# IRON BIOFORTIFICATION POTENTIAL OF FIELD PEA (PISUM SATIVUM L.)

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**Title**

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

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## ABSTRACT

Iron (Fe) deficiency affects more than 3 billion of the global population. The objectives of this study were to (1) determine the genetic and environmental variation of seed Fe concentration and food matrix factors that govern Fe bioavailability in field peas (*Pisum sativum* L.) grown in North Dakota, USA in 2010 and 2011, and (2) determine the genetic variation of Fe uptake by field pea grown under greenhouse conditions with different Fe treatments. Seed Fe concentration in field pea samples from the field study ranged between 46-53 mg/kg with a mean of 51 mg/kg. Mean concentrations of the food matrix factors in those field peas were as follows: phytic acid=5.1 mg/g, xanthophyll=17.3 mg/100 g, canthaxanthin=86.8 mg/100 g, beta-carotene=516.8 µg/100 g, kestose=1697 mg/100g, quercetin=54.3 mg/100 g, and ferulic acid=46.9 mg/100 g. DS Admiral and CDC Golden showed high concentrations of Fe promoter compounds and low concentrations of phytic acid. DS Admiral showed high Fe uptake with increasing Fe fertilizer rates in the greenhouse study. Therefore, DS Admiral and CDC Golden could be potential field pea genotypes for future Fe biofortification efforts.


Key words: biofortification, iron, food matrix factors, field pea

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Last but not least, I express my heartfelt gratitude to my beloved mother, father, and sister for the support given throughout my life.

## DEDICATION

To my beloved parents and teachers

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# 1. INTRODUCTION

Iron (Fe), one of the most abundant elements in the earth, comprises 4.2% of the earth's crust (Vose, 1982) and is an essential element for both plants and humans. Iron facilitates oxygen transportation and is a major component/cofactor in enzymatic reactions (Dallman, 1986). The recommended daily allowances (RDA) of Fe for adult males and females (ranging from 19-50 years of age) are 8 and 18 mg/day, respectively. Long term inability to supply the adequate daily Fe requirement may result in Fe deficiency (NIH, 2012).

Iron deficiency affects more than 3 billion people worldwide, and most affected are women and pre-school children in south-east Asia and Africa (WHO, 2012). Iron deficiency reduces the physical and mental development of infants, pre-school children, and school-aged children. In addition, Fe deficiency increases the maternal mortality and stillbirths during pregnancy (WHO, 2001). Several approaches (e.g., Fe supplementation, Fe fortification) have been implemented in the past to reduce Fe deficiency; however, none of these approaches were effective as a result of infrastructure, cost, inability to reach large populations, and less cultural acceptance. Biofortification, which is the development of micronutrient-enriched staple food crops through traditional plant breeding and modern biotechnology, is a novel concept introduced as a sustainable approach to reduce Fe deficiency (Welch and Graham, 2002).

Biofortification research on wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and common bean (*Phaseolus vulgaris* L.) suggests that there is considerable genetic variation in the total seed Fe concentration (wheat: 28.8-56.5 mg/kg; rice: 7.5-24.4 mg/kg; common bean: 34-89 mg/kg) (Graham et al., 1999; Graham et al., 2001; Welch and Graham, 2004). In addition, biofortification research on lentil (*Lens culinaris* L.) shows that the total Fe concentration in lentil varies between 73-90 mg/kg with a high potential for Fe biofortification (Thavarajah et al.,

2009a). This research also showed that a 100 g-serving of lentils provides 91-113% of the minimum RDA of Fe for males and 41-50% of the minimum RDA of Fe for females.

Field pea (*Pisum sativum* L.) is a pulse crop that originated in Middle East countries approximately 9000 years ago. Currently, the global field pea production exceeds 10.2 million tonnes of which the USA is the 6$^{th}$ major field pea producer in the world (FAOSTAT, 2012). The field pea production in the USA is approximately 0.7 million tonnes (FAOSTAT, 2012), and it is mainly exported to south and south-east Asia and sub-Saharan Africa (Jansen et al., 2006). Pulses, such as lentil, field pea, and chickpea (*Cicer arietinum* L.), are commonly consumed staple foods by south Asian populations who are deficient in Fe. Therefore, development of Fe-rich pulses may be a food-based solution to combat the global micronutrient malnutrition. Research on the genetic and environmental variation of Fe concentration in the field pea is limited. This study will provide the baseline information on the biofortification potential of the field pea grown in North Dakota, USA.

The objectives of this study were (1) to determine the genetic and environmental variation of total seed Fe concentration and food matrix factors that govern the Fe bioavailability in field pea genotypes grown in North Dakota, USA in 2010 and 2011 and (2) to determine the genetic variation of Fe uptake by field pea genotypes grown under greenhouse conditions with different Fe fertilizer treatments.

# 2. LITERATURE REVIEW

## 2.1. Iron

Iron is one of the most abundant elements on the earth. It is an important element for many living organisms for their cellular functions such as oxidation-reduction processes (Bothwell et al., 1979), physiological functions (NIH, 2012), and oxygen transportation. Iron also acts as a major co-enzyme/co-factor in enzymatic functions, and it is an essential element for cell growth and differentiation (Dallman, 1986).

More than 60% of the total body-Fe in humans exists as hemoglobin, a heme-protein that transports oxygen in the human body. Twenty-five percent of the total body-Fe is present as readily mobilizable Fe stores, 10% as myoglobin – another heme-protein that transports and stores oxygen for use during muscle contraction, and the remaining 5% of the total body-Fe is present in enzymes (Dallman, 1986).

## 2.2. Presence of Fe in the soil

The most common form of Fe found in the earth is hematite (Shacklette et al., 1984). The mean Fe percentage in soils of the conterminous USA has been reported as 1.8%. The mean Fe percentages in soils of the western and eastern USA have been reported as 2.1% and 1.4%, respectively (Shacklette et al., 1984). Generally, Fe availability to the plant is influenced by soil factors such as ion contents (e.g., sodium $\{Na^+\}$, chloride $\{Cl^-\}$, magnesium $\{Mg^{2+}\}$, calcium $\{Ca^{2+}\}$, and carbonate $\{CO_3^{2-}\}$), moisture, temperature, and pH. Studies indicated that the Fe uptake by plants increased in soils with low concentrations of $Na^+$, $Cl^-$, $Mg^{2+}$, $Ca^{2+}$, and $CO_3^{2-}$ (Olsen and Watanabe, 1979; Franzen and Richardson, 2000). In northern Great Plains, where soils can have high soluble salts, free lime, and free gypsum in soil, plants showed a low Fe uptake (Franzen and Richardson, 2000). Salts (e.g., $Na^+$, $Cl^-$) are additional stresses to the plant,

and they may limit the soil Fe availability, resulting Fe chlorosis in the plant (Franzen and Richardson, 2000).

**2.3. Iron uptake in plants**

Plants require $10^{-4}$-$10^{-8}$ M ferric ($Fe^{3+}$) ions; however, $Fe^{3+}$ ions are insoluble and are less bioavailable to plants. There are two mechanisms for Fe uptake by plants: a) Strategy I and b) Strategy II (Romheld and Marschner, 1986a). Strategy I is used by all higher plants except graminaceous monocots (Romheld and Marschner, 1986). Strategy-I plants utilize three basic steps for Fe uptake as follows: 1) acidification of the rhizosphere by $H^+$ Adenosinetriphosphatase, 2) reduction of $Fe^{3+}$ to ferrous ($Fe^{2+}$) by nicotineamide-adenine-dinucleotide (reduced form) reductase, and 3) uptake of Fe by an Fe transporter in the root cell cytoplasm (Chaney et al., 1972; Romheld et al., 1984; Romheld and Marschner, 1986a). Once Fe is absorbed into the root cell, it is further transported to the shoot through the xylem as chelates of siderophores or of citrate (Cataldo et al., 1998). Fe absorption by the leaf cell occurs through a mechanism which is similar to Strategy-I (Bruggeman, et al., 1993).

Strategy II is used by graminaceous species. These plants release Fe-chelating compounds that are known as phytosiderophores. The release of phytosiderophores is stimulated by an Fe-deficiency in the plant (Takagi et al., 1984). Phytosiderophores readily solubilize inorganic Fe compounds that are sparingly soluble (e.g., iron hydroxide). The reduction of $Fe^{3+}$ to $Fe^{2+}$ is of marginal importance for Fe solubilization by phytosiderophores in Strategy-II plants (Takagi et al., 1984). Once solubilized, $Fe^{3+}$ phytosiderophores are taken up at high rates by the plant roots (Romheld and Marschner, 1986b).

## 2.4. Iron deficiency in humans

Iron deficiency is the condition where mobilizable Fe stores (i.e. hemosiderin and ferritin) in the human body are depleted. A more severe stage of Fe deficiency is known as anemia (WHO, 2001). Populations affected by anemia are classified using World Health Organization (WHO) hemoglobin threshold levels. These hemoglobin threshold levels may vary between 110-130 g/L of blood depending on the age and the physiological condition of the human (WHO, 2001). More than 60% of the global population is Fe deficient (WHO, 2012). Between 30-80% of infants in developing countries suffer from severe Fe deficiency (WHO, 1992). Also, it is estimated that approximately 52% of pregnant women in developing countries and 23% of pregnant women in industrialized countries suffer from Fe deficiency anemia (WHO, 1992). A common reason for diet related Fe deficiency around the world is the staple foods that are low in bioavailable Fe (WHO, 2001). Use of plant breeding and biotechnology to produce staple food crops with high Fe bioavailability is an approach to increase the Fe intake via foods.

## 2.5. Biofortification

Plant breeding and improved modern biotechnologies have significantly contributed to the increase in global food production. For example, the Green Revolution improved agriculture and breeding techniques to increase the grain yield in many cereal crops (Welch and Graham, 2002). As a result, sufficient amounts of calories and moderate amounts of proteins were supplied to malnourished populations around the world (Welch and Graham, 2002). This situation led to the negative impacts on the micronutrient security of the people in developing countries (Welch and Graham, 2002). With the expansion of these new cropping systems, the consumption and production of traditional micronutrient-rich pulse crop production has declined (Welch et al., 1997). Scientists have suggested that the development of micronutrient-enriched

staple food crops through traditional plant breeding and modern biotechnology would be an effective solution for micronutrient malnutrition (Welch and Graham, 2004). Consumption of biofortified staple food crops significantly improves the micronutrient concentrations in targeted populations (Welch and Graham, 1999). Also, biofortification is highly cost effective, sustainable, and feasible for agricultural communities that are dependent on fewer resources (Nestel et al., 2006). Therefore, many efforts have been taken to biofortify different food crops with micronutrients.

### 2.5.1. Iron biofortification

The genetic variability in the Fe concentration in different cereal and pulse crops has been determined by different research organizations {e.g., Consultative Group on International Agricultural Research (CGIAR) and HarvestPlus} (Senadheera et al., 1998; Thavarajah et al, 2009a; HarvestPlus, 2012). A study in Mexico showed that the Fe concentration in wheat cultivars varied between 28.8-56.5 mg/kg, with a mean Fe concentration of 37.2 mg/kg (Graham et al., 2000). This study showed that the genetic variation in Fe concentration was limited among cultivated tetraploid and hexaploid varieties of wheat (Graham et al., 2000). However, this study found that the genetic variation in Fe concentration was higher in wild diploid and tetraploid wheat cultivars compared to modern wheat cultivars (Monasterio and Graham, 2000; Balint et al., 2001; Cakmak et al., 2004; Grusak and Cakmak, 2005).

Researchers at the International Rice Research Institute (IRRI) have been evaluating the genetic variability of Fe in rice over the past two decades (Graham et al., 1999). A total of 939 germplasm collections, which included traditional and improved lines, IRRI breeding lines, tropical japonicas, commercial lines, and parents were evaluated for the genetic variability of Fe. The overall Fe concentration among these samples ranged between 7.5-24.4 mg/kg. There was a

four-fold difference in the Fe concentrations within the genotypes tested. Moreover, this study showed that the aromatic rice genotypes had high Fe concentrations (18-22 mg/kg). Further studies showed that the trait for aroma could be used to screen for genotypes that have high Fe concentration (Welch and Graham, 2002).

A study on Southern African germplasm of maize (*Zea mays* L.) showed that the Fe concentration ranged between 16.4-22.9 mg/kg (mean Fe concentration = 19.6 mg/kg) (Bunziger and Long, 2000). Another study that evaluated early maturing maize lines grown in West Africa showed that the Fe concentration varied between 15.5-19.1 mg/kg. Both studies showed that the Fe concentration is naturally very low in maize. Thus, it has been suggested that maize should be genetically modified to develop Fe-rich maize cultivars.

Researchers at the Centro Internacional de Agricultura Tropical (CIAT) have studied the degree of genetic variability for Fe concentration in common beans (Graham et al., 1999). They evaluated a core collection of common bean accessions and found that the Fe concentration varied between 34-89 mg/kg with a mean concentration of 55 mg/kg. Also, they found that some bean accessions from Peru contained high concentrations of Fe (mean Fe = over 100 mg/kg). These data suggested that there was sufficient genetic variation to increase the Fe concentration by approximately 80% in common beans. Also, the researchers found that the interaction between location and genotype was significant ($P<0.05$). This significance suggested that the environment affects the seed Fe concentration (Welch and Graham, 2002).

Micronutrient research on lentils indicated that lentils can be used as a potential candidate for Fe biofortification (Thavarajah et al., 2009a). The total Fe concentration in lentils varied between 73-90 mg/kg with a mean concentration of 81 mg/kg. The broad sense heritability estimate of Fe for the lentil germplasm was approximately 64%, indicating a possible genetic

influence on the Fe uptake by lentil plants. This research also indicated that a 100 g serving of dry lentils could provide 91-113% of the minimum RDA of Fe for males and 41-50% of the minimum RDA of Fe for females (Thavarajah et al., 2009a).

### 2.5.2. Food matrix factors that affect the Fe bioavailability

Bioavailable Fe is defined as the amount of Fe that is absorbed from a meal and utilized for metabolic processes in the body (Welch and Graham, 2004). The bioavailability of Fe in a diet is mainly governed by food matrix factors (Graham et al., 2001). Food matrix factors in plant based foods can be categorized into two groups (Fe promoters and Fe inhibitors) based on whether they enhance or limit the Fe absorbance by the human gut. Selected food matrix factors that affect the mineral bioavailability are presented in **Table 2.1.** Promoter compounds are organic acids (e.g., vitamin C, fumarate, malate, and citrate), carotenoids (xanthophyll, canthaxanthin, and beta-carotene), pre-biotic carbohydrates (inulin and other fructooligosaccharides), and amino acids (methionine, cysteine, histidine, and lysine) (Delzenne et al., 1995; Ohta et al., 1995; Gracia-Casal et al., 2000). Concentrations of promoter compounds in plants are influenced by the environment and the genotype (Welch, 2001). Carotenoids (e.g., xanthophyll, canthaxanthin, and beta-carotene) are essential for humans particularly because of their roles in eye health and photo-protection apart from the provitamin A activity. Carotenoids are abundant in orange and dark green fruits and vegetables. Research shows that beta-carotene has a direct effect on the Fe uptake by Caco-2 cells (Gracia-Casal et al., 2000). Furthermore, beta-carotene can prevent the inhibitory actions of potential inhibitors (e.g., polyphenols) on Fe bioavailability (Garcia-Casal et al., 1998).

Prebiotic carbohydrates are defined as nondigestible food ingredients that selectively stimulate the growth and activity of gut micro flora (Cummings et al., 2001). Common prebiotic

carbohydrates include fructooligosaccharides such as kestose and nystose. These compounds occur naturally in abundant levels in plants such as Jerusalem artichoke (*Helianthus tuberosus* L.), asparagus (*Asparagus officinalis* L.), garlic (*Allium sativum* L.), leek (*Allium ampeloprasum* L.), onion (*Allium cepa* L.), and chicory roots (*Cichorium intybus* L.) (Gorski, 1997; Tungland and Meyer, 2002). Prebiotics create a favorable environment in the gut that promotes the reduction of $Fe^{3+}$ to $Fe^{2+}$, and this process may increase the Fe bioavailability (Yeung et al., 2005).

Table 2.1. Food matrix factors that affect the mineral bioavailability.

| **Food matrix factors** | **Biological importance** | **Elements** |
|---|---|---|
| Ascorbic acid | Antioxidant properties, formation of collagen, maintenance of teeth, bones and gums | Fe and Zn |
| Amino acids (methionine, cysteine, histidine, lysine) | Protein synthesis, body growth and development | Fe, Zn, and Se |
| β-carotene | Vitamin A precursor | Fe, Zn, and Se |
| Inulin | Prebiotic function | Fe, Zn, and Ca |
| Fructooligosaccharides | Prebiotic function | Ca, Fe, and Zn |
| Phytic acid | Antioxidant and anticarcinogenic properties | Fe and Zn |
| Phenolics | Anticarcinogenic properties | Fe, Zn, Ca, and Mg |

Sources: Welch, 2002; Thavarajah and Thavarajah, 2012

Phytic acid (1,2,3,4,5,6-hexakis myo-inositol) and its salts are present in legumes, cereal grains, oil seeds, and nuts at levels of 0.5-5% (Graf et al., 1987; Raboy, 2002; Pilu et al., 2003; Crea et al., 2008) . Phytic acid binds to mineral cations such as calcium (Ca), Fe, and zinc (Zn), which reduces their bioavailability in the human gut (Welch, 2002). Several attempts have been carried out to breed for low phytate cultivars of maize, barley (*Hordeum vulgare* L.), rice, and

soybean (*Glycine max* L.) (Raboy, 2002). Also, research on lentils showed that phytic acid is naturally low in lentils (2.5-4.4 mg $g^{-1}$) (Thavarajah et al., 2009b).

**2.6. Field pea**

Field pea is one of the oldest pulse crops that originated in the Middle East approximately 9000 years ago and has been cultivated in Europe for thousands of years (Welch and Graham, 2002). Currently, it is grown in all climatic conditions, including the tropical countries at high elevations (Saskatchewan Pulse Growers, 2000). The global field pea production area is approximately one hectare (McKay, 2003).

The major field pea producing countries are Canada, Russian Federation, France, China, India, and USA **(Table 2.2.)**. The annual global field pea production exceeds 9.0 million tonnes, and the USA production is approximately 0.7 million tonnes (FAOSTAT, 2012; USA Dry Pea & Lentil Council, 2011). In 2009, Canada exported the highest quantity of field pea (2.6 million tonnes) followed by USA (0.5 million tonnes) and Ukraine (0.3 million tonnes). India, Bangladesh, and China are the top importers of field pea, and these countries imported 1.7, 0.5, and 0.4 million tonnes of field pea, respectively in 2009 (FAOSTAT, 2012). USA grown field pea is mainly imported by South-east and South Asian countries and Sub Saharan African countries (Jansen et al., 2006). India, Pakistan, Bangladesh, Sri Lanka, China, and Philippines are the major consumers of field pea around the world (American Pulse Association, 2010).

Field pea had been a best paying cash crop in the early 1900's in Wisconsin in USA (Oelke et al., 1991). Gradually, it became a major crop in Washington and Idaho. In the 1990's the cultivation of field pea expanded to the northern tier of USA such as North Dakota, South Dakota, Montana, and Minnesota (McKay et al., 2003). According to the 2010 statistics from the

US Dry Pea & Lentil Council, North Dakota (0.5 million tons) had the highest production of field peas followed by Montana (0.2 million tons) and Washington (0.08 million tons).

Table 2.2. Major field pea growing countries and their annual production in 2010.

| **Country** | **Production (million tonnes)** |
|---|---|
| Canada | 2.86 |
| Russian Federation | 1.22 |
| France | 1.10 |
| China | 0.99 |
| India | 0.70 |
| USA | 0.65 |

Source: FAOSTAT, 2012

The field pea is an annual cool-season pulse crop, well adapted to the semi-arid climate of the Northern Plains (Field Pea Production, 2003). Field peas are grown on a wide range of soil types, from sandy and sandy loamy soils to clay. Higher yields of field pea are obtained when grown in high organic matter containing clay soils compared to other soil zones (Saskatchewan Pulse Growers, 2000). Furthermore, field peas can be grown in no-till cropping system (Saskatchewan Pulse Growers, 2000).

Field peas are seeded in a narrow row spacing of 15-31 cm during early-April to mid-May. The recommended seeding depth is 3-8 cm, and the soil should be well moist. The recommended plant density is 88 plants/$m^2$. Field pea fixes atmospheric nitrogen and produces the nitrogen it requires from atmospheric nitrogen. At planting, field pea seeds can be inoculated with *Rhizobium leguminosarum* to ensure effective nitrogen fixation. The nitrogen fixation rate of field pea is about 40 kg N $ha^{-1}$ (Saskatchewan Pulse Growers, 2000; Field Pea Production, 2003).

Field pea crops flower within 60 days after seeding, and the maturity is completed by 90-100 days. Flowering occurs in the plant at about the $12^{th}$-$16^{th}$ node. Each flowering node produces 1-3 flowers, and the flowers self-pollinate before opening. The plant reaches its maximum height (60 cm-120 cm) at early pod fill. The mature seed pods are 4-10 cm long and about 1.3 cm wide. One pod contains 6-10 seeds. The average seed yield in dryland is around 1400-2900 kg/ha (Field Pea Production, 2003).

Field pea is a good source of complex carbohydrates, protein (20-25%), dietary fiber (5%), essential amino acids (e.g. lysine and tryptophan), and a wide range of micronutrients (Field pea production manual, 2003; Thavarajah et al., 2010; 2011a). Field pea is also an excellent source of folic acid and mineral micronutrients such as Fe, Zn, selenium (Se), Ca and potassium (K). Research shows that field pea has high concentrations of Se (457 µg $kg^{-1}$) and Fe (54 mg $kg^{-1}$) compared to cereals (Thavarajah et al., 2011a). A serving of 100 g of dry field pea could potentially provide 68-94% of the RDA of Fe for adults (Thavarajah et al., 2010).

# 3. MATERIALS AND METHODS

## 3.1. Study 1 – Field study

### 3.1.1. Objectives

1) Determine the genetic and environmental variation of the total seed Fe concentration in field pea genotypes grown in North Dakota, USA in 2010 and 2011.
2) Determine the genetic and environmental variation of food matrix factors that govern the Fe bioavailability in field pea genotypes grown in North Dakota, USA in 2010 and 2011.

### 3.1.2. Hypothesis

The concentrations of a) total Fe concentrations and b) food matrix factors (phytic acid, carotenoids, fructooligosaccharides, and phenolics) in field pea vary with the genotype and the environment.

### 3.1.3. Null hypothesis

The concentrations of a) total Fe and b) food matrix factors (phytic acid, carotenoids, fructooligosaccharides, and phenolics) in field pea do not vary with the genotype and the environment.

### 3.1.4. Materials

Chemical standards, chemicals, and high purity solvents used for the experiment were purchased from Alfa Aesar-A Johnson Matthey Company (Ward Hill, MA, USA), VWR International LLC (Batavia, IL, USA), and Sigma-Aldrich Chemical Company Inc., (Allentown, PA, USA) and used without further purification. Water (distilled and deionized; dd$H_2O$) was purified by a Milli-Q Water System (Millipore, Milford, MA, USA) to a resistance of 18.5 mΩ or greater.

### 3.1.5. Field pea seed samples

Field pea samples from the regional variety trials conducted by the NDSU pulse breeding program across North Dakota in 2010 and 2011 were used in this experiment. Field pea samples from the 2010 growing season were collected from 7 locations (Cass, Divide, McKenzie, Mountrail, Sheridan, Ward, and Williams counties). Field pea samples from the 2011 growing season were collected from 3 locations (Divide, Sheridan, and Ward counties). Each location in both years had six commercial field pea genotypes (Agassiz, CDC Golden, CDC Striker, Cruiser, DS Admiral, and Majoret) with three replicates for each genotype **(Table 3.1.)**. A total of 180 samples were used in this experiment. Sub-samples (10-20 g) of field pea seeds were randomly collected from each replicate plot from all locations for 2010 and 2011 growing years. These samples were dried at 40 °C for 2 days, cleaned, and finely ground (UDY Mill, Unholtz Dickie Corporation, CT, USA).

Table 3.1. Field pea genotypes and their market classes used in the field study.

| Field pea genotype | Market class |
|---|---|
| Agassiz | Yellow field pea |
| CDC Golden | Yellow field pea |
| CDC Striker | Green field pea |
| Cruiser | Green field pea |
| DS Admiral | Yellow field pea |
| Majoret | Green field pea |

**Table 3.2.** shows the mean temperature and the total rainfall during the growing season (April-August) in the past five years (2007, 2008, 2009, 2010, and 2011) in the study locations. **Table 3.3.** shows the organic matter, pH, N, K and phosphorous (P) contents in soils in three counties (Divide, Williams, and Mountrail) across North Dakota in 2010.

Table 3.2. Mean temperature and total rainfall during the growing season (April-August) from 2007 to 2011 for the study locations.

| Parameter | County | 2007 | 2008 | 2009 | 2010 | 2011 |
|---|---|---|---|---|---|---|
| Mean temperature (°C) | Cass | 17 | 16 | 15 | 17 | 16 |
| | Divide | 15 | 14 | 14 | 14 | 15 |
| | Mountrail | 15 | 14 | 14 | 14 | 14 |
| | McKenzie | 16 | 15 | 15 | 15 | 15 |
| | Sheridan | 15 | 14 | 14 | 15 | 14 |
| | Ward | 16 | 14 | 14 | 15 | 15 |
| | Williams | 16 | 15 | 15 | 15 | 15 |
| Total precipitation (mm) | Cass | 473 | 558 | 255 | 515 | 437 |
| | Divide | 297 | 292 | 210 | 449 | 494 |
| | Mountrail | 246 | 288 | 317 | 472 | 455 |
| | McKenzie | 231 | 210 | 244 | 366 | 455 |
| | Sheridan | 359 | 452 | 218 | 431 | 355 |
| | Ward | 396 | 402 | 298 | 472 | 468 |
| | Williams | 247 | 194 | 235 | 339 | 366 |

North Dakota State Climate Office, 2012

Table 3.3. Organic matter, pH, nitrogen (N), phosphorous (P), and K contents in soils in three counties across North Dakota in 2010.

| County | pH | Organic matter (%) | Concentrations | | |
|---|---|---|---|---|---|
| | | | N kg/ha | P kg/ha | K kg/ha |
| Divide | 7.1 | 4.1 | 78 | 31 | 897 |
| Williams | 7.2 | 2.7 | 49 | 29 | 717 |
| Mountrail | 6.9 | 3.3 | 92 | 27 | 381 |

The soil data was obtained from the Williston Research Extension Center, North Dakota. Data is not available for the following locations and years: 2010-Cass, 2010-Mckenzie, 2010-Sheridan, 2010-Ward, 2011-Divide, 2011-Sheridan, and 2011-Ward.

### 3.1.6. Seed Fe concentration

Total Fe concentration in field pea seed samples was determined using modified $HNO_3$-$H_2O_2$ method (Thavarajah et al., 2009a) where 500 mg from the powdered field pea were weighed into digestion tubes. Initially, 6 mL of concentrated (70%) nitric acid ($HNO_3$) were added to the digestion tubes, and the digestion tubes were heated at 90 °C for one hour with occasional shaking of the tubes followed by the addition of 3 mL of 30% w/w hydrogen peroxide ($H_2O_2$) were added to the digestion tubes. The tubes were kept for 15 min at the same

temperature. Finally, 3 mL of 6 M hydrochloric acid (HCl) were added to the digestion tubes, and the tubes were kept in the digestion block for 5 minutes. Upon complete digestion, the tubes were removed from the digestion block, and the volume was adjusted to 10 mL, and filtered (Whatman No. 1 filter papers) using a vacuum system (Gardener Denver Thomas Inc., Welch Vacuum Technologies, LA, USA). Measurement of total Fe concentration was validated using the National Institute of Standards and Technology (NIST) standard reference (1567a Wheat Flour) (14.1 ±0.5 mg/kg). The total Fe concentration was determined using ICP-EMS (Inductively Coupled Plasma Emission Spectrometry) (ICP-ES; ICP-6500 Duo, Thermo Fisher Scientific, PA, USA).

**3.1.7. Phytic acid**

Phytic acid analysis was carried out as previously described by Thavarajah et al. (2009b). Briefly, 100 mg of powdered field pea seeds were weighed into a 15 mL polystyrene conical tube and 10 mL of 0.5 M HCl was added, the mixture then vortexed for 30 s, and heated in a hot water bath at 80 °C for 5 min. The tubes were centrifuged (GPR Centrifuge, Beckman, NY, USAA) at 2000×*g* for 3 min. The supernatant was collected and the extracted phytic acid was de-complexed by adding 1.5 mL of 12 M HCl. Phytic acid was measured using High Performance Anion Exchange (HPAE) system with conductivity detection with a detection limit of 0.5 ppm. Omnipac Pax-100 anion exchange column (250×4 mm i.d.) along with Omnipac Pax-100 (8 µm) guard column (Dionex Corporation, CA, USA) and an anion suppressor (ASRS 300, 4mm – Dionex) was used in the analysis. Deionized water/isopropanol (50:50, v/v), 130 mM sodium hydroxide (NaOH), and water were used as the mobile phase in a gradient system. The flow rate used was 1.0mL/minute with a total run time of 15 minutes. The phytic acid:Fe molar ratio was calculated using the following formula:

Phytic acid (μg $g^{-1}$/660.04 g $mol^{-1}$)/Fe (μg $g^{-1}$/55.845g $mol^{-1}$)

### 3.1.8. Carotenoids

Xanthophyll, canthaxanthin and β-carotene concentrations in field pea seed samples were determined using the method described by Nells and De Leenheer (1983). Five grams of finely ground field pea seeds were weighed into amber glass bottles and extracted in 10 mL of n-hexane. The samples were shaken for one hr. using an incubating-mini shaker (VWR International, PA, USA) for complete carotene extractions. The supernatant was decanted into a 25 mL beaker and evaporated with nitrogen. Upon complete removal of n-hexane, 1 mL of additional n-hexane was added, and the solution was filtered through 0.45 μm cellulose acetate filters (Sterile syringe filter, VWR International). The extracted carotenes were analyzed using a High Performance Liquid Chromatography system (HPLC) with Photo Diode Array Detection (PAD) (7000 series, Agilent Technologies, CA, USA) with the following detection limits (xanthophyll: 0.6 ppm, canthaxanthin: 1.6 ppm, and beta-carotene: 0.01 ppm). The mobile phase consisted of acetonitrile, dichloromethane, and methanol in a ratio of 70:20:10. A C 18 column (250×4.60 mm) (Phenomenex, CA, USA) was used along with a guard column (30×4.60 mm) (Phenomenex, CA, USA), and the three carotenoids (Xanthophyll, Canthaxanthin, and β-carotene) were measured at a wavelength of 450 nm (Nells and De Leenheer, 1983).

### 3.1.9. Fructooligosaccharides

Kestose and nystose concentrations in field pea seed samples were determined as previously described by Feinberg et al. (2009). Five hundred milligrams of finely ground field pea samples were weighed into 15 mL Falcon tubes (17×120 mm) and mixed with 10 mL of Millipore water. The solution was vortexed for 30 s, kept in a hot water bath (80 °C) for 1 hour, and centrifuged for 5 min at 2000×*g*. One mL of the supernatant was diluted (1:11) and filtered

through 0.45 µm cellulose acetate filters. Kestose and nystose sugars were measured using HPAE with Pulse Amperometric Detection (PAD) (Dionex Corporation, CA, USA). The detection limit for both kestose and nystose were 1 ppm. The column used for analysis was CarboPac PA1 (4×250 mm). The mobile phases used were 100 mM NaOH/600 mM sodium acetate, 200 mM NaOH, and 18 MΩ deionized water.

### 3.1.10. Phenolics

Phenolic profile in field pea seed samples was determined as described by Dueñas et al. (2002). One gram of finely ground field pea seeds was weighed into 15 mL Falcon tubes, and 10 mL of a mixture of methanol:water:acetic acid (899:100:1) were added. This solution was vortexed for 30 s and shaken for one hour using the incubating-mini shaker. Finally, the tubes were centrifuged for 5 min at 2000×*g*. The supernatant was filtered through 0.45 µm filters and the extracted phenolics were analyzed using HPLC system with photo diode array detection. The detection limits for gallic, caffeic, catechin, quercetin, and ferullic were 0.1 ppm, 0.2 ppm, 0.1 ppm, 3 0ppm, and 0.1 ppm, respectively. A gradient consisting of mobile phases A (water:acetic acid, 98:2) and B (water:acetonitrile:acetic acid, 78:20:2) was applied at a flow rate of 1.0 mL/min. A C 18 column (250×4.60 mm) was used along with a guard column (30×4.60 mm), and the different phenolic acids (gallic, caffeic, catechin, quercetin and ferullic) were measured at 280 nm wavelength.

### 3.1.11. Statistical design

The experimental design was an incomplete block design with three replicates for six field pea genotypes. The location and year were considered together as a treatment combination. Data from all treatment combinations were tested for homogeneity using Bartlett's test of homogeneity of variance. Replicates and the treatment combinations were considered as random

factors, and genotype was considered as the fixed factor. Replicates, genotypes, and treatment combinations were considered as class variables. Mixed model analysis of variance was performed using the PROC GLM procedure of SAS version 9.3 (SAS Institute SAS User's Guide, 2008). Means were separated by Fisher's protected least significant difference (LSD) at $P<0.05$.

### 3.2. Study 2 - Greenhouse study

#### 3.2.1. Objective

To determine the genetic variation of Fe uptake by field pea genotypes grown under greenhouse conditions with different Fe fertilizer treatments.

#### 3.2.2. Hypothesis

The Fe uptake of commercial field peas varies with the genotype and the Fe treatment.

#### 3.2.3. Null hypothesis

The Fe uptake of commercial field peas does not vary with the genotype and the Fe treatment.

#### 3.2.4. Greenhouse experiment

Pots (500 mL) were filled with 300-320 g of peat-perlite-vermiculite mixture (soil mixture: sunshine no.1), saturated with deionized water, and allowed to drain overnight (Thavarajah, 2006). Three field pea seeds were directly planted in each pot. At planting, pots were kept at 70% (w/w) moisture capacity. At seedling stage, modified Hoagland solution with the Fe treatments was added to all the pots (Thavarajah, 2006). The three Fe treatments were as follows: 1) control treatment (0 ppm), 2) regular Fe treatment (1.1 ppm), and 3) additional Fe treatment (2.8 ppm). The fertilizer solutions were added to plants every week. Each plant received 250 mL of the Hoagland solution at each application. The greenhouse conditions were

as follows: 16 hr. photoperiod, 22/20° C day/night temperature, 50-60% relative humidity, and 300 µ mol $m^{-2}$ $s^{-1}$ light intensity. The whole greenhouse experiment was repeated twice.

Upon complete maturity, the pods were harvested, and seeds were threshed. The harvested field pea samples were dried at 40 °C for 2 days, cleaned, and the total seed Fe concentration was determined as described previously in 3.1.3 section.

### 3.2.5. Statistical design

The experimental design was a three-factor factorial randomized complete block design. Runs, treatments, and the genotypes were considered as the three factors. Data from the two runs were combined and data error variances were tested for homogeneity using Bartlett's test of homogeneity of variance. Replicates, runs, and field pea genotypes were considered as random factors, and the Fe fertilizer treatment was considered as the fixed factor. Replicates, field pea genotypes, treatments, and runs were considered as class variables. Mixed model analysis of variance was performed using the PROC GLM procedure of SAS version 9.2. Means were separated by Fisher's protected LSD at $P<0.05$.

# 4. RESULTS AND DISCUSSION

## 4.1. Study 1 - Field study

### 4.1.1. Iron

**Table 4.1.** indicates the analysis of variance for the Fe concentration for six field pea genotypes grown in North Dakota in 2010 and 2011. The analysis of variance showed that the genotype and the locations(year) were significant ($P<0.05$) for the Fe concentration in field peas **(Table 4.1.)**. The Fe concentration in field peas ranged between 46-53 mg/kg with a mean value of 51 mg/kg. The highest Fe concentration was observed in Cruiser (53 mg/kg) **(Table 4.2.)**. DS Admiral showed the lowest Fe concentration (46 mg/kg). It was shown that a 50 g-serving of field peas could provide 29-33% of the RDA of Fe for males and 13-15% of the RDA of Fe for females **(Table 4.2.)**.

Among the field peas grown in 2010, the highest Fe concentration was observed in those grown in Cass County (59 mg/kg), while the lowest was observed in those grown in McKenzie County (44 mg/kg) **(Table 4.3.)**. In addition, Fe concentrations in field peas grown in Sheridan and Divide counties in 2011 were significantly lower ($P<0.05$) than those in field peas grown in Sheridan and Divide counties in 2010 (Sheridan County: 2010=54 mg/kg and 2011=47 mg/kg; Divide County: 2010=53 mg/kg and 2011=44 mg/kg).

The Fe uptake by plants can be affected by the type of soils (e.g., calcareous and alkaline soils) (Franzen and Richardson, 2000; Department of Agriculture and Food, Government of Western Australia, 2012). Calcareous and alkaline soils produce high concentration of bicarbonate ions, which in turn immobilize soil Fe and lower the Fe uptake by plants (Mengel and Kirkby, 1982; Ag Communications Center, University of Idaho, 2012). Moreover, the Fe uptake by plants can be affected by several environmental and soil factors including soil

Table 4.1. Analysis of variance for Fe, xanthophyll, canthaxanthin, beta-carotene, and phytic acid concentrations in six field pea genotypes grown in different counties across North Dakota in 2010 and 2011.

| Source | df | Mean Square | | | | |
|---|---|---|---|---|---|---|
| | | Fe | Xanthophyll | Canthaxanthin | Beta-carotene | Phytic acid |
| Genotype | 5 | * | * | * | * | * |
| Location(Year) | 9 | * | * | * | * | * |
| Replicate (Year, Location) | 20 | * | NS | NS | NS | * |
| Location(Year) × Genotype | 45 | NS | * | * | * | * |

* Mean square was significantly different at *P<0.05*. NS=Non-significant. Field pea samples from 2010 growing season were collected from seven counties, and field pea samples from 2011 growing season were collected from three counties (N=180).



Table 4.2. Mean Fe concentrations and the %RDA provided by 50 g-serving of six field pea genotypes grown in different counties across North Dakota in 2010 and 2011.

| Genotype | Fe concentration† (mg/kg) | % RDA by 50 g serving (dry weight basis)# | |
|---|---|---|---|
| | | Males (8mg/day) | Females (18 mg/day) |
| Agassiz | 50 a | 32 | 14 |
| CDC golden | 51 a | 32 | 14 |
| CDC striker | 52 a | 33 | 15 |
| Cruiser | 53 a | 33 | 15 |
| DS Admiral | 46 b | 29 | 13 |
| Majoret | 51 a | 32 | 14 |
| Mean | 51 | | |
| SE‡ | 0.4 | | |

† Means within a column followed by different letters are significantly different at *P<0.05*. ‡ SE=Pooled standard error of the mean calculated from the mean square of the ANOVA for Fe concentration in the six field pea genotypes grown in different counties across North Dakota in 2010 and 2011 (N=180). # The percentage RDA provided by 50 g serving of field pea was calculated based on the RDAs for adults between 19-50 years of age (NIH, 2012).

Table 4.3. Mean Fe concentrations for the field peas grown in different counties across North Dakota in 2010 and 2011.

| Year | Location | Mean Fe[†] (SE)[‡] (mg/kg) |
|---|---|---|
| 2010 | Cass County | 59 (1.6) a |
| | Divide County | 53 (1.1) bc |
| | McKenzie County | 44 (4.2) d |
| | Mountrail County | 51 (1.3) c |
| | Sheridan County | 54 (2.4) bc |
| | Ward County | 47 (1.1) d |
| | Williams County | 56 (2.3) b |
| 2011 | Divide County | 44 (0.9) d |
| | Sheridan County | 47 (1.2) d |
| | Ward County | 52 (0.8) c |

[†] Means within a column followed by different letters are significantly different at $P<0.05$.
[‡] SE=Pooled standard error of the mean calculated from the mean square of the ANOVA for Fe concentration in the field peas grown in each county across North Dakota in 2010 and 2011 (N=18).

moisture content, temperature, and soluble salt levels (Inskeep and Bloom, 1986; Franzen and Richardson, 2000; Department of Agriculture and Food, Government of Western Australia, 2012). Past studies showed that high soil moisture contents limited the Fe uptake by plants (Inskeep and Bloom, 1986).

Data from the National Weather Service Cooperative Network and North Dakota Agricultural Weather Network showed that the precipitation in Sheridan and Divide counties during the 2011-growing season (April-September) was higher than that during the 2010-growing season (North Dakota State Climate Office, 2012). High soil moisture in Sheridan and Divide counties during the 2011 growing season would have limited the Fe uptake by field pea plants. Therefore, Fe concentrations in field peas grown in Divide and Sheridan counties in 2011 were lower compared to those in field peas grown in Divide and Sheridan counties in 2010. Ward County had a drier growing season in 2011 compared to 2010 (North Dakota State Climate Office, 2012). The low moisture content in soils in Ward County during the 2011 growing

season would have facilitated increased Fe uptake. As a result, the Fe concentration in field peas from Ward County in 2011 was higher than that in field peas from Ward County in 2010.

The Fe concentration in cereals and pulses has been studied over the past years. Pulses such as lentil, field pea, chickpea, and common bean have been identified as excellent sources of Fe (Beebe et al., 1999; Thavarajah et al., 2009a; Cvitanich et al., 2010). High Fe concentrations observed in these pulse crops could be due to the high Fe concentration in the soils (Abrahams, 1997; Hooda et al., 2002). Thavarajah et al. (2009a) showed that the Fe concentration in lentils ranged between 73-90 mg/kg depending upon growing location, soil type, and weather conditions. Beebe et al. (1999) indicated that the Fe concentration in common beans ranged from 34-89 mg/kg with a mean of 55 mg/kg. A separate study on common beans grown in Colombia showed that the seed Fe concentration in seven common bean genotypes varied between 45-78 mg/kg (Cvitanich et al., 2010). A recent study conducted by Tidemann-Anderson et al. (2011) in east-Uganda indicated that Fe concentrations in common beans ranged between 64-90 mg/kg. The present study results are similar to the results reported by Thavarajah et al. (2009a), Beebe et al. (1999) and Cvitanich et al. (2010).

Total Fe concentrations in cereals (e.g., rice and wheat) are generally lower than those values reported in pulses. For example, a study conducted by the International Rice Research Institute (IRRI) showed that the Fe concentration in rice varied between 7.5-24.4 mg/kg with a mean value of 12.1 mg/kg (Senadhira et al., 1998). Another study conducted at CIMMYT on spring wheat showed that the Fe concentration ranged between 29-57 mg/kg with a mean value of 37 mg/kg. Gawalko et al. (2009) reported that Fe concentrations in wheat, barley (*Hordeum vulgare* L.), and oat (*Avena sativa* L.) grown in Canada were 41, 47, and 38 mg/kg, respectively.

These studies clearly show that Fe concentrations in cereals were lower than that from the field peas in my study.

Rice and wheat are staple foods consumed by more than 50% of the world's population; however, these staple foods may not be able to provide the daily Fe requirement to these Fe-deficient populations (FAOSTAT, 2012; WHO, 2012). Combining Fe-rich pulses with these staple foods may provide the daily Fe requirement for Fe-deficient populations.

**4.1.2. Phytic acid**

The analysis of variance showed that the genotype, location(year), and the interaction between the genotype and the location(year) were significant ($P<0.05$) for phytic acid present in field peas grown in different counties in 2010 and 2011 **(Table 4.1.)**. The phytic acid concentration among the field peas ranged between 0.3-17.5 mg/g with a mean concentration of 5.1 mg/g **(Table 4.4.)**. The highest mean phytic acid concentration was observed in Cruiser (5.8 mg/g), and the lowest was observed in DS Admiral (4.3 mg/g).

The phytic acid concentration in field pea seeds varied significantly with the location and the year **(Table 4.5.)**. Among the field peas from the 2010 growing season, the highest phytic acid concentration was observed in those grown in Cass County (12.8 mg/g), and the lowest was observed in those grown in Sheridan County (1.4 mg/g). Field peas from the 2011 growing season had lower phytic acid concentration compared to those from the 2010 growing season **(Table 4.5.)**. For example, field peas that were grown in Divide County in 2011 had significantly lower ($P<0.05$) phytic acid concentration than in those grown in Divide County in 2010 (2010-Divide County: 4.9 mg/g and 2011-Divide County: 4.1 mg/g). This similar observation was seen for field peas that were grown in Ward County in 2010 and 2011 (2010-Ward County: 4.4 mg/g; 2011-Ward County: 1.7 mg/g). However, phytic acid concentrations in field peas grown in

Sheridan County did not differ significantly (*P>0.05*) between the two years (2010-Sheridan County: 1.4 mg/g; 2011-Sheridan County: 1.8 mg/g).

Table 4.4. Mean phytic acid concentration of six field pea genotypes grown in different counties across North Dakota in 2010 and 2011.

| Genotype | Mean phytic acid concentration† (mg/g) |
|---|---|
| Agassiz | 5.4 ab |
| CDC Golden | 4.9 c |
| CDC Striker | 4.9 c |
| Cruiser | 5.8 a |
| DS Admiral | 4.3 d |
| Majoret | 5.1 bc |
| Mean | 5.1 |
| SE‡ | 0.1 |

† Means within a column followed by different letters are significantly different at *P<0.05*.
‡ SE=Pooled standard error of the mean calculated from the mean square of the ANOVA for total phytic acid concentration in the six field pea genotypes grown in different counties across North Dakota in 2010 and 2011 (N=180).

Table 4.5. Mean phytic acid concentrations for the field peas grown in different counties across North Dakota in 2010 and 2011.

| Year | Location | Phytic acid† (SE) ‡ (mg/g) |
|---|---|---|
| 2010 | Cass County | 12.8 (0.6) a |
| | Divide County | 4.9 (0.2) cd |
| | McKenzie County | 7.5 (0.3) b |
| | Mountrail County | 5.4 (0.3)c |
| | Sheridan County | 1.4 (0.2) f |
| | Ward County | 4.4 (0.4) de |
| | Williams County | 6.9 (0.3) b |
| 2011 | Divide County | 4.1 (0.1) e |
| | Sheridan County | 1.8 (0.1) f |
| | Ward County | 1.7 (0.1) f |

† Means within a column followed by different letters are significantly different at *P<0.05*.
‡ SE=Pooled standard error of the mean calculated from the mean square of the ANOVA for total phytic acid concentration in the field peas grown in each county across North Dakota in 2010 and 2011 (N=18).

The temperature variation during seed maturation causes increase in phytic acid concentration in lentil seeds (Thavarajah et al., 2010). My study shows that the phytic acid concentration was high in Cass County in 2010, which had higher temperatures compared to other counties during that growing season (North Dakota Agricultural Weather Network, North

Dakota State Climate Office, 2012). The increased temperature during the seed maturation could have led to increased production of phytic acid in field peas grown in Cass County.

Phytic acid is naturally found in cereals, legumes, and nuts, and it is formed during seed maturation (Konietzny and Greiner, 2003). Phytic acid binds to minerals such as Fe, zinc (Zn), calcium (Ca), and magnesium (Mg) and forms insoluble cation-phytate complexes (Morris, 1986). These complexes reduce the mineral bioavailability in humans and animals (Turnlund et al., 1984; Lonnerdal et al., 1989; Davidson et al., 1995; Hurrell et al., 2003; Bohn et al., 2004). However, recent epidemiological and animal studies indicated that phytic acid is a protective agent against colon cancer and renal stone formation in humans (Jenab and Thompson, 1998; Curhan et al., 2004).

Research has been carried out to determine the phytic acid concentration in different pulse crops. Nikolopoulou et al. (2007) indicated that the phytic acid concentration in field peas grown in different locations in Greece ranged between 2.1-11.2 mg/g. It is clear that the present study data is similar to the results reported by Nikolopoulou et al. (2007). The high phytic acid concentration in field peas grown in a specific location can be attributed to the high phosphorous (P) concentration in the soil (Nikolopoulou et al., 2006). Most of the plant P is stored as phytic acid P; therefore, plants grown in high P soil tend to produce high concentration of phytic acid (Nikolopoulou et al., 2006).

Wang and Daun (2004) reported that field peas grown in Canada showed a wide range of phytic acid concentration (3-13 mg/g) with a high mean value of 8.6 mg/g. However, the validity of this data is quite debated, since the phytic acid concentration had been estimated using a colorimetric method, which would have led to 27% over-estimation of phytic acid (Talamond et al., 2000).

Researchers have developed low-phytate mutant lines of field pea (e.g., Line 1-2347-144 and Line 1-150-81) after a series of experiments (Rehman et al., 2012). These lines have displayed the low-phytate phenotype (i.e., high inorganic phosphorous concentration) uniformly in these experiments. Yet, more research needs to be conducted to develop these lines into improved cultivars.

Past research indicated that the phytic acid:Fe molar ratio and the relative Fe absorption in the gut are inversely related (Glahn et al., 2002; Hurrell, 2003). Glahn et al. (2002) showed that the maximum inhibition of Fe uptake by phytic acid occurred when the phytic acid:Fe molar ratio was greater than 10. Another study suggested that the relative Fe absorption could be increased up to 50% when the phytic acid:Fe molar ratio was reduced below l.0 (Hurrell, 2003). Several studies have been carried out to determine the phytic acid:Fe molar ratio in different crops. A study on sorghum (*Sorghum bicolor* L.) showed that the phytic acid:Fe molar ratio ranged from 6.7 to 8.7 (Afify et al., 2011). These ratios are quite similar to what I observed in my study. The phytic acid/total Fe molar ratios for each genotype were as follows **(Table 4.6.)**: Cruiser=9.3, Agassiz=9.1, Majoret=8.5, CDC Golden=8.1, CDC Striker=8.0, and DS Admiral=7.9. All these genotypes showed low phytic acid:Fe molar ratio indicating moderate to high Fe bioavailability (Hurrell, 2003).

Table 4.6. Iron and phytic acid concentrations, and the phytic acid:Fe molar ratio in the six field pea genotypes grown across different counties in North Dakota in 2010 and 2011.

| Genotype | Total Fe (mg/kg) | Phytic acid (mg/g) | Phytic acid:Fe molar ratio[†] |
|---|---|---|---|
| Agassiz | 50 | 5.4 | 9.1 |
| CDC Golden | 51 | 4.9 | 8.1 |
| CDC Striker | 52 | 4.9 | 8.0 |
| Cruiser | 53 | 5.8 | 9.3 |
| DS Admiral | 46 | 4.3 | 7.9 |
| Majoret | 51 | 5.1 | 8.5 |

[†] The phytic acid:Fe molar ratio was determined using the formula Phytic acid (μg $g^{-1}$/660.04 g $mol^{-1}$)/Fe (μg $g^{-1}$/55.845g $mol^{-1}$).

### 4.1.3. Carotenoids

The analysis of variance showed that the genotype, location(year), and the interaction between genotype and location(year) were significant at $P<0.05$ for all three carotenoids **(Table 4.1.)**. Xanthophyll, canthaxanthin, and beta-carotene concentrations in field peas ranged between 4.0-26.4 mg/100 g, 3.1-259.8 mg/ 100 g, and 14.9-1838.2 µg/100 g, respectively. The mean concentrations for xanthophyll, canthaxanthin, and beta-carotene for all genotypes were 17.3 mg/100 g, 86.8 mg/100g, and 516.8 µg/100 g, respectively **(Table 4.7.)**. The highest mean xanthophyll and canthaxanthin concentrations were observed in CDC Golden (xanthophyll: 18.8 mg/100 g; canthaxanthin: 119.4 mg/100 g), and the highest mean beta-carotene concentration was observed in Cruiser (1140.0 µg/100 g). DS Admiral had higher xanthophyll (18.0 mg/100 g) and canthaxanthin (109.9 mg/100 g) concentrations compared to the overall mean xanthophyll and canthaxanthin concentrations of all field pea genotypes. However, DS Admiral showed lower beta-carotene concentration (141.4 µg/100 g) compared to Cruiser, CDC Striker, and Majoret (Cruiser: 1138 µg/100 g, CDC Striker: 917.5 µg/100 g, and Majoret: 755.6 µg/100 g). Agassiz showed lower concentrations for all three carotenoids (xanthophyll: 15.3 mg/100 g; canthaxanthin: 67.0 mg/100 g; beta-carotene: 22.3 µg/100 g) compared to all other genotypes. Overall, it was observed that green field pea genotypes (Cruiser, CDC Striker, and Majoret) had high beta-carotene concentrations (Cruiser: 1138.0 µg/100 g, CDC Striker: 917.5 µg/100 g, and Majoret: 755.6 µg/100 g), whilst yellow field pea genotypes (DS Admiral, CDC Golden, and Agassiz) had low beta-carotene concentrations (DS Admiral: 141.4 µg/100 g, CDC Golden: 125.8 µg/100 g, and Agassiz: 22.3 µg/100 g).

Xanthophyll and canthaxanthin concentrations in field peas grown in 2010 were higher than those in field peas grown in 2011 **(Table 4.8.)**. Highest concentrations of canthaxanthin and

Table 4.7. Mean xanthophyll, canthaxanthin, and beta-carotene concentrations in the six field pea genotypes grown in different counties across North Dakota in 2010 and 2011.

| Genotype | Xanthophyll† (mg/100 g) | Canthaxanthin† (mg/100 g) | Beta-carotene† (µg/100 g) |
|---|---|---|---|
| Agassiz | 15.3 d | 67.0 c | 22.3 e |
| CDC Golden | 18.8 a | 119.4 a | 125.8 d |
| CDC Striker | 16.9 bc | 72.1 c | 917.5 b |
| Cruiser | 18.7 a | 86.4 b | 1138.0 a |
| DS Admiral | 18.0 ab | 109.9 a | 141.4 d |
| Majoret | 15.8 cd | 66.1 c | 755.6 c |
| Mean | 17.3 | 86.8 | 516.8 |
| SE‡ | 0.2 | 1.4 | 7.3 |

† Means within a column followed by different letters are significantly different at $P<0.05$. ‡ SE=Pooled standard error of the mean calculated from the mean square of the ANOVA for total xanthophyll, canthaxanthin, and beta-carotene concentrations in the six field pea genotypes grown in different Counties across North Dakota in 2010 and 2011 (N=180).



Table 4.8. Mean xanthophyll, canthaxanthin, and beta-carotene concentrations in the field peas grown in different counties across North Dakota in 2010 and 2011.

| Year | Location | Mean concentration† (SE) ‡ | | |
|---|---|---|---|---|
| | | Xanthophyll (mg/100 g) | Canthaxanthin (mg/100 g) | Beta-carotene (µg/100 g) |
| 2010 | Cass County | 13.2 (0.9) d | 63.9 (8.1) d | 333.1 (65.7) d |
| | Divide County | 21.5 (0.9) a | 109.8 (6.6) c | 605.5 (142.3) b |
| | McKenzie County | 18.5 (1.1) c | 124.4 (13.8) b | 647.3 (132.5) ab |
| | Mountrail County | 20.9 (0.8) ab | 116.2 (6.8) bc | 657.0 (143.6) ab |
| | Sheridan County | 19.1 (0.9) bc | 125.9 (10.3) b | 498.1 (117.3) c |
| | Ward County | 20.6 (0.8) ab | 117.6 (10.6) bc | 500.0 (106.3) c |
| | Williams County | 19.4 (0.9) bc | 155.2 (10.5) a | 673.3 (118.3) a |
| 2011 | Divide County | 10.4 (0.8) e | 6.8 (0.5) f | 507.7 (106.9) c |
| | Sheridan County | 14.9 (0.5) d | 24.3 (3.4) e | 133.6 (48.9) e |
| | Ward County | 14.1 (0.4) d | 24.0 (4.9) e | 609.6 (135.6) ab |

† Means within a column followed by different letters are significantly different at $P<0.05$. ‡ SE=Pooled standard error of the mean calculated from the mean square of the ANOVA for total carotenoids' concentrations in the field peas grown in each county across North Dakota in 2010 and 2011 (N=18).

beta-carotene were observed in field peas grown in Williams County in 2010 (155.2 mg/100 g and 673.3 µg/100 µg, respectively), and the highest xanthophyll concentration was observed in field peas grown in Divide County in 2010 (21.5 mg/100 g). Cass County, from the 2010 growing season, showed the least concentrations of carotenoids (xanthophyll: 13.2 mg/100 g; canthaxanthin: 63.9 mg/100 g; beta-carotene: 333.1 µg/100 g).

Carotenoids are a group of pigmented compounds that are naturally found in colored food crops. Carotenoids are categorized into two major groups based on the presence or absence of oxygen in their structures: 1) Xanthophyll (e.g., zeaxanthin, lutein, spirilloxanthin, echinenone, antheraxanthin) and 2) Carotenes (e.g., beta-carotene and lycopene) (Goodwin, 1980). Carotenoids, especially, xanthophyll and beta-carotene have been identified as compounds with high antioxidant capacity (Martin et al., 1999).

Carotenoids promote the Fe bioavailability in the human gut (Welch, 2002). Gracia-Casal et al. (2000) showed that beta-carotene directly affected the Fe uptake using Caco-2 cell as a model to test the Fe bioavailability in the human gut. It has been shown that the Fe uptake by Caco-2 cells increased by 100% when beta-carotene was incorporated at concentrations of 3 and 6 µmol/L. The high Fe uptake could be a result of the chelating effect of beta-carotene that increased the Fe solubility and availability (Gracia-Casal et al., 2000). In addition, Gracia-Casal et al., (1998) indicated that beta-carotene prevented the inhibitory effects of polyphenols on Fe absorption (Gracia-Casal et al., 1998). Also, Layrisse et al., (1997) showed that vitamin A and beta-carotene bound with Fe during food digestion and formed chelating agents that prevented inhibitory effects of polyphenols on non-heme Fe absorption.

Several studies have been carried out to determine the carotenoids' concentration in legume crops (Chavan et al., 1999; Holden et al., 1999; Kandlakunta et al., 2008). Field peas

grown in Saskatchewan, Canada showed a mean beta-carotene concentration of 120 µg/100g (Chavan et al., 1999). Another study that was conducted on green peas grown in USA showed that the mean beta-carotene concentration in those green peas was 485 µg/100g (Holden et al., 1999). Past studies showed that the carotenoids' concentration in a crop varied with location. Common beans grown in USA had very low beta-carotene concentration (26 µg/100g); however, those grown in India had high beta-carotene concentration (554 µg/100g) (Holden et al., 1999; Kandlakunta et al., 2008). The beta-carotene concentrations observed in field peas in the current study are similar to what was observed by Kandlakunta et al. (2008).

Research conducted in India suggested that the major legumes grown in India contained low xanthophyll concentrations (Mamatha et al., 2011). Lutein (a major component in xanthophyll group) concentrations in cowpea (*Vigna unguiculata* L.), whole chickpea, green gram (*Vigna radiaata* L.), split red gram (*Cajanus cajan* L.), and split lentil were 0.09, 0.20, 0.09, 0.19, and 0.10 mg/100 g, respectively. Zeaxanthin (another major component in xanthophyll group) concentration was very low (less than 0.01 mg/100g) in all the crops. Compared to the xanthophyll concentration in the above mentioned legumes, the xanthophyll concentration in field peas grown in North Dakota was higher.

Previous studies have shown that the carotenoids' concentration in field peas depended on environmental conditions (Iturbe-Ormaetxe et al., 1998). The biosynthesis of carotenoids is, especially, dependent on temperature and moisture (Koskitalo and Ormrod, 1972; Iturbe-Ormaetxe et al., 1998). Koskitalo and Ormrod (1972) showed that temperatures between 10-30 °C are favorable for the production of carotenoids. Iturbe-Ormaetxe et al. (1998) showed that soil-water deficit inhibited the production of carotenoids. In this study the regular irrigation was withheld until the leaf water potential reduced up to -1.30 MPa and -1.93 MPA. The carotenoids

production did not reduce significantly when the leaf water potential was reduced up to -1.30 MPa. However, when the leaf water potential was reduced up to -1.93 MPa, the carotenoids production reduced by 21-38%. My data showed that field peas grown in Cass County had the lowest carotenoids' concentrations in 2010. Cass County had a drier growing season in 2010; thus the limited water availability for field pea plants might have reduced the production of carotenoids.

Our study showed that the beta-carotene concentration was higher in green field peas when compared with the yellow field peas. The beta-carotene concentration in green field peas ranged from 755.6-1140.0 µg/100 g, whilst in yellow peas it varied from 22.3-141.4 µg/100 g. The same observation (i.e. the significant variation of the beta-carotene concentration between green peas and yellow peas) was observed by Holasova et al. (2009) where the beta-carotene concentration in green peas (100-200 µg/100 g) was 10-fold greater than those observed in yellow peas.

### 4.1.4. Fructooligosaccharides

The two fructooligosaccharides analyzed in this study were kestose and nystose. The statistical analysis showed that field peas had higher kestose concentration compared to the nystose concentration **(Table 4.9.)**. The mean kestose concentration was 1697 mg/100 g **(Table 4.10.)**. The highest kestose concentration was observed in DS Admiral (2160 mg/100 g), and the lowest kestose concentration was observed in Majoret (1162 mg/100 g). The studied field pea genotypes had low nystose concentrations with a mean value of 40 mg/100 g. Cruiser (107 mg/100 g) had the highest nystose concentration followed by CDC Golden (63 mg/100 g), CDC Striker (30 mg/100 g), and Agassiz (30 mg/100 g). The lowest nystose concentrations were observed in Majoret (4 mg/100 g) and DS Admiral (3 mg/100 g).

**Table 4.11.** shows how the concentration of fructooligosaccharides varied in different locations. Amongst all the counties, the kestose concentration was the highest in field peas grown in Divide County (3255 mg/100 g). The kestose concentration was lower in field peas grown in Mountrail and Ward counties (661 mg/100 g and 425 mg/100 g) compared to other locations. Nystose concentration was highest in field peas grown in Williams County (142 mg/100 g), and lowest in those grown in McKenzie County (3 mg/100 g).

Table 4.9. Analysis of variance for kestose and nystose concentrations in six field pea genotypes grown in seven counties across North Dakota in 2010.

| Source | df | Mean Square | |
|---|---|---|---|
| | | Kestose | Nystose |
| Genotype | 5 | NS | ** |
| Location | 6 | * | * |
| Replicate (Location) | 14 | * | NS |
| Location × Genotype | 30 | * | * |

**Mean square was significantly different at *P<0.1*. *Mean square was significantly different at *P<0.05*. (N=126).

Fructooligosaccharides are prebiotics that selectively stimulate the growth and the activity of specific species of bacteria (e.g., *Bifidobacteria* and *Lactobacilli*) in the human intestine (Cummings et al., 2001). Prebiotics and their products of microbial fermentation have been suggested to increase the Fe absorption by the human intestine (Delzenne et al., 1995; Ohta et al., 1995). In an extensive study of the fructooligosaccharides in foods and feeds Campbell et al. (1997) indicated that field peas contained 20 mg/100 g of kestose and nystose. These fructooligosaccharides were not detected in green beans (*Phaseolus vulgaris* L.) and soybeans (*Glycine max* L.); however, only nystose was present in kidney beans (*Phaseolus vulgaris* L.) at a mean concentration of 10 mg/100 g. Lentils grown in North Dakota contain high concentrations of fructooligosaccharides, especially, kestose (Thavarajah and Thavarajah, 2011). The kestose concentration in the genotypes evaluated ranged between 1320-2088 mg/100 g with a mean concentration of 1580 mg/100 g. This observation shows that the kestose concentration

observed in North Dakota-grown field peas are quite similar to that in North Dakota-grown lentils.

Table 4.10. Mean kestose and nystose concentrations in the six field pea genotypes grown in seven counties across North Dakota in 2010.

| Genotype | Kestose† (mg/100 g) | Nystose† (mg/100 g) |
|---|---|---|
| Agassiz | 1954 ab | 30 c |
| CDC Golden | 1461 c | 63 b |
| CDC Striker | 1564 c | 30 c |
| Cruiser | 1879 b | 107 a |
| DS Admiral | 2160 a | 3 d |
| Majoret | 1162 d | 4 d |
| Mean | 1697 | 40 |
| SE‡ | 36.9 | 2.2 |

† Means within a column followed by different letters are significantly different at *P<0.05*.
‡ SE=Pooled standard error of the mean calculated from the mean square of the ANOVA for kestose and nystose concentrations in the six field pea genotypes grown in seven counties across North Dakota in 2010 (N=126).

Table 4.11. Mean kestose and nystose concentrations in the six field pea genotypes grown in seven counties across North Dakota in year 2010.

| Location | Mean concentration† (mg/100 g) (SE) ‡ | |
|---|---|---|
| | Kestose | Nystose |
| Cass County | 1777 (227) c | 71 (26) b |
| Divide County | 3255 (240) a | ND |
| McKenzie County | 1679 (179) cd | 3 (1) d |
| Mountrail County | 551 (47) e | 11 (1) d |
| Sheridan County | 1490 (229) d | 51 (28) c |
| Ward County | 425 (101) e | ND |
| Williams County | 2701 (253) b | 142 (25) a |

† Means within a column followed by different letters are significantly different at *P<0.05*.
‡ SE=Pooled standard error of the mean calculated from the mean square of the ANOVA for total kestose and nystose concentrations in the field peas grown in each county across North Dakota in 2010 (N=18). ND=Not detected.

### 4.1.5. Phenolics

The analysis of variance showed that the genotype, location(year), and the interaction between location(year) and genotype were significant (*P<0.*1) for caffeic acid, catechin, and quercetin **(Table 4.12.)**. For gallic acid, the genotype and location(year) were significant (*P<0.05*), and for ferulic acid, location(year) and the interaction between genotype and

location(year) were significant (*P<0.05*). Quercetin and ferulic acid concentrations were high in field peas, compared to other phenolic compounds. The highest concentration of quercetin was observed in DS Admiral (62.6 mg/100 g), followed by CDC Golden (55.7 mg/100 g), Majoret (51.9 mg/100 g), Agassiz (41.3 mg/100 g), and CDC Striker (40.7 mg/100 g) (**Table 4.13.)**. The lowest concentration of quercetin was observed in Cruiser (29.2 mg/100 g). The highest concentration of ferulic acid was observed in CDC Golden (68.8 mg/100 g) and the lowest was observed in Cruiser (36.3 mg/100 g).

Caffeic acid, another strong antioxidant (Brown et al., 1989), is also present in field peas. The mean caffeic acid concentration in field pea was 2.4 mg/100 g. Higher concentrations of caffeic acid were observed in Majoret, CDC Striker, CDC Golden, and Cruiser (2.7, 2.6, 2.6, and 2.6 mg/100 g) compared to DS Admiral and Agassiz (2.4 mg/100 g and 1.4 mg/100 g). Gallic acid and catechin were also present in field peas in smaller concentrations. The mean concentrations of gallic acid and catechin in field peas were 3.4 and 1.7 mg/100 g, respectively. All phenolic compounds varied significantly between locations in both years **(Table 4.14.)**. The quercetin concentration was higher in all three counties in 2011 compared to all the counties in 2010.

Phenolics are a group of naturally occurring phytochemicals in plants (Larson 1997; Cadenas and Packer, 2002) that are considered as antioxidants. It is known that some phenolics may be beneficial to Fe absorption in the human body (Brown et al., 1990; Dueñas et al., 2006). For example, quercetin, ferulic acid, and caffeic acid are strong antioxidants (Brown et al., 1989; Dueñas et al., 2006), which may increase the Fe bioavailability. On the other hand some phenolic compounds (e.g., gallic acid and catechin) act as inhibitors and decrease the Fe absorption in humans (Brune et al., 1989; Khokhar and Apenten, 2003).

Table 4.12. Analysis of variance for phenolic compounds' concentrations in six field pea genotypes grown in different counties across North Dakota in 2010 and 2011.

| Source | df | Mean Square | | | | |
|---|---|---|---|---|---|---|
| | | Gallic acid | Caffeic acid | Catechin | Quercetin | Ferulic acid |
| Genotype | 5 | * | * | * | * | NS |
| Location(Year) | 9 | * | * | * | ** | * |
| Replicate (Year, Location) | 20 | * | NS | NS | NS | NS |
| Location(Year) × Genotype | 45 | NS | * | * | * | * |

**Mean square was significantly different at *P<0.1*. *Mean square was significantly different at *P<0.05*. NS=Non-significant. Field pea samples from 2010 growing season were collected from seven counties, and field pea samples from 2011 growing season were collected from three counties (N=180).



Table 4.13. Mean concentration of phenolic acids in the six field pea genotypes grown in different counties across North Dakota in 2010 and 2011.

| Genotype | Mean concentration of phenolic acids† (mg/100 g) | | | | |
|---|---|---|---|---|---|
| | Gallic acid | Caffeic acid | Catechin | Ferulic acid | Quercetin |
| Agassiz | 3.5 a | 1.4 c | 1.6 b | 54.7 c | 41.3 c |
| CDC Golden | 3.5 a | 2.6 a | 1.1 c | 68.8 a | 55.7 b |
| CDC Striker | 3.2 b | 2.6 a | 2.6 a | 58.9 b | 40.7 c |
| Cruiser | 3.6 a | 2.6 ab | 1.6 b | 36.3 d | 29.2 d |
| DS Admiral | 3.3 b | 2.4 b | 0.8 d | 54.4 c | 62.6 a |
| Majoret | 3.3 b | 2.7 a | 2.4 a | 52.5 c | 51.9 b |
| Mean | 3.4 | 2.4 | 1. 7 | 54.3 | 46.9 |
| ‡ SE | 0.1 | 0.1 | 0.1 | 0.6 | 0.6 |

† Means within a column followed by different letters are significantly different at *P<0.05*. ‡ SE=Pooled standard error of the mean calculated from the mean square of the ANOVA for phenolic acid concentrations in the six field pea genotypes grown in different counties across North Dakota in 2010 and 2011 (N=180).

Table 4.14. Mean concentrations of phenolic acids in the field peas grown in different counties across North Dakota in 2010 and 2011.

| Year | Location | Mean concentration of phenolic acids[†] (mg/100 g) (SE)[‡] | | | | |
|---|---|---|---|---|---|---|
| | | Gallic acid | Caffeic acid | Catechin | Ferulic acid | Quercetin |
| 2010 | Cass County | 3.6 (0.1) bc | 2.4 (0.3) c | 1.0 (0.1) d | ND | 31.7 (5.7) c |
| | Divide County | 3.4 (0.1) d | 2.0 (0.2) de | 2.6 (0.3) c | 73.7 (4.2) c | 57.8 (4.2) a |
| | McKenzie County | 3.7 (0.1) ab | 2.6 (0.1) bc | 3.1 (0.5) b | 93.5 (3.7) b | 53.2 (11.8) a |
| | Mountrail County | 3.9 (0.1) a | 3.5 (0.1) a | 4.6 (0.5) a | 112.9 (4.4) a | 20.5 (4.1) d |
| | Sheridan County | 3.5 (0.1) cd | 2.5 (0.2) bc | 0.7 (0) e | 28.1 (9.6) g | 43.1 (3.4) b |
| | Ward County | 3.5 (0.1) cd | 2.0 (0.1) d | 2.6 (0.3) c | 70.6 (9.3) cd | 42.4 (5.0) b |
| | Williams County | 3.6 (0.1) bc | 2.7 (0.1) b | 0.4 (0) e | ND | 53.4 (4.4) a |
| 2011 | Divide County | 3.3 (0) d | 1.8 (0.1) de | 0.6 (0.1) e | 53.6 (6.4) e | 53.6 (4.4) a |
| | Sheridan County | 3.0 (0.1) e | 2.6 (0.3) bc | 0.5 (0.1) e | 66.7 (7.3) d | 55.1 (3.8) a |
| | Ward County | 2.4 (0.1) f | 1.7 (0.2) e | 0.5 (0.1) e | 43.7 (2.5) f | 58.1 (5.6) a |

[†] Means within a column followed by different letters are significantly different at $P<0.05$. [‡] SE=Standard error of the mean calculated from the mean square of the ANOVA for each phenolic acid concentration in field peas grown at each county in North Dakota in 2010 and 2011 (N=18). ND=Not detected.

### 4.2. Study 2 - Greenhouse study

Combined statistical analysis showed that genotypes were significantly different for seed Fe concentration at *P<0.1,* and Fe fertilizer treatments were significantly different for seed Fe concentration at *P<0.05* **(Table 4.15.)**. The highest mean Fe concentration was observed in field pea cultivars that received the additional Fe treatment (31 mg/kg). Mean Fe concentrations of field pea genotypes that received the regular and the control Fe treatments were 28 mg/kg and 27 mg/kg, respectively **(Table 4.16.)**.

Within control and additional Fe treatments the genotypes were significant; however, within regular Fe treatment the genotypes were not significant for the Fe concentration **(Table 4.16.)**. For control Fe treatment, CDC Golden showed the highest Fe concentration (31 mg/kg) followed by Agassiz (29 mg/kg), DS Admiral (27 mg/kg), Majoret (26 mg/kg), Cruiser (25 mg/kg), and CDC Striker (23 mg/kg). For regular Fe fertilizer treatment, DS Admiral showed the highest Fe concentration (30 mg/kg), followed by CDC Golden (29 mg/kg), CDC Striker (29 mg/kg), Majoret (28 mg/kg), Agassiz (27 mg/kg), and Cruiser (27 mg/kg). For additional Fe fertilizer treatment, CDC Golden showed the highest Fe concentration, (34 mg/kg) followed by Cruiser (33 mg/kg), DS Admiral (31 mg/kg), Agassiz (29 mg/kg), CDC Striker (29 mg/kg), and Majoret (28 mg/kg). Among all the three treatments, CDC Golden and DS Admiral showed higher Fe uptake compared to all other tested genotypes.

Research based on the Fe uptake of legumes under controlled conditions is limited. The only studies that have been conducted have focused on how different plant traits (e.g., biomass, chlorosis symptoms, root and shoot Fe concentration) are affected by different Fe treatments. Longnecker and Welch (1990) showed that soybean plants treated with Fe (+Fe) produced more biomass than the control. The Fe concentrations in shoots and roots from each treatment were also recorded at different growth stages of the plant. Initially, the control plants had higher shoot

Fe concentration than the +Fe treated plants. However, as the Fe treatment continued, the shoot Fe concentration in the control plants declined rapidly. Also, it was observed that the root Fe concentration in the +Fe plants and -Fe plants did not differ significantly (*P>0.05*). Moreover, this study indicated that in soybeans the Fe absorption rate in -Fe plants were higher than that in +Fe plants.

Table 4.15. Summary of combined analysis of variance for seed Fe concentration of six field pea genotypes grown under greenhouse conditions with three Fe fertilizer treatments.

| Source | df | Mean square[†] |
|---|---|---|
| Run | 1 | * |
| Treatment | 2 | * |
| Genotype | 5 | ** |
| Replicate | 2 | NS |
| Genotype × Treatment | 10 | NS |

[†] Mean square was significantly different at **, *P<0.1*, and *, *P<0.05*. (N=108). NS=Non significant.

Table 4.16. Comparison of the Fe concentrations in six field pea genotypes grown under greenhouse conditions with three Fe fertilizer treatments.

| Genotype | Total Fe[†] (mg/kg) | | |
|---|---|---|---|
| | Control (0 ppm) | Regular Fe (1.1 ppm) | Additional Fe (2.8 ppm) |
| Agassiz | 29 ab | 27 a | 29 b |
| CDC Golden | 31 a | 29 a | 34 a |
| CDC Striker | 23 c | 29 a | 29 b |
| Cruiser | 25 c | 27 a | 33 a |
| DS Admiral | 27 abc | 30 a | 31 ab |
| Majoret | 26 bc | 28 a | 28 b |
| Mean | 27 | 28 | 31 |
| SE[‡] | 1 | 1 | 1 |

[†] Means within a column followed by different letters are significantly different at *P<0.05*.
[‡] SE, pooled standard error of the mean calculated from the mean square of ANOVA for each treatment (n=36).

A separate study showed that the accumulation of Fe in mature soybean seeds was not affected by the varying levels of Fe fertilization (Welch and Campen, 1975). In that study two treatments of Fe were applied to soybean plants at the rates of 0.4 ppm and 1.0 ppm. The seed Fe concentration in plants treated with 0.4 ppm and 1.0 ppm of Fe were 58 and 51 mg/kg, respectively, and these seed Fe concentrations were not significantly different from each other.

This observation agrees with what we observed in our study. We observed that the effect of Fe fertilizer treatment rate was not significant in some genotypes (e.g., Agassiz, CDC Golden, DS Admiral, and Majoret).

A study on lentil and chickpea showed that the biomass of lentil was significantly affected by adding Fe fertilizer (30 µmol of Fe) (Mahmoudi et al., 2005). However, the biomass of chickpea was not significantly affected by Fe addition. Also, they found that lentil was more sensitive to Fe fertilization when compared to chickpea. Another study showed that chickpea treated with different Fe treatments (0 ppm and 20 ppm) had significant variation of chlorophyll symptoms between genotypes (Mahmoudi et al., 2007). Also, it was shown that the total Fe concentration was lower in the plants that did not receive Fe fertilizer when compared with those that received Fe fertilizer. Similarly, in our study too, we observed that that the seed Fe concentration in field pea varied with different levels of Fe treatment.

# 5. CONCLUSION & FUTURE DIRECTIONS

The field study showed that there is genetic potential to increase the Fe concentration in field peas. All the genotypes tested had phytic acid:Fe molar ratios that were lower than 10, indicating that field peas are rich in bioavailable Fe. Compared to other genotypes, DS Admiral contained lower concentrations of phytic acid and higher concentrations of carotenoids, fructooligosaccharides, and phenolics such as quercetin and ferulic acid. Because of the higher concentrations of promoters of Fe, DS Admiral may have high bioavailable Fe. Similarly, CDC Golden showed higher concentrations of Fe promoter compounds including carotenoids, kestose, and quercetin. Thus, DS Admiral and CDC Golden are better candidates for Fe biofortification research. Therefore, selection of appropriate genotypes in conjunction with the environment will assist further Fe biofortification studies on pulses.

The greenhouse study showed that Fe uptake varied significantly among the six genotypes of field pea. CDC Golden showed higher Fe uptake compared to the other tested genotypes, especially when treated with the control and the additional Fe fertilizer treatments. Overall, DS Admiral and CDC Golden have more bioavailable Fe than the other genotypes.

This study was a baseline study to determine the potential for Fe biofortification in field peas. However, future studies can be conducted to determine how much Fe is going to be bioavailable for humans. Bioavailability studies using Caco-2 cell cultures, animals, or humans can be conducted for genotypes such as DS Admiral and CDC Golden, genotypes that have high Fe uptake and contain more bioavailable Fe.

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