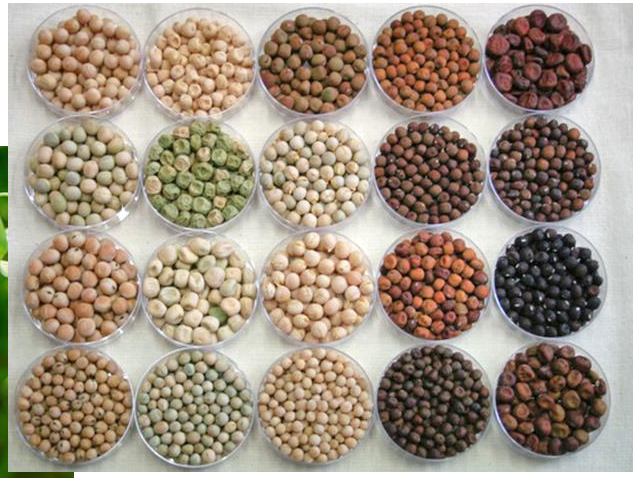
Genome wide association study for Fe, Zn, and Se concentration in field pea (*Pisum sativum* L.)



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Introduction

- Iron (Fe), Zinc (Zn) and Selenium (Se) are among the twenty essential nutrients which comprise the basis of all human nutrition (WHO).

- According to FAOSTAT (2012):
  - ✓ 60% Fe deficient
  - ✓ 30% Zn deficient
  - ✓ 15% Se deficient

- Micronutrient malnutrition?

Strategies to overcome the micronutrient malnutrition

- ✓ Diet supplementation
- ✓ Food fortification
- ✓ Bio-fortification:

  Bio-fortification is the practice of deliberately increasing the content of an essential nutrient in a crop through agronomic practices, conventional plant breeding, or modern biotechnology.

Biofortification potential of Field pea

- Pulses including field pea contain significant proportions of the recommended daily allowance (RDA) of micronutrients (Ray et al. 2014).

- Field pea (*Pisum sativum* L.) is one of the major pulse crops in the world with annual production of 10 million tonnes (FAOSTAT 2012).

- Canada is at the top of the field pea producing countries.

Objectives

1) Evaluation of diverse pea accessions for Fe, Zn and Se concentration.

2) Genome wide association study (GWAS) to identify SNPs associated with Fe, Zn and Se concentration.

3) Validation of identified trait-associated SNP markers

Methodology

1) Phenotyping

- Table 1 : Details of seed samples for evaluation of Fe, Zn, and Se concentration - 177 pea accessions Developed at the Crop Development Centre (CDC), University of Saskatchewan.

Year	Fargo, North Dakota	Saskatoon, Saskatchewan	Rosthern, Saskatchewan	Sample number
2013	-	2 replicates	-	177 x 2 = 354
2014	2 replicates	-	-	177 x 2 = 354
2015	-	2 replicates	-	177 x 2 = 354
2016	-	2 replicates	2 replicates	177 x 4 = 708

- Grinding the seed samples:- using the cyclone sample mill (UDY Corporation, Fort Collins, Colorado-USA).

- Sample digestion:- using a Vulcan-84 automatic digester (Thavarajah et al. 2007).

- Micronutrient concentration analysis:- using an atomic absorption spectrophotometer (Diapari et al. 2015).

2) Soil Analysis

- Correlation analysis of Fe, Zn, and Se concentration in seeds with Fe, Zn, and Se concentration in soil.

- 2017 GWAS panel in Saskatoon, SK and Rosthern, SK.
- 10 randomly selected accessions
- 2 biological replicates
- 3 depths (A, B, C horizons)
- soil pH, texture, particle size, organic and inorganic C, plant available nutrients including N, P, and K.

3) Association Study

- Genotyping: Genotyping-by-sequencing* (Elshire et al. 2011).
- Population structure: (Software : STRUCTURE)
- Kinship, GWAS: (Software : TASSEL)
- Identifying SNPs associated with Fe, Zn, and Se concentration.

*Gali et al. (unpublished)

Results - Phenotyping

Zinc

Table 2 : Summary of the analysis of variance (ANOVA) of zinc (Zn) concentration in seeds of field pea across Rosthern, SK Saskatoon, SK and Fargo, ND in 2013, 2014, 2015, and 2016.

Trait	Year	Location	Mean ± SD (ppm)	CV (%)	Range (ppm)	Effects		F value		pr
						y	L	G	G*Y	G*L
Zn	2013	Saskatoon	28.34 ± 3.98	14.0	16.78-39.89	.	.	4.1***	.	.
	2014	Fargo, ND	23.22 ± 4.66	20.1	12.83-43.46	.	.	3.24***	.	.
	2015	Saskatoon	29.62 ± 3.66	12.3	16.64-44.08	.	.	2.53***	.	.
	2016	Saskatoon	32.79 ± 4.49	13.7	19.75-44.33	.	.	4.28***	.	.
	2016	Rosthern	33.55 ± 4.92	14.7	21.97-51.47	.	.	5.16***	.	.
	2016	Ros + Sas	33.17 ± 4.72	14.2	19.75-51.47	.	10.58***	6.55***	.	1.03
	2013/2015/2016	Saskatoon	30.26 ± 4.46	14.8	16.64-44.33	209.27***	.	5.72***	1.28	.

Y –Year; L – Location; G – Genotype; CV – Coefficient of variation; Pr - Probability
Pr < 0.001 is represented with ***

Iron

Table 3: Summary of the analysis of variance (ANOVA) of iron (Fe) concentration in seeds of field pea across Rosthern, SK Saskatoon, SK and Fargo, ND in 2013, 2014, 2015, and 2016.

Trait	Year	Location	Mean ± SD (ppm)	CV%	Range (ppm)	Effects		F value		pr
						y	L	G	G*Y	G*L
Fe	2013	Saskatoon	45.95 ± 6.46	14.1	31.41-68.96	.	.	4.51***	.	.
	2014	Fargo, ND	52.95 ± 8.97	17.0	36.72-90.53	.	.	5.05***	.	.
	2015	Saskatoon	48.80 ± 6.16	12.6	37.4-74.21	.	.	2.05***	.	.
	2016	Saskatoon	50.81 ± 6.68	13.2	29.22-74.76	.	.	1.97***	.	.
	2016	Rosthern	53.81 ± 6.66	12.4	39.73-74.52	.	.	3.31***	.	.
	2016	Ros + Sas	52.31 ± 6.83	13.1	29.22-74.76	.	61.39***	4.03***	.	0.98
	2013/2015/2016	Saskatoon	48.51 ± 6.74	13.9	29.22-74.76	86.06***	.	4.84***	1.03	.

Y –Year; L – Location; G – Genotype; CV – Coefficient of variation; Pr - Probability
Pr < 0.001 is represented with ***

Selenium

Table 4: Summary of the analysis of variance (ANOVA) of selenium (Se) concentration in seeds of field pea across Rosthern, SK Saskatoon, SK and Fargo, ND in 2013, 2014, 2015, and 2016.

Trait	Year	Location	Mean ± SD (ppm)	CV%	Range (ppm)	Effects		F value		pr
						y	L	G	G*Y	G*L
Se	2013	Saskatoon	1.09 ± 0.85	78.3	0.25-6.12	.	.	0.98	.	.
	2014	Fargo,ND	0.41 ± 0.09	22.6	0.22-0.88	.	.	2.04***	.	.
	2015	Saskatoon	1.73 ± 0.76	44.2	0.27-8.75	.	.	1.07	.	.
	2016	Saskatoon	0.96 ± 0.37	39.0	0.33-3.28	.	.	0.98	.	.
	2016	Rosthern	0.58 ± 0.3	51.8	0.06-1.9	.	.	0.8	.	.
	2016	Ros/Sas	0.77 ± 0.39	50.5	0.06-3.28	.	230.5***	0.99	.	0.82
	2013/2015/2016	Saskatoon	1.26 ± 0.77	61.2	0.25-8.75	126.98***	.	1	0.97	.

Y –Year; L – Location; G – Genotype; CV – Coefficient of variation; Pr - Probability
Pr < 0.001 is represented with ***

Results – Soil Analysis

Table 8: Pearson's correlation coefficient (r) between the micronutrient concentration of soil and seeds for Se, Zn, and Fe in A, B, C, and the total depth of A,B, and C horizons in Rosthern and Saskatoon, 2017.

Location	Micronutrient	A horizon	B horizon	C horizon	Average
Rosthern	Se	-0.30	0.36	0.57*	0.47*
	Zn	0.03	0.43	-0.26	0.31
	Fe	0.25	0.26	0.21	0.25
Saskatoon	Se	-0.07	-0.05	0.76***	0.20
	Zn	0.50	0.44	-0.13	0.53*
	Fe	0.62	0.49	0.40	0.59**

Significance: * at 0.05, ** at 0.01, *** at 0.001

Results - Association study

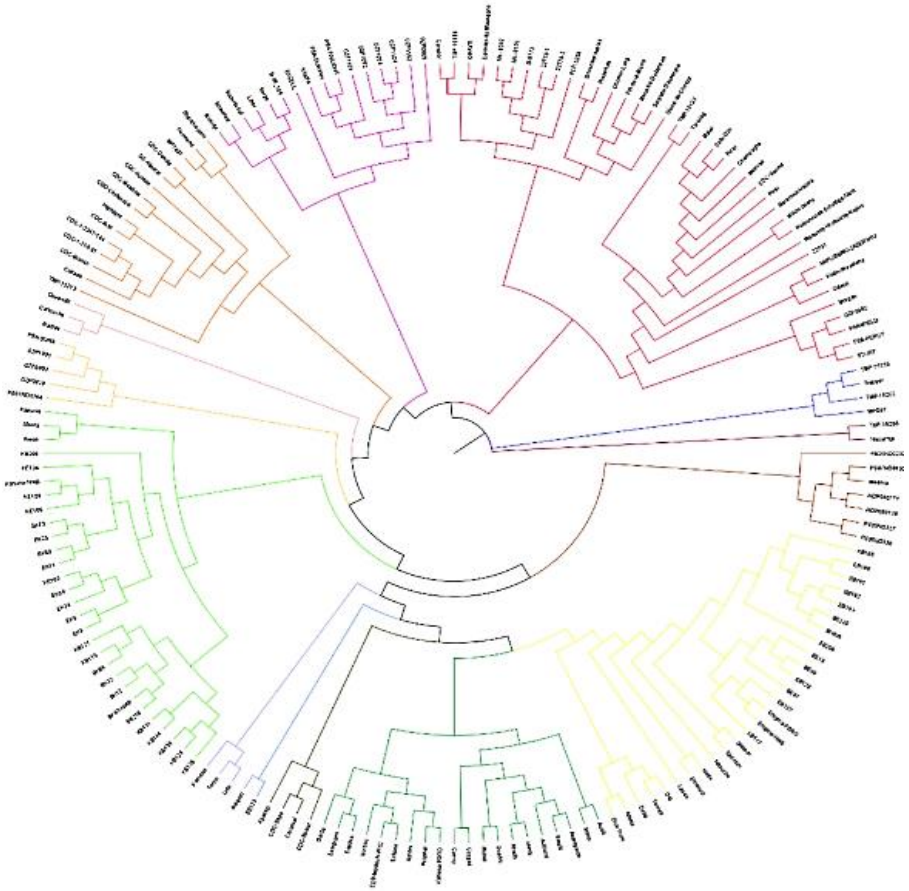

Fig 1: Phylogenetic clustering of 175 GWAS accessions based on 14391 SNPs*

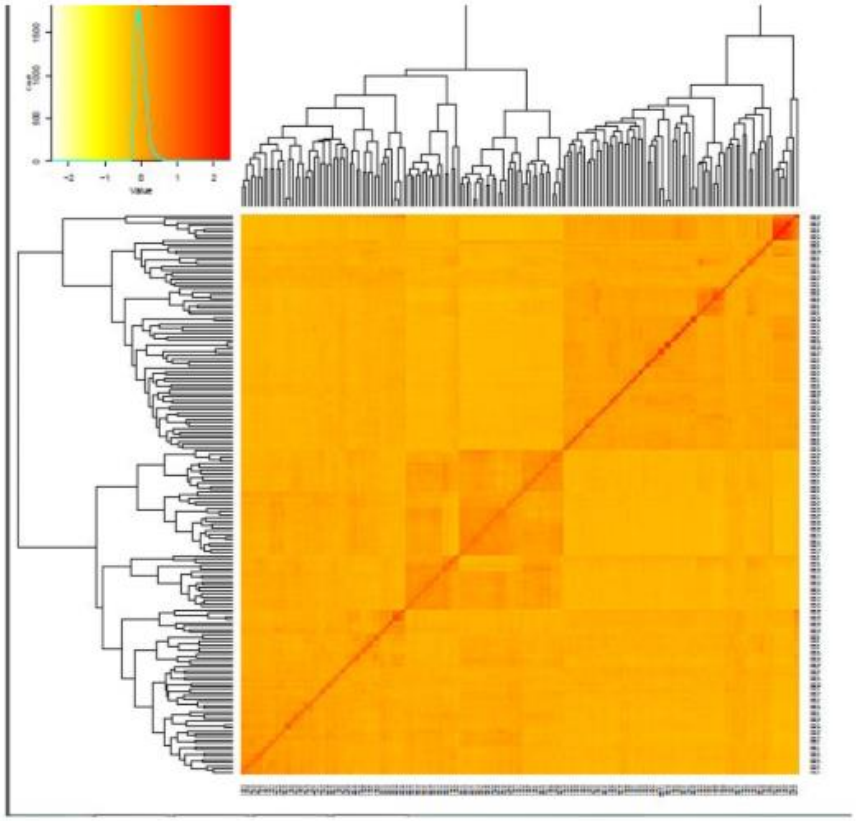

Fig 2: Heat map image based on Kinship analysis of 175 GWAS accessions*

*Gali et al. (unpublished)

Table 7: SNP markers associated with Fe and Zn concentration in seeds of field pea across Rosthern, SK Saskatoon, SK and Fargo, ND in 2013, 2014, 2015, and 2016 (after Bonferroni correction was applied)

Trait	Marker	P value	-log 10
Fe	Sc_219_42293	5.57E-07	6.25**
	Sc_219_42326	5.57E-07	6.25**
	Sc_6182_63782	2.15E-06	5.67*
Zn	Sc_8032_15394	1.44E-07	6.84**
	Sc_1512_36017	1.57E-06	5.80*
	Sc_1340_418746	3.28E-06	5.48*

Significance: at 0.05* - >5.46
at 0.01** > 6.16
at 0001*** >7.16

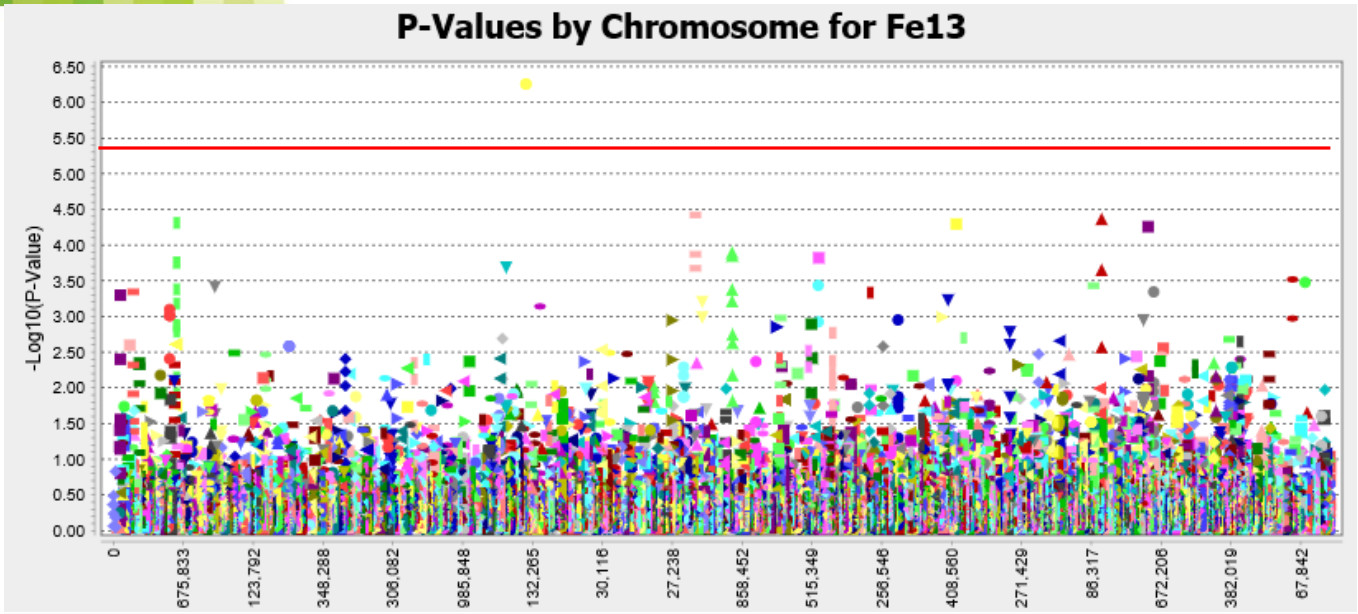

Fig 3: Manhattan plot of Fe in Saskatoon,2013

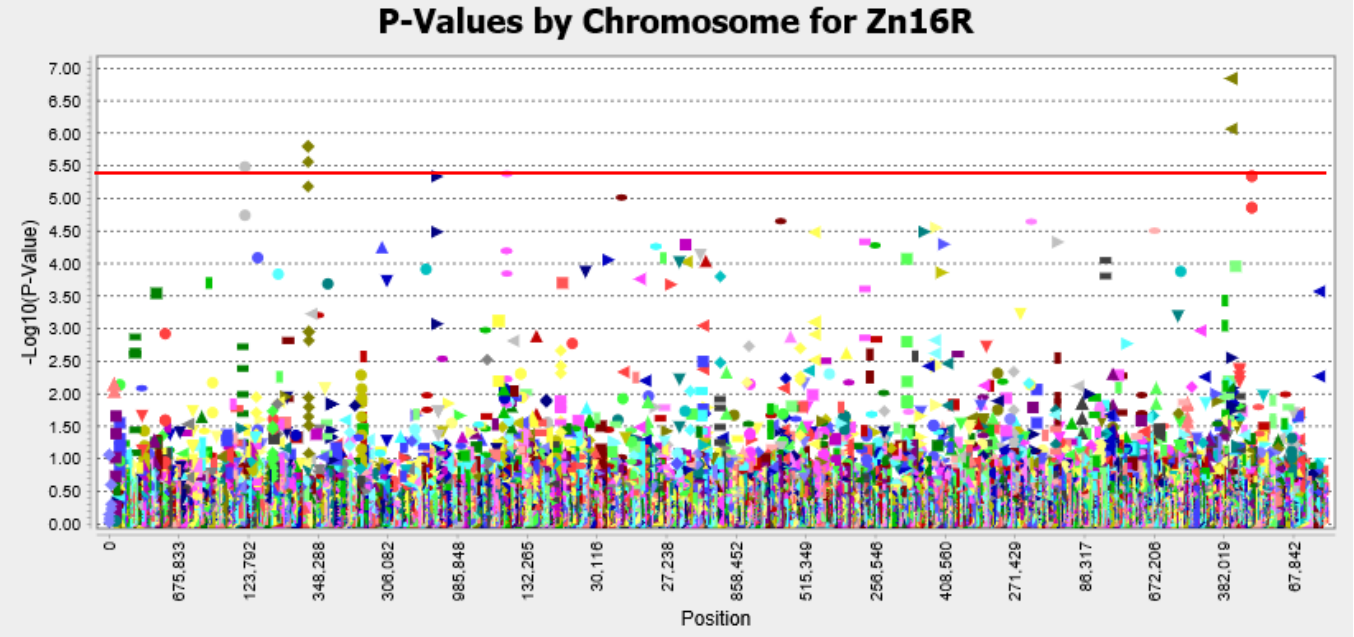

Fig 4: Manhattan plot of Zn in Rosthern,2016

Conclusions

- Genotypes differed significantly (P=0.001) for Fe and Zn in all location-years, but not for Se.
- Soil and seed Fe and Zn concentration is significantly correlated (P=0.05) in Saskatoon but not in Rosthern. Soil and seed Se concentration is significantly correlated (P=0.05) in Rosthern but not in Saskatoon.
- After Bonferroni correction, 3 markers were detected for Fe concentration and 3 markers for Zn concentration (P=0.05). Few other less significant markers – for all three micronutrients

Future work

- Convert identified SNPs to KASP markers

- Validation of identified SNP markers using two recombinant inbred line populations segregating for seed micronutrient concentration

  - PR-02
  - PR-07

Acknowledgement

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Thank you!

Questions?

Comments?