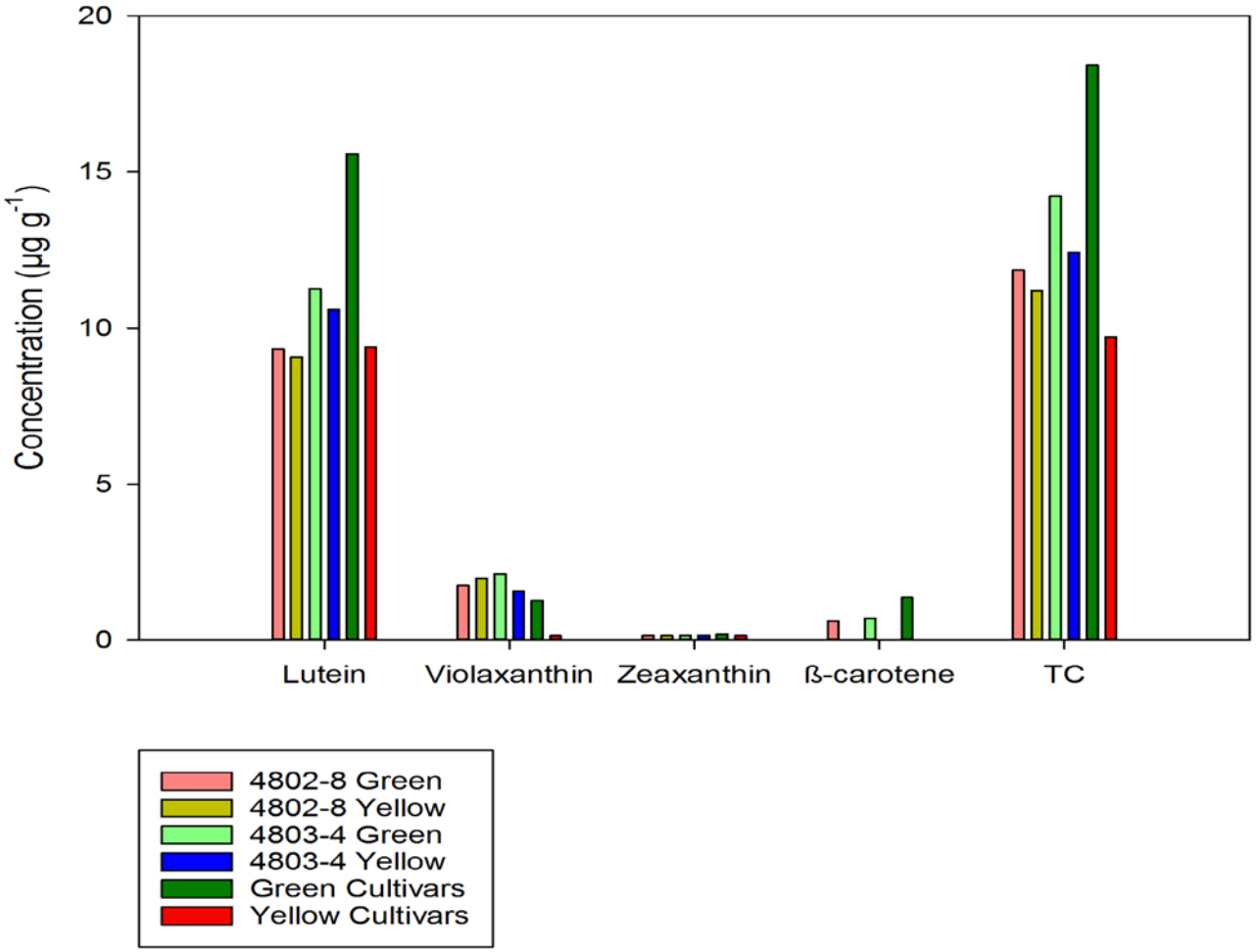


Figure: Comparison of lutein, violaxanthin, zeaxanthin, β-carotene and total carotenoid (TC) concentrations between cultivars (green and yellow cotyledon) used by Kaliyaperumal et al (2014) and sub-lines (4802-8 and 4803-4)

Conclusion

- a significant positive correlation between lutein and iron bioavailability in the 4802-8 sub-lines.
- a significant negative correlation between phytate and iron bioavailability in both sub-lines.
- The pattern of change in iron bioavailability with change in molar ratio of PA:Fe was not significant.
- Substantial contrast for carotenoid concentration was not observed in present study

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Saskatchewan Pulse Growers

Government of Saskatchewan
Ministry of Agriculture

Thank You!