

Genome wide association study (GWAS) on selected micronutrients in field pea (*Pisum sativum* L.)



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Outline

- Introduction
- Hypotheses
- Project objectives
- Methodology
- Results



Introduction

- Iron (Fe), Zinc (Zn) and Selenium (Se) are among the twenty essential nutrients which comprise the basis of all human nutrition (WHO 1999).

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



There are several options to overcome the above issue.

✓ Increased food production

✓ Diet supplementation

✓ Bio-fortification:

Bio-fortification is the practice of deliberately increasing the content of an essential nutrients in a food, so as to improve the nutritional quality of the food supply (WHO)

- 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.

Phenotypic data

Genotypic data



Genome wide association study(GWAS)



Identifying significant Single Nucleotide Polymorphisms



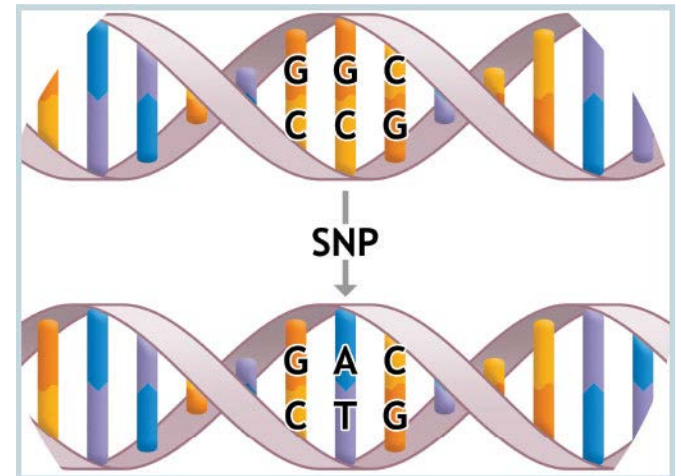
Development of markers for Marker Assisted Selection



Bio-fortification

What is GWAS?

- Also known as whole genome association study.
- is an examination of a genome-wide set of genetic variants in different individuals to see if any variant is associated with a trait.
- Typically the genetic variant is the SNPs.



Objectives

- 1) Evaluation of diverse pea accessions for Fe, Zn and Se concentration.
- 2) GWAS study to identify SNPs associated with Fe, Zn and Se concentration.



Hypotheses

- 1) Substantial variation in Fe, Zn and Se concentration will be observed in a pea Genome Wide Association Study (GWAS) panel.
- 2) Single nucleotide polymorphisms (SNPs) associated with Fe, Zn and Se concentration in pea can be identified by GWAS.

Methodology

- 1) Evaluation of diverse pea accessions for Fe, Zn and Se concentration
 - A GWAS panel of 177 pea accessions developed at the Crop Development Centre (CDC), University of Saskatchewan will be evaluated for Fe, Zn, and Se concentration using atomic absorption spectrophotometer (AAS).
 - A total of 1770 seed samples derived from field trials conducted in Saskatchewan and North Dakota will be used.

Table 1 : Details of seed samples for evaluation of Fe, Zn, and Se concentration

Year	Fargo, North Dakota	Saskatoon	Rosthern	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

Total number of samples - **1770**

- Seed samples will be ground using the cyclone sample mill [UDY Corporation, Fort Collins, Colorado-USA].
- Ground samples will be digested (Thavarajah et al. 2007) using a Vulcan-84 automatic digester.
- Digested samples will be analyzed using an atomic absorption spectrophotometer (Diapari et al. 2015).

Preliminary test

- to justify the seed sample size that should be used for AAS.
- ✓ Three seed sample sizes (10,60,120)
- ✓ Three cultivars (CDC Striker, CDC Dakota and CDC Meadow)
- ✓ Two locations (Saskatoon and Rosthern)
- ✓ Two biological replicates from each location
- ✓ Three technical repeats(digestions) for each sample.

2) GWAS to identify SNPs associated with Fe, Zn, and Se concentration

- Genotypic data:
 - ✓ Genotyping-by-sequencing (Gali et al., unpublished data, 2016)
 - ✓ Illumina Golden Gate assay (Stonehouse et al., unpublished data, 2016)

- Population structure → “Structure” software



- Association mapping → TASSEL software
- ✓ Association between SNP markers against each of Fe, Zn, and Se concentration.
- ✓ Significant SNPs will be identified.

Results

Table 2 - Preliminary test-Least square mean in ppm (LSM), Analysis of Variance(ANOVA), mean, and coefficient of variance of Fe, Zn and Se concentrations for three sample sizes in cultivars of CDC Striker, CDC Dakota and CDC Meadow obtained from two locations of Rosthern and Saskatoon with two biological replicates per location in 2016.

	Se	Zn	Fe
LSM_10	1.0	29.4	48.5
LSM_60	1.0	29.1	48.9
LSM_120	1.0	29.9	49.8
Mean	1.0	29.4	49.1
ANOVA (F value)	1.18 ^{ns}	1.21 ^{ns}	1.32 ^{ns}
*Sample_size	0.01 ^{ns}	0.91 ^{ns}	0.97 ^{ns}
*Techrep	0.01 ^{ns}	0.02 ^{ns}	0.4 ^{ns}
CV%	76.28	9.11	8.07

ns – not significant , CV – Coefficient of variation *at 5% level of significance

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Questions?
Comments?