PROJECT CODE BRE1119
TITLE **Iron Biofortification of Lentils: Defining Current Production of high Fe Lentils and Development of Enhanced Nutritional Quality**
INVESTIGATORS
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FUNDER(S)
Saskatchewan Pulse Growers; Simpson Seeds contributed samples
TYPE OF STUDY
GENETIC IMPROVEMENT - MICRONUTRIENTS
OBJECTIVES
1. Evaluate current lentil production to determine what percent of lentils were already high in Fe concentration and establish their bioavailability range. Establish correlation between the *in vitro* bioavailability model and a poultry feeding trial for Fe bioavailability. 2. Establish some of the fundamental information required to develop a breeding strategy to enhance Fe concentration and bioavailability of lentils.
WHY STUDY NEEDED
Iron deficiency is the leading nutritional deficiency in the world, estimated to affect a third of the world's population and prevalent mostly in women and children. A major cause of Fe deficiency is low Fe concentration and bioavailability in diets high in staple food crops such as maize, rice, and wheat. Pulse crops such as lentils are significantly higher in Fe concentration and therefore have potential to provide better Fe nutrition. Enhancing the levels of Fe concentration and bioavailability through plant breeding and agricultural practices is known as Fe biofortification and is considered to be an effective and sustainable approach. Previous research indicated that approximately 10-15% of lentils produced in southern Saskatchewan were already "biofortified" with Fe (more than 90 µg Fe/g seed). However, the environmental and genetic factors that interact to influence Fe concentration and bioavailability are only poorly understood. Research is needed to consistently produce Fe biofortified lentils. Such an achievement would provide enhanced product

quality value to both consumers and stakeholders in lentil production.

STUDY DESIGN

Objective 1.

Initially, lentil samples were tested for iron level and bioavailability, and the most promising taken forward to a poultry feeding trial. Simpson Seeds (Moose Jaw, SK) provided samples from shipments of Saskatchewan lentils. Samples were analyzed for Fe concentration, using Atomic Absorption Spectrometry (AAS), and bioavailability, using a previously developed bioassay. This study was designed to show the range of Fe concentration and bioavailability in current field lentils grown with a variety of soil type, location, and weather. Samples were cooked, cooled, and freeze-dried prior to the *in vitro*/Caco-2 cell model assay.

Samples identified as promising in this bioassay were examined in a poultry feeding trial. Six diets with red lentil varieties (Rouleau, Imax, Maxim, dehulled or whole, Impact 26101, and a mixture of Red Rider dehulled with added red seed coat) were selected for poultry feeding trials using up to 90% lentil, with corn oil, and supplements. Groups of 10 birds were fed *ad libitum* for six weeks. Body weight and hemoglobin concentrations were measured weekly. After the six-week feeding trial, liver and intestinal samples were taken for measurement of iron stores, liver ferritin, and gene expression of key proteins involved in Fe absorption.

Objective 2.

Results from the *in vitro* experiments were used as screening tools to search through mapping populations of lentils and identify the molecular markers associated with Fe bioavailability and concentration. This should result in a breeding approach that allows sustainable enhancement of Fe nutritional quality.

The lentil association mapping (LAM) populations (143 lines) were sown at two locations in 2012. Seed samples of LAM were harvested and Fe concentration was quantified as previously. The accessions were then genotyped using 1293 single nucleotide polymorphism (SNP) markers (based on the 1536-SNP Illumina Golden Gate Assay). Fe bioavailability of the LAM populations was evaluated.

FINDINGS

Objective 1.

Iron concentration of the commercial whole lentil samples was relatively high (72.8 ±10.8 μg Fe/g) among the 24 varieties tested, ranging from 53.4 to 96.7 μg Fe/g, indicating that some of the lines could be considered “biofortified” with Fe, with an arbitrary minimum of 90 μg Fe/g. Relative Fe bioavailability of the whole lentils ranged from 8% in whole lentils to 28% of a dehulled CDC Robin control sample. Dehulling is known to decrease the Fe concentration of the sample while increasing its bioavailability, and this study confirmed the effect. A zero-tannin lentil tested in a small-scale trial, however, had high bioavailability with or without the hull. Both genotype and location of origin of the samples influenced the Fe concentration.

In the feeding trial, birds gained weight faster on dehulled lentil of the same cultivar (Maxim), but differences among cultivars were larger, and the correlation of hemoglobin with lentil iron or bioavailable iron was weak. The data suggest an additional factor in some cultivars. Phytate, a known iron-binding compound in seed, was examined but no correlation with the feeding trial results was apparent.

Objective 2.
Fe concentration of the 2012 LAM ranges from 46 µg Fe/g to 100 µg Fe/g. Association mapping analysis revealed that seven unmapped SNPs were significantly associated with Fe concentration. Association mapping analysis was also conducted using the Illumina Golden Gate assay, but showed no significant association between any of the SNPs and in vitro Fe bioavailability of lentil. This lack of association is likely explained by the insufficient numbers of accessions tested, combined with high variability among experimental replicates.

Next steps: The LAM populations were also grown in two locations in both 2013 and 2014. Fe concentration ranged from 34 to more than 120 µg Fe/g in 2013 trials. Association mapping analysis with the combined 2013 and 2014 LAM data will be conducted to confirm if the seven SNPs are truly associated with higher Fe concentration in lentil. Subsequently, the seven loci will be mapped using the lentil genome assembly, expected to be available in early 2015.

Individual samples with consistently high Fe concentration from 2013 and 2014 trials will be selected for further testing. Development of a recombinant inbred population that varies significantly in Fe concentration is currently in progress.

SIGNIFICANCE OF STUDY

The study confirms that iron concentrations in Saskatchewan lentils can be high enough to be considered biofortified, particularly the dehulled lentils. Evidence suggests that tannins or other components of seed coat reduce the bioavailability of iron. However, within the cotyledon, current research suggests that levels of kaempferol-glycoside are positively correlated with Fe bioavailability. Efforts to map genes or genome regions which contribute most to high iron content have not succeeded to date, but are continuing.

PUBLICATIONS, PRESENTATIONS, EDUCATIONAL MATERIALS PRODUCED

Peer Reviewed Papers:

DellaValle, D.M., Vandenberg, A. and Glahn, R.P. 2013. Seed coat removal improves iron bioavailability in cooked lentils: Studies using an in vitro digestion/Caco-2 cell culture model. J. Agric. Food Chem. 61: 8084-8089.

Dellavalle D.M., Thavarajah, D., Thavarajah, P., Vandenberg, A. and Glahn, R.P.

2013. Lentil (*Lens culinaris* L.) as a candidate crop for iron biofortification: Is there genetic potential for iron bioavailability? Field Crops Research 144: 119-125.
VALUE TO PRODUCERS
Identification and development of cultivars and conditions which lead to increased nutritional quality of iron in lentils could lead to an identity-preserved marketing of lentils which meet the conditions, providing a highly acceptable means of reducing iron deficiency in areas where it is prevalent.