GENETIC AND ENVIRONMENTAL VARIATION ON MINERAL NUTRIENT CONCENTRATION IN PEA AND LENTIL SEED

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Genetic and environmental variation on mineral nutrient concentration in pea and lentil seed

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ABSTRACT

Current food supply is expected to be insufficient to support the growing population both in quantity and nutritional quality; therefore, the need to breed for higher yield and greater nutritional quality is urgent. Twenty-five dry pea genotypes, 25 Turkish red lentil and 23 green lentil genotypes were tested across different locations of North Dakota to quantify the nutrient concentration and to characterize the genotype and the environmental factors affecting nutrient concentration. Significant genotypic, environmental and genotype-by-environment interaction was present for Ca, Cu, Fe, K, Mg, Mn, P and Zn analyzed in dry pea and lentil. A range of correlations among and/or between mineral elements and seed yield parameters was observed. This suggests that breeding for quantitative trait like mineral elements is possible through conventional breeding however; multi-location testing is very crucial for analyzing the genetic and environmental effect on mineral concentration in the seeds.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

More than one-half of the global population is suffering from mineral nutrient deficiency due to consumption of food with lower levels of essential mineral nutrients (White and Broadley, 2009). More than 800 million people in food insecure regions are affected by calorie and protein deficiencies whereas more than 2 billion people worldwide are affected by micronutrient deficiency, and this number is ever increasing especially in developing countries (Welch and Graham, 2002; Yip and Scanlon, 1994; Welch and Graham, 2002). Micronutrient malnutrition is known as "hidden hunger" and leads to a lower immune response in the body and makes individuals vulnerable to diseases and serious health issues. The wide spread occurrence of micronutrient malnutrition is a concern among under privileged people in developed and developing countries (Buyckx, 1993; Ramalingaswami, 1995). Mathers et al. (2009) estimated that a total of 3.9 million deaths among children and 144 million disabled children 5 years of age and younger are due to micronutrient deficiency, underweight and suboptimal breastfeeding. In addition, 41% of pregnant woman and 27% of pre-school children have anemia due to iron (Fe) deficiency worldwide (Mathers et al., 2009). Nearly two-thirds of all childhood deaths are associated with nutritional deficiencies and micronutrient deficiency is one of the main reasons (Caballero, 2002). Mineral element deficiency not only increases the mortality and morbidity rate in children but also reduce the cognitive and learning abilities. In adults mineral deficiencies reduce productivity in the work place and overwhelming immune deficiency can impact the nation's economy.

Zinc (Zn), iron and vitamin A deficiency are the most common nutritional disorders in most developing countries. More than 60% of the world's population is deficient in Fe and more than 30% is Zn deficient (Kennedy et al., 2003). Summary of mineral nutrient deficiency and the associated major health disorder is presented in Table 1.1.

Table 1.1. Summary of mineral nutrient deficiencies and the associated major health disorder.

Micronutrients	Deficiency Prevalence	Major Deficiency Disorder
Vitamin A	254 million pre-school children	Night blindness, xerophthalmia, increased risk of mortality in children and pregnant women.
Iron	There are an estimated 2 billion cases of anemia worldwide. Iron deficiency is estimated to be responsible for around 50% of all anemia cases	Reduced cognitive performance, lower work performance and endurance, impaired iodine and vitamin A metabolism, increased risk of maternal mortality and child mortality
Zinc	Insufficient data but prevalence of deficiency is likely to be high in developing countries especially in Africa, South-East Asia and Western Pacific.	Marginal deficiency may result poor pregnancy outcome, impaired growth (stunting), genetic disorders, decreased resistance to infectious diseases. Severe deficiency results in dermatitis, retarded growth, diarrhea, mental disturbance, delayed sexual maturation and/or recurrent infections
Calcium	Insufficient data but low intake very common	Decreased bone mineralization increased risk of osteoporosis in adults Increased risk of rickets in children

Source: Allen et al., 2006; Tulchinsky, 2010.

To control this global health issue in deficiency prone areas, government and global health organizations started food fortification by adding the minerals directly to the food and/or providing nutrient supplementation (Yip, 1997). Food fortification was found effective for comparatively small groups of people but when it comes to addressing global deficiency, it failed to have a significant impact (Welch and Graham, 1999; 2004). Adequate financial support for large populations, timely and consistent availability of fortified or supplemented food to the targeted population, affordable manpower and resources to carry out these operations are some of the major constraints limiting food fortification and supplementation measures.

Plant breeding is the art and science of manipulating plants for human benefit through selection and hybridization. Hybridization is an important evolutionary phenomenon in plants, animals, and fungi which can result in new species of the same ploidy level or different ploidy

levels, transfer adaptive traits between species, and, in general, "release" of genetic constraints on phenotypic evolution and generating genetic novelty within populations (Whitney et al., 2010). As plant breeding has proved to be an important agricultural measure during the green revolution to reduce starvation and famine, it can again be a used as a powerful measure to fight mineral deficiency worldwide through breeding for nutrient dense crops, also known as bio-fortification (Welch and Graham, 2002). Biofortification is a new public health approach to increase the amount of different essential mineral nutrients in seed, especially Vitamin A, iron and zinc in staple crops through traditional plant breeding or modern biotechnology to cope with nutritional disorders especially in economically challenged countries (Nestel et al., 2006).

North Dakota is one of the leading pulse crop producing states in the U.S. and limited research has been done to assess mineral element (Ca, Cu, Fe, K, Mg. Mn, P, Zn) accumulation in available germplasm (including advanced breeding lines and commercial varieties). Therefore, the objectives of this research were to 1) quantify the mineral nutrient (Ca, Cu, Fe, K, Mg, Mn, P, and Zn) concentration in dry pea and lentil seed and 2) characterize the genotype x environment interaction affecting the mineral nutrient concentration in the seed. This information is necessary to develop mineral nutrient-dense dry pea varieties which can play an important role in fighting nutrient deficiency.

1.2. Literature Review

1.2.1. Crop Importance and Present Scenario

Pea and lentil are nutrient-dense grain legumes grown in temperate regions usually in rotation with cereal crops (Duke 1981; Materne and Siddique, 2009; Ali et al., 2009). These pulse crops, along with chickpea are the major staple food legume consumed with cereals to provide micronutrients and protein in developing countries (Erskine et al., 2009) and has been a part of the daily diet for vegetarians' worldwide (Muehlbauer and McPhee, 1997). Pulses are

important sources of protein, complex carbohydrates, fiber, vitamins and minerals and have high nutritional value to fulfill the needs of health conscious consumers (Reichert and MacKenzie, 1982; Wang and Daun, 2004). Dry pea production in the United States (U.S.) was 493,150 MT in 2012 (FAOSTAT, 2012) whereas in 2013 the U.S. ranked 5th in world dry pea production with the production of 708,512 MT (FAOSTAT, 2013). With the production of 240,495 MT in 2012 (FAOSTAT, 2012) and 227,658 MT in 2013 (FAOSTAT, 2013) U.S. lentil production ranks 5th worldwide lentil production. North Dakota was the second largest pulse crop producer in 2012 and 2013 with in the U.S. In 2012, North Dakota ranked number one in production of dry pea (203,436 MT) in the U.S. and Montana (199,353 MT) and Washington (58,967 MT) ranked second and third, respectively. In 2013, Montana produced 322,504 MT of dry pea and ranked ahead of North Dakota (260,362 MT) and Washington (82,418 MT). In the case of lentil production, North Dakota ranked second after Montana in 2012 and also in 2013. In 2012 North Dakota produced 87,453 MT of lentil whereas Montana produced 97,295 MT and Washington produced 38,328 MT and became the third largest lentil producing state in the U.S. Although U.S. lentil production dropped in 2013 compared to 2012, North Dakota still ranked second for lentil production with production of 80,013 MT. Montana was the leading lentil producer with 87,770 MT and Washington ranked third with 42,184 MT (Crop production summary 2013, USDA). The Northern Great Plains and the Pacific Northwest are the main pulse producing regions in the United States. Although, Montana is the largest pea and lentil producing state, North Dakota produces the greatest yield per acre of pea and lentil (USDA-NASS, 2013).

1.2.2. Environment and Soil Influence on Nutrient Concentration in Seed

Soil and environmental conditions affect micronutrient accumulation in crops (Grusak, 2009). Research in chickpea by Frimpong et al. (2009) suggested that to improve seed quality in individual seeds a wide range of environmental testing was necessary because selection

strategies were influenced by genotype and genotype × environment interaction. Environmental conditions within each growing region, including variation in climate, soils, and their interaction, directly or indirectly affect lentil productivity and quality (Duke, 1981). Nezamuddin (1970) reported that there was genetic variation in root growth patterns which influenced adaptation to different soil types within the Indian Subcontinent. In his research he found that lentil root ecology differs with soil conditions to take up mineral nutrients required for the plant. He further reported that in heavy black soils of central India where large cracks and rapid loss of moisture from the top soil layer is prominent, lentil plants developed a deep root system to cope with moisture deficiency and the branches were thin and widely spaced with comparatively larger seeds.

Thavarajah et al. (2010) reported that the micronutrient content in lentil depends upon the geographical location of production because of the influence of soil factors, temperature, photoperiod and other growing conditions. Erskine (1997) reported that there are differences in temperature requirements during the reproductive period for the small and large seed market classes of lentil. According to him large seeded lentils were high yielding in cooler seasons as they needed a longer seed filling period. According to Materne and Siddique (2009) large seeded greeiin lentils are more prominent in colder environments where planting in summer gives them a cooler temperature during maturity whereas red lentils are produced in winter growing areas where the temperature is warmer during maturity. However, all lentils are severely affected by the extreme temperatures during growth and reproduction phase (Materne and Siddique, 2009).

Soil fungi which form symbiotic relationships with legume crops and fix nitrogen are also susceptible to high soil temperature (Malhotra and Saxena, 1993). Thavarajah et al. (2008) reported that lentil grown in the dark brown and brown soil zone of western Canada had high Se concentration in the seed (425-672µg/kg). Panadian et al. (2011) reported environmental

effects in mineral nutrient concentration in the rice (*Oryza sativa*) kernel, particularly; Zn, Fe, Mn, and Cu concentration were influenced by environmental factors like soil pH, mineral concentration in the soil, phosphorous content and electrical conductivity.

Past research on factors affecting soil available micro and macro elements shows that natural and anthropogenic activities are involved in creating high or low mineral concentration in the soil (Mermut et al., 1996; Aguilar et al., 1988). Most of the trace elements are derived from soil parent materials and partially from anthropogenic activities. Parent materials differ in mineral composition which is shaped for years by the surrounding environment and the climatic conditions. Human activities like fertilizer application, crude oil drilling, sewage-slurry, industrial waste, and many developmental activities increase the mineral elements in the soil. Percent clay content was also found to have a role in changing the amount of mineral elements present in soil; higher clay content was associated with higher content of mineral elements in the soil (Mermut et al., 1996; Aguilar et al., 1988). Furthermore, Panadian et al. (2011) reported that the carbon content in soil regulates Mn and Cu content in the rice kernel. Garrett et al. (2013) also reported that 25% of Se variation in field pea was contributed by soil edaphic factors where organic carbon and pH played a vital role. Furthermore differential response in Selenium (Se) concentration was also seen with the great group soils, arid climate, and weather conditions during the growing season (Garrett et al., 2009). Since clay particles predominately in the northern Great Plains are negatively charged particles and the trace elements are positively charged ions, there is an attraction allowing the clay to hold more mineral elements. As plants derive nutrients from the soil so if they are grown on nutrient deficient soils, they are more likely to be nutrient deficient reducing their dietary contribution to human health.

Legumes are a key component of sustainable agriculture systems with many economic and environmental benefits from their ability to fix nitrogen in the root nodules in a symbiotic

interaction with soil rhizobia (Manchanda and Garg, 2008). Lentil improves the soil nutrient status for sustainable crop production systems (Erskine et al., 2009). Quinn (2009) reported that the rhizobia-plant symbiosis limits micronutrient accumulation in lentil to a greater extent than soil fertility. Borucki and Sujkowska (2007) reported that salinity influences on growth and nodulation of pea plants where he found that NaCl induced salinity reduced the root nodule formation in pea compared to (without NaCl) control. Furthermore Rao et al. (2002) reported that salinity not necessarily affects rhizobia colonization around roots; however, the growth of new nodules and the efficiency of fully formed nodules (developed earlier under non-saline conditions) were reduced. This results in a lower number of active N_2-fixing nodules in the legume crop hampering the overall growth and development. Lower nodulation resulted because "salinity caused disturbances in bacterial release from the infection threads during nodulation. The plant on the other hand synthesizes and deposits electron dense material (phenolics) in pea nodules to safe guard against DNA damaging reactive oxygen species produces due to salinity" (Borucki and Sujkowska, 2007). Reactive oxygen species at higher level damage cellular macromolecules (such as DNA and RNA) and participate in programmed cell death (Borucki and Sujkowska, 2007).

Root architecture plays a major role in micronutrient accumulation in plants. Phosphorous concentration in the plant is associated with root growth and architecture. Deficiency of phosphorus in the plant system results in reduced root area which ultimately results in reduced nutrient absorption (Hopkins and Hunter, 2008). Welch and Shuman (1995) reported that the micronutrient uptake mechanism can be increased by increasing the absorption area of root cells and also by enhancing root-cell processes which modify micronutrient solubility and movement to root surfaces. This can be done by changing the microenvironment around the root by stimulating ion concentration through acidification, reduction of ions to an absorbable form, presence of chelating compounds as ion binders, and

increasing root absorptive surface area by increasing the number of fine roots and root hairs (Hopkins and Hunter, 2008). Increased micronutrient uptake can also be accomplished through regulation of root cell plasma membrane absorption mechanisms (transporters and ion channels) allowing sufficient accumulation and efficient translocation of micronutrients once they enter the root cells from the soil (Palmer and Guerinot, 2009).

1.2.3. Physiology of Mineral Nutrient Uptake and Concentration in Seeds

Micronutrient uptake mechanisms are more complex than macronutrient uptake mechanisms. Since micronutrients function at low concentration their contribution to total dry weight of the seed is also low (Grusak et al., 1999). Nutrient uptake, accumulation and regulation are dynamic phenomenon which should be optimum, avoiding deficiency and toxicity. Muehlbauer and McPhee (1997) reported that 1000 kg of dry pea seed contains up to 43 kg N, 4.2 kg P, 9.2 kg K, and 1.2 kg Mg. However, the concentration varies among genotype and the environment in which they are grown. Most of the processes are strictly regulated; uptake of essential micronutrients is controlled by the plant root-shoot system as they play a major physiological role in the plant. Garrett et al. (2013) reported that "the pea plant controls uptake of bio-essential micronutrients Ca, Fe, K, Mn, Mg, P and Zn homeostatically." Se and Cd have no physiological role in plant health (McLaughlin and Singh, 1999; Djanaguiraman et al., 2005); therefore, plants accumulate these elements from the soil and translocate them to different physiological sites including the seed (Garrett et al., 2013).

Studies on Fe uptake mechanisms in dicotyledonous plants have identified many root and plant physiology mechanisms that are responsible for increasing the ability of the plant to obtain Fe induced deficiency. Collectively it is known as the strategy I mechanism in dicotyledonous plants (Marschner and Romheld, 1994). Dicotyledonous plants uptake the ferrous (Fe^{2+}) form of Fe from the soil (Chaney et al., 1972) and this form of Fe is less abundant than the ferric form. Due to this nature of Fe availability the dicot plant has to use the strategy

I mechanism specifically; increasing the Fe solubility and/or reducing the ferric form of Fe to the ferrous form. Iron is more soluble at low pH; therefore, acidification of the rhizhospheric region is made possible by releasing organic acids and or phenolics which are able to chelate the Fe (Romheld and Marschner, 1983) and also through plasmalemma proton pumps (Romheld et al., 1984) which release H^+ ATPases in dicots. Depending on the Fe availability the plant regulates the process and root-shoot communication plays a vital role in up and down regulation of strategy I (Grusak and Pezeshgi, 1996).

Plants absorb and uptake mineral nutrients from the soil solution in the form of ions (eg. K^+, NO_3^-) through two possible pathways in the roots: (i) the apoplasm (cell walls and intercellular spaces) and (ii) cell-to-cell movement through the symplasm (through the living cells). These ions diffuse until they reach the casparian band through the epidermal and cortical pores (Kochian, 1991). "Casparian bands" (deposits of hydrophobic materials) block the apoplasmic pathway at the endodermis of cell walls (Kochian, 1991; Grusak et al., 1999). Blockage of the apoplasmic pathway forces water and ions to cross the plasmalemma or cortical cells and to follow symplasmic pathways. This band consists of specific ion transporter proteins and lipid bilayers embedded in the membranes of plant cells which prevent the free movement of substances in and out of cells. The transporter proteins are highly specific for the transfer of different ions across the membrane. Therefore, regulation of internal nutrient composition and concentration is maintained by these transporter proteins (Hopkins and Hunter, 2008). Radial transport of the ions across the root faces some difficulty due to the presence of high cation exchange capacity compounds in cell wall pores of the root epidermis and cortex (Clarkson, 1988). As soon as the ions enter the symplasmic pathways they are unloaded into xylem vessels where they are moved up to the shoot. Transport of ions within the xylem is partially driven by root pressure and the water potential gradient between roots and shoots (Grusak et al., 1999). The distribution in the whole plant system differs widely within or remobilizing from certain

parts to the seeds. The membrane bound, longitudinal network of living cells called phloem is an important factor in nutrient partitioning in the whole plant. Phloem transports nutrients from source organs to the sinks including young leaves, root systems, and developing reproductive tissue (Grusak et al., 1999). Phloem sap loading (influx) and unloading (efflux) are important hydrostatic pressure gradients, driving source to sink micronutrient accumulation in seeds and grains. However, not all the micronutrients are mobile which affects the remobilization of the nutrients. For example, Zn was reported to be highly mobile from leaves in deficiency (Pearson and Rengel, 1994), and Fe was reported to be less mobilized compared to Zn (Miller et al., 1993) whereas Mn was not mobilized regardless of deficiency (Pearson and Rengel, 1995). Further in depth research is needed to understand the mechanism of phloem loading and unloading to understand the whole plant partition of different mineral ions.

A study done by Hocking (2008) in semi-dwarf spring wheat grown under typical irrigation farming conditions showed that most of the dry-matter production and nutrient uptake occurs by anthesis. He reports that 75–100% of the final Mg, Cu, chloride, S, P, N, and K content uptake happened pre-anthesis. He also found that the concentrations of phloem-mobile nutrients, such as N and P, decreased in the leaves and stems throughout the season, whereas concentrations of phloem-immobile nutrients, such as Ca and Fe, generally increased. Mineral element concentrations differ with the age of the plant. A major proportion of most of the elements in young plants was found in leaves whereas it was found more in stems at anthesis (Hocking, 2008). However, Saxena and Hawtin (1981) reported that the stem continues to act as an active photosynthate sink even after flowering suggesting plant nutrient accumulation might continue during reproductive growth. Mineral element distribution pattern differs from element to element (Birsin et al., 2010). As redistribution of mineral elements also depends on the mobile and/or immobile nature of the elements; therefore, the concentration of mineral elements differs accordingly within the plant. Hocking (2008) reported that 100% of the K, 68–

72% of the N and P, and 33–48% of the Zn, Cu, Mg, and S accumulated in the grain of spring wheat could have been redistributed from stems and leaves. According to Hocking (2008) over 70% of the N and P, and 15 to 51% of the Mg, K, Cu, S, and Zn was redistributed from stems and leaves to developing seed whereas negligible amounts of Ca, Na, Cl, Fe, and Mn were redistributed from vegetative organs in spring wheat. "The capacity of plants to redistribute dry matter and nutrients to grain is a valuable trait when nutrient uptake is severely restricted in the post-anthesis period" (Hocking, 2008).

1.2.4. Genotype X Environment Interactions Influence on Seed Mineral Concentration

Both conventional and modern biotechnology breeding techniques can be used to increase the concentration and bioavailability mineral elements in lentil (Welch, 2002). However, selection and identification of genes having favorable alleles for increased micronutrient concentration in germplasm is a prerequisite for breeding. Several studies have shown that there is significant variability for micronutrient concentration in lentil germplasm (Thavarajah et al., 2009, 2011; Karakoy et al., 2012). Despite the genetic variability that is available genetic progress is limited by genotype by environment interactions. These interactions are widely recognized in many crop species, including lentil.

Hood-Niefer (2012) reported a three-way interaction of genotype x location x year in concentration and physiochemical properties of starch in pea. Genotypic variation in pea was reported for starch, protein concentration (Hood-Niefer, 2012), and Fe concentration at differential temperature conditions (Thavarajah et al., 2010). Amarakoon et al. (2012) reported wide diversity among U.S. grown field pea varieties for mineral concentration (Fe =46 to 54mg/kg, Zn =39 to 63mg/kg, Mg=1350 to 1427mg/kg, Ca=622 to 1219mg/kg, and P=3.5mg/kg). Ray et al. (2014) reported that the location-year was more important than the cultivar itself although, cultivar showed a significant effect on mineral nutrient accumulation in bean. These results suggest that genotype and environment impact overall composition of

crops and implies that there is enough variation and scope to exploit this variability through breeding and agronomy to improve the nutrient concentration in the seed. Thavarajah et al. (2010) reported a significantly higher mean total Zn concentration (69mg/kg) in lentil genotypes grown at rising temperatures during the seed filling stage compared to the mean total Zn concentration (61mg/kg) at falling temperatures at the same growth stage. In addition, Ray et al. (2014) reported significant genetic variation for Ca, Cu, Fe, K, Mg, Mn, Se, and Zn in lentil varieties grown in Saskatchewan, Canada.

Micronutrient biofortification in the soil–plant system can be defined as increasing the density and bioavailability of micronutrients in the edible parts of crop plants. This can be done through both plant biotechnology and nutrition management of the soil–plant system with the aim of improving human nutrition and health. This approach is more sustainable, generates environmentally well adapted novel crops, and involves an adjustment of the flow of micronutrients from soils to humans. Research suggests that micronutrient uptake and translocation are homeostatically controlled phenomenon and has identified large genotypic differences in the micronutrient content of the edible parts within the same plant species (Graham et al. 1999; Welch and Graham 1999, 2004). Pandian et al. (2011) to evaluate the genetic variability and genotype × environmental interaction in milled rice and showed significant genotype by environment interaction for Zn, Fe, Cu, and Mn uptake. Fe and Zn concentration in lentil were found to be influenced by environmental conditions such as soil type, rainfall, temperature, and varying cultural practices (Thavarajah et al., 2009b; Thavarajah et al., 2009a). The fact that growing environment and soil condition play a role in nutrient concentration in crops was further demonstrated by Thavarajah et al. (2011) where lentils from Nepal, Australia, or Canada had greater Se concentration and Syria had the lowest concentration with 22µg/kg and Nepal was the highest with 180µg/kg.

Location plays a crucial role in micronutrient accumulation. Soil and the environment in which the crop is grown is an integral part of the plant life cycle. Thavarajah et al. (2008) reported significant location effects in Canada grown lentil. Significant location and location x genotype effects were reported for both Fe and Zn concentrations in bean, suggesting that environment influences the concentration of Fe and Zn (Gregorio, 2001; Beebe et al., 2000). Amarakoon et al. (2012) reported that the selection of specific varietal and location combinations further augmented mineral micronutrient content in U.S. grown field pea demonstrating that genetic biofortification of field pea as a food-based solution to global micronutrient malnutrition is possible. Ray et al. (2014) reported significant location effect on mineral nutrient concentration in field pea. Year, location and cultivar had a significant contribution in mineral nutrient concentration in chickpea (Ray et al., 2014). Furthermore, Ray et al. (2014) found that the Fe, Zn, and Cu were greatly affected by year and location in chickpea, whereas Mn and Se were greatly affected by location. In the same report a significant location and location x year x cultivar effect was observed in most of the mineral nutrient concentrations analyzed in lentil suggesting the importance of genotype, environment, year and the interaction effect in selection and breeding for mineral-dense pulse crops.

1.3. Objectives

The objectives of this research are to:

Broad Objective:

Improve micronutrient concentration in pea and lentil through conventional breeding.

Specific Objectives:

1. Quantify the mineral nutrient (Ca, Cu, Fe, K, Mg, Mn, P and Zn) concentration in dry pea and lentil advanced yield trial lines.
2. Characterize the genotype and the environmental factors affecting the mineral nutrient concentration in pea and lentil crops.

CHAPTER 2. GENETIC AND ENVIRONMENTAL VARIATION ON MINERAL NUTRIENT CONCENTRATION IN PEA SEED

2.1. Introduction

Environmental influence on the genetic control of mineral nutrient accumulation in pea and lentil is important in breeding for improved cultivars. Individual performance is influenced by the genotype x environment interaction which complicates selection of the best genotype in a specific environment (Sabaghnia et al., 2006). Green revolution of the 1960's drastically increased total production and productivity of major cereal crops which then greatly impacted the global food demand, expanding cereal crop production worldwide. Focus on cereal crops in the green revolution lead to categorical negligence of other nutritionally important crops like pulses (Singh et al., 2013). Cereal-based diets are deficient in many essential mineral elements and vitamins and have played a negative role in making society vulnerable to many nutritional deficiencies (Welch and Grahm, 2002). According to the WHO about two billion people suffer from anemia, a nutritional disorder caused by iron deficiency resulting from hemoglobin concentration below threshold levels. This might be the result of deficiency in folate, vitamin A or vitamin B_{12} and/or due to infectious diseases like malaria, hookworm, schistosomiasis and other genetically inherited diseases like thalassaemia (Allen et al., 2006). Calcium, magnesium (White and Broadley, 2005) zinc, manganese, copper, and selenium deficiencies are also broadly prevalent in society (Mathers et al., 2009). This alarming health concern demands a better way to ensure a balanced nutrient supply to the needy people in a continuous, adequate, affordable and timely manner (Welch et al., 1997; Combs et al., 1997). Finding germplasm that is high in particular mineral elements is a very important part of biofortification through breeding so that selection and hybridization can improve mineral element concentration in the seed (Graham and Welch, 1996; Bouis, 2003; Rocheford et al., 2014). Information on genetic

potential, environmental effect and the interaction of these two factors in nutrient accumulation in specific varieties at specific locations is crucial in breeding for these traits.

Domestication of pea was thought to have occurred from 7000-6000 B.C. in the Near East (Zohary and Hopf, 1973; Smartt, 1990; Muehlbauer and McPhee, 1997). Zohary and Hopf, (1973) reported that domestication of pulse crops like pea, lentil and chickpea started together with or shortly after the domestication of cereal crops like emmer and einkorn wheat, barley, bitter vetch and flax during Neolithic Agricultural Revolution. Pea production in the western region, especially in Canada, has increased significantly in recent years (Muehlbauer and McPhee, 1997). Historically, Wisconsin, Minnesota, and Washington were the leading producers of processing peas and Idaho was the leading producer of dry peas in the U.S. (Muehlbauer and McPhee, 1997). This pattern of pulse crop production in U.S. has recently changed with increasing area and yields per acre in Montana, North Dakota, and Idaho (USDA-NASS, 2013). According to Smartt (1990), the wild relative of the pea had seed dormancy and pod shattering. Zohary and Hopf, (1973) reported that the wild relative of *P. sativum* is genetically diverse and has a rough seed coat with comparatively small seed size.

Pea production is concentrated between the Tropic of Cancer and 50° N with a growing season varying from 80 to 100 days in semi-arid regions and up to 150 days in humid and temperate areas (Davies et al. 1985). Pea was originally reported to be cultivated as a winter annual crop in the Mediterranean region (Smartt, 1990). Peas can be grown at higher altitude since the crop requires a cool, humid climate with a temperature from 7°C to 30°C (Duke, 1981). Spring sown peas are more common in the temperate area because of the harsh winter condition limiting survivability. Winter types are cultivated as a fall-sown crop in Montana and the U.S. Pacific Northwest. Winter pea crops have the capacity to survive in harsh climatic conditions and provide flexibility to agronomic production. The nitrogen fixing ability of pea through symbiotic association with Rhizobium allows the crop to be planted on less fertile land

and minimizes the cost of nitrogenous fertilizer application (Duke, 1981; Muehlbauer and McPhee, 1997).

Most pea varieties have indeterminate growth characteristics and are broadly classified as dry pea and fresh pea. Fresh peas are produced for fresh market consumption, including the canning, frozen, and fresh market pea (Muehlbauer and McPhee, 1997). Canning peas have light green testa whereas frozen peas have dark green testa (Muehlbauer and McPhee, 1997). Canning and frozen peas are harvested when the tenderometer (an instrument to measure the stage of maturity) reading is in between 95-105. Dry peas are also commonly known as field peas which include smooth green, smooth yellow, Austrian winter, smooth red, and marrowfat types (Muehlbauer and McPhee, 1997). Smooth yellow, red or green cotyledon peas have starchy seeds have indeterminate growth habit and are consumed as whole or split. Split seeds are commonly consumed in soups. Marrowfat peas were believed to have originated from England and are used to prepare 'mushy peas' or roasted and marketed as a snack food in Southeast Asian countries. Marrowfat peas are large, flattened, dark green colored and somewhat dimpled in shape. Marrowfat pea quality and appearance are unique compared to other dry pea types. Varieties of marrowfat pea are typically dwarfed with short heavy vines and very large leaves. Austrian winter peas are winter hardy peas that have pigmented stems, flowers and seed (Muehlbauer and McPhee, 1997). Most of the production of this type is in the U.S. Pacific Northwest. These winter types are primarily used for green manure and pigeon feed, however, recently released varieties have human food qualities (Muehlbauer and McPhee, 1997)

Dry pea contains 37-49% starch, 21-33% protein, 2.4% lipids, and 4.6-7% fiber (Duke, 1981). Amarakoon et al. (2012) reported 46-54mg/kg Fe, 39-63mg/kg Zn in U.S. grown field pea. Fresh green peas contain 32mg Ca, 102mg P, 1.2mg Fe, 6mg Na, 350mg K, 405µg per 100g, wide range of vitamins proteins and carbohydrate (Duke, 1981). Dry pea contains greater

protein fat and carbohydrate than fresh pea (Duke, 1981; Hulse, 1994). Pea flour contains 22.8g protein, 4.2g fiber, 2.8g ash, 72mg Ca, 338mg P, 11.3mg Fe, 0.86mg thiamine, 0.18mg riboflavin, and 2.8mg niacin per 100g (Duke, 1981).

Thavarajah (2012) reported that dry pea cultivars vary with respect to mineral nutrient level present in the seed. Amarakoon et al. (2012) reported "a single serving of field pea grown in the USA could provide 28 to 68% of the recommended daily allowance (RDA) of Fe, 34 to 46% of the RDA of Mg, 6 to 12% of RDA of Ca and 36 to 78% of the RDA of Zn and is also naturally low in phytic acid (1.4 to 2mg/g).

According to Muehlbauer and McPhee (1997) consumption patterns of pulse crops differ among developed and developing countries. The pea crop is produced for fresh green seeds, tender green pods, dried seeds, and foliage. Developed countries consume succulent types of peas as canned or frozen whereas dry pea is consumed predominantly more in developing countries due to its ease of storage. Increasing the mineral concentration in the seeds of the crops that are widely grown and consumed in the area of malnutrition is vital in case of acceptance and reaching to the targeted population at low cost. Dry pea is also one of those crop species that is consumed worldwide and especially in developing countries. Therefore this research aimed to categorize factors interfering nutrients concentration in dry pea seed and also to quantify the amount of mineral nutrients present in the seed which is crucial information in recommending this crop for human benefit.

2.2. Materials and Methods

2.2.1. Dry Pea Genotypes

Twenty-five genotypes of advanced pea breeding lines including check varieties were planted in North Dakota for two years (2012-2013). Fifteen genotypes were common across all locations and years (Table 2.1) and only these common genotypes were used for the analysis of variance and Pearson's correlation coefficient analysis.

Table 2.1. List of Advanced Dry Pea Yield Trial genotypes planted in North Dakota in 2012 and 2013.

2012	Market Class	2013	Market Class
AC AGASSIZ¥	Large yellow	AC AGASSIZ¥	Large yellow
ARAGORN¥	Large yellow	ARAGORN¥	Large yellow
CDC GOLDEN¥	Large yellow	CDC GOLDEN¥	Large yellow
CDC STRIKER¥	Large green	CDC STRIKER¥	Large green
COOPER¥	Large green	COOPER¥	Large green
DS ADMIRAL¥	Large yellow	DS ADMIRAL¥	Large yellow
MAJORET¥	Large green	MAJORET¥	Large green
NDP080111¥	Large green	NDP080111¥	Large green
NDP080114¥	Large green	NDP080114¥	Large green
PS07ND0190¥	Large yellow	PS07ND0190¥	Large yellow
PS07ND0189¥	Large green	PS07ND0189¥	Large green
PS07ND0102¥	Large green	PS07ND0102¥	Large green
PS07ND0110¥	Large yellow	PS07ND0110¥	Large yellow
PS07100716¥	Large green	PS07100716¥	Large green
PS07ND0163¥	Large green	PS07ND0163¥	Large green
NDP100140	Large green	NDP080138	Large green
NDP100144	Large green	NDP080142	Large yellow
NDP100146	Large green	PS04100722	Large yellow
NDP100595	Large green	PS05ND0232	Large green
NDP100624	Large yellow	PS07100165	Large green
NDP100720	Large yellow	PS07100450	Large green
NDP101132	Large green	PS07100452	Large green
NDP101144	Large green	PS07100470	Large green
NDP101154	Large green	PS07100892	Large yellow
NDP101171	Large yellow	PS07ND0164	Large yellow

¥ indicates common genotypes in the 2012 and 2013 trials.

2.2.2. Field Experiments

Statewide dry pea trials were conducted in North Dakota in 2012 and 2013 at six locations, i.e. Hettinger Research Extension Center (HREC), Williston Research Extension Center (WREC), North Central Research Extension Center (NCREC), Prosper research site near Fargo, Langdon Research Extension Center (LREC), and Carrington Research Extension Center (CREC). Trials at each research location were grown under rain fed conditions and established using a randomized complete block design with four replications. Plot size ranged from 6.9-11.6 square meters per plot and 6-8 rows per plot depending on the equipment available at each location. Row spacing was 18cm and the targeted seed density of 70 plants per square meter. Grassy and broad leaf weeds were controlled at each location according to

local management practices while in crop weeds were controlled manually by hand weeding when necessary.

2.2.3. Mineral Nutrient Analysis in Seed

Fifty grams of whole seed from each replicate plot was thoroughly cleaned removing off-types, debris and broken seeds. The cleaned sample was milled using a UDY mill to pass through a ~0.5mm sieve. The finely ground samples were acid digested and mineral nutrient concentration was analyzed in the USDA-ARS Children's Nutrition Laboratory, Houston, Texas (Farnham et al., 2011).

2.2.4. Soil Sampling and Mineral Analysis

Four representative soil samples were collected from each trial site in North Dakota. Soil cores representing the 0-15 and 16-30cm range were taken and stored separately until they were analyzed. The mean concentration of each mineral nutrient sampled from 0-15 cm and 15-30 cm region was taken for the analysis. The samples were analyzed for eight mineral elements (Ca, Cu, K, Fe, Mg, Mn, P, and Zn) at the North Dakota State University soil testing lab following the extraction method outlined in the "Recommended Chemical Soil Test Procedures for the North Central Region" (Brown, 1998).

2.2.5. Statistical Analysis

Analysis of variance (ANOVA) was performed to partition the environmental, genotype, and genotype x environment variance components for Ca, Cu, Fe, K, Mg, Mn, and Zn concentration in the seed using the PROC-MIXED command of SAS (release 9.3, SAS Institute, Inc., Cary, NC). Location, environment and year were considered as random effects and genotype as a fixed effect. Pearson's Correlation Coefficient at each location was analyzed to identify the relationship between and among mineral nutrients and yield parameters. The yield parameters used in this research are seed yield, test-weight and thousand seed weight.

2.3. Results

Plant available soil mineral nutrients analyzed from six locations (Table 2.2) in 2013 showed that soil pH varied from slightly acidic to slightly alkaline and ranged from 6.3 at HREC to 7.8 at LREC (Table 2.2). Calcium (6270ppm) and Cu (1.33ppm) were highest at Prosper, Fe was highest at Langdon (302.5ppm), K was highest at HREC (418ppm), Mg was more abundant at Prosper (1260ppm), P was highest in HREC (25ppm) and Zn was high at Prosper (1.45ppm). Seed mineral analysis of the advanced dry pea yield trial grown at six locations in North Dakota in 2013 showed that seed from the CREC and NCREC had the greatest concentration of Ca in the seed (Table 2.2). Dry peas grown at NCREC and Prosper had the greatest accumulation of Cu. Fe was high in seed grown at NCREC and CREC whereas K was high in seed grown at CREC and NCREC. High concentration of Mg was found in seed grown at Prosper and NCREC whereas Mn and P were high in seed grown at NCREC and LREC. Pea seed grown at Prosper and HREC had the highest Zn concentration in 2013.

Analysis of variance was conducted across years and locations for the Advanced Dry Pea Yield Trial sown at six locations in North Dakota in 2012-2013 (Table 2.3). Variety main effects were highly significant for all the mineral nutrients. Location main effects were significant for K (P=0.0171) and Mn (P=0.0011). The effect of year was not significant for any of the mineral nutrients analyzed. The year x location effect was significant for all the minerals except K (P=0.1216). The block (Yr x Loc) effect was highly significant for all minerals analyzed. The variety x location effect was significant for all minerals except Cu (P=0.2316) and Mn (P=0.4558). The year x variety effect was significant only for Mg (P=0.0254). The three way interaction, variety x location x year, was highly significant for Ca (P=0.0001), Cu (P=0.0001), K (P=0.0006), and Mg. (P=0.0001), moderately significant for Fe (P=0.0026), Mg (P=0.0206) and P (P=0.0164) while the three-way interaction was not significant for Zn (P=0.6714).

Table 2.2. Plant available soil mineral nutrients analyzed from top 0-30cm and location effect estimates for seed mineral concentration for 15 dry pea genotypes grown at six locations in North Dakota in 2013.

Locations Soil analysis	Ca (ppm)	Cu(ppm)	Fe(ppm)	K(ppm)	Mg(ppm)	Mn(ppm)	P(ppm)	Zn(ppm)	pH
CREC	4870	0.57	12.3	215	400	4.8	24	1.32	7.7
HREC	4000	1.20	46.3	418	450	23.7	25	0.75	6.3
LREC	5980	0.44	302.5	9	770	7.5	5	0.30	7.8
NCREC	5650	0.98	55.0	283	660	21.9	4.5	0.30	6.6
Prosper	6270	1.33	26.7	278	1260	12.1	21	1.46	7.5
Williston	3930	1.08	39.8	325	390	23.5	16	0.35	6.4
Location estimates for seed concentration									
CREC	64.00	-2.07	5.88	397.89	11.367	-1.78	130.16	-4.04	
Prosper	-14.77	1.20	3.38	56.96	143.89	-2.64	252.53	9.43	
HREC	-50.71	-0.37	-3.82	-204.26	-83.90	-0.11	-158.57	3.34	
LREC	-33.75	-0.96	-2.73	-136.56	-143.8	0.22	-302.97	-2.98	
NCREC	69.68	2.14	7.59	168.61	152.41	4.41	255.59	0.12	

Note: PROC-MIXED analyzed mean estimates (y-intercept) of 2013 location seed Ca concentration =699, Cu=6.4, Fe=58, K=8925, Mg=1348, Mn=14, P=3836, and Zn=35.

Table 2.3. Analysis of Variance for the Advanced Dry Pea Yield Trial grown in North Dakota in 2012 and 2013.

Sources of Variation	Ca mg/kg DW	Cu µg/g DW	Fe µg/g DW	K mg/kg DW	Mg mg/kg DW	Mn µg/g DW	P mg/kg DW	Zn µg/g DW
Variety	<.0001***	0.0133*	0.0024**	<.0001***	<.0001***	0.0013**	0.0003***	<.0001***
Location	0.4129	0.3226	0.3274	0.0171*	0.0751	0.0011**	0.1684	0.2183
Year	0.2132	0.0758	0.4411	0.0854	0.4397	0.8079	0.0636	0.148
Yr x Loc	<.0001***	<.0001***	<.0001***	0.1216	<.0001***	0.0155*	<.0001***	<.0001***
Block	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***
Var x Loc	0.0124*	0.2316	0.0003***	0.0272*	0.0122*	0.4558	0.0212*	0.0369*
Var x Yr	0.2315	0.7657	0.1314	0.2774	0.0254*	0.3574	0.729	0.4915
Var x Yr x Loc	<.0001***	<.0001***	0.0026**	0.0006***	0.0206*	<.0001***	0.0164**	0.6714

Note: $P<0.05$ = *, $P<0.01$ = **, and $P<0.001$ = ***

The LS means for varieties across locations and years showed a broad range of variation for all minerals (Table 2.4). The range of mineral concentration among genotypes across years and locations was 542 to 1041mg/kg DW for Ca, 6.6 to 7.5µg/g DW for Cu, 49.4 to 56.8µg/g DW for Fe, 8259 to 9278mg/kg DW for K, 1257 to 1454 mg/kg DW for Mg, 12.8 to 15.9mg/g DW for Mn, 3783 to 4341mg/kg DW for P, and 33.6 to 44.3µg/g DW for Zn.

DS Admiral (1040.94mg/kg DW), PS07100716 (1006.45mg/kg DW) and NDP080111 (845.61mg/kg DW) had the highest Ca accumulation across both years and locations (Table 2.4). PS07ND0190 (7.47µg/g DW), PS07ND0163 (7.40µg/g DW) and PS07ND0102 (7.30µg/g DW) had the highest Cu concentration across both years and locations (Table 2.4). PS07100716 (56.84µg/g DW), CDC Striker (56.05µg/g DW) and Aragorn (55.46µg/g DW) had the highest Fe concentration across years and locations (Table 2.4). PS07ND0163 (9277.70mg/kg DW), PS07ND0102 (9191.32mg/kg DW) and Agassiz (9171.61mg/kg DW) had the highest K concentration across years and locations (Table 2.4). PS07ND0110 (1453.89mg/kg DW), NDP080111 (1412.92mg/kg DW) and PS07100716 (1408.05mg/kg DW) had the highest Mg concentrations across years and locations (Table 2.4). Aragorn (15.76 µg/g DW), Admiral (15.46µg/g DW) and NDP080111 (15.33µg/g DW) had the highest Mn concentration across years and locations (Table 2.4). Aragorn (4341.05mg/kg DW), PS07ND0163 (4265.47mg/kg DW) and Cooper (4239.24mg/kg DW) had the highest P concentrations across years and locations (Table 2.4). Cooper (44.29µg/g DW), Aragorn (39.97µg/g DW) and Majoret (39.69µg/g DW) had the highest Zn concentration across years and locations (Table 2.4).

Analysis of seed mineral nutrient concentration for 15 dry pea genotypes grown at six locations in 2012 and 2013 (Table 2.5) showed that seed grown at CREC and WREC had the highest concentration of Ca while seed grown at NCREC and WREC had the highest concentration of Cu. Iron was highest in dry pea seed grown at CREC and NCREC. Potassium

was highest in dry pea seed grown at CREC and NCREC while Mg was highest in dry pea seed grown at NCREC and Prosper. Manganese was highest in seed grown at NCREC and HREC and P was highest in seed grown at CREC and Prosper. Zinc was highest in dry pea seed grown at HREC and Prosper.

Table 2.4. LS means of 15 dry pea genotypes planted at six locations in North Dakota in 2012 and 2013.

Genotypes	Ca (mg/kg DW)	Cu (µg/g DW)	Fe (µg/g DW)	K (mg/kg DW)	Mg (mg/kg DW)	Mn (µg/g DW)	P (mg/kg DW)	Zn (µg/g DW)
AGASSIZ	696.48	6.66	54.40	9171.61	1356.22	15.25	4005.45	37.36
ARAGORN	819.69	7.26	55.46	8815.43	1299.36	15.76	4341.05	39.97
CDC	541.89	6.88	53.81	9069.73	1280.99	12.89	4061.37	38.85
CDC	747.89	7.26	56.05	9044.99	1347.26	14.38	4094.64	36.65
COOPER	794.48	6.75	52.81	8791.31	1260.16	13.13	4239.24	44.29
DS	1040.94	6.61	49.43	8259.07	1390.54	15.46	3830.29	35.36
MAJORET	752.55	7.08	53.80	8456.87	1317.91	13.48	4041.25	39.69
NDP080111	845.61	7.28	54.49	8818.21	1412.92	15.33	4181.77	37.85
NDP080114	652.72	6.92	52.21	8875.16	1285.25	13.19	3997.64	35.92
PS07100716	1006.45	6.91	56.84	8396.35	1408.05	14.56	3782.67	36.10
PS07ND0102	605.29	7.30	55.20	9191.32	1330.57	12.79	4188.44	38.84
PS07ND0110	842.13	7.12	51.62	8795.88	1453.98	14.61	4106.88	38.19
PS07ND0163	675.79	7.40	53.90	9277.70	1257.44	13.82	4264.47	37.03
PS07ND0189	689.22	6.62	50.78	8955.75	1266.30	12.83	3942.81	33.64
PS07ND0190	834.15	7.47	54.31	8987.18	1311.38	13.46	4140.67	37.25

Table 2.5. Location effect estimates generated from PROC MIXED analysis for seed mineral nutrient concentration for 15 genotypes grown at six locations in North Dakota in 2012 and 2013.

Parameters	Ca	Cu	Fe	K	Mg	Mn	P	Zn
Y-intercept	747.89	7.25	56.04	9044.99	1347.26	14.38	4094.64	36.65
CREC	9.34	-0.73	2.50	352.07	37.50	-2.02	326.95	-3.76
HREC	-6.96	0.17	-0.24	-124.76	-62.30	0.15	-93.11	3.35
LREC	-13.75	-0.14	-0.49	7.51	-68.81	-0.20	-175.11	-0.85
NCREC	7.87	0.27	0.77	279.43	96.10	5.16	33.70	-1.12
Prosper	-5.22	0.17	-0.03	-91.40	59.52	-2.98	228.03	3.07
WREC	8.71	0.26	-2.51	-422.84	-62.01	-0.10	-320.45	-0.68

Pearson's correlation coefficients among mineral elements and between mineral elements and seed yield (SY), test weight (TW) and one thousand seed weight (TSW) for 15 dry pea genotypes grown at the CREC research location in 2012 showed (Table 2.6) that

none of the 8 mineral elements tested had a significant positive or negative correlation with seed yield; however, one thousand seed weight and seed yield showed a significant positive correlation with test-weight at $P<0.05$ ($r = 0.613$). Zinc showed a significant positive correlation with P ($r = 0.699$, $P <0.01$) and a significant negative correlation with Mg ($r = -0.584$) at $P<0.05$. Potassium showed a significant positive correlation with Fe ($r = 0.575$, $P <0.05$) and a significant negative correlations with P ($r = -0.690$ $P <0.01$). Positive correlation between Fe and Zn is seen.

Pearson's correlation coefficients between/among mineral elements and yield (SY) parameters i.e. seed yield (SY), test weight (TW) and one thousand seed weight (TSW) in 15 common genotypes of dry pea planted in HREC research location in 2012 showed (Table 2.7) that only one thousand seed weight and Zn ($r = 0.624$) had a significant positive correlation at $P <0.05$. Calcium showed a significant negative correlation with K ($r = -0.701$), a significant positive correlation with Mg ($r = 0.692$) at $P <0.01$, whereas significant positive correlation with Mn at $p<0.05$ (r= 0.609). Significant positive correlation was also found between Zn with P ($r = 0.602$) and Mn ($r = 0.548$) at $P <0.05$. Phosphorous showed a significant positive correlation with K at $P <0.01$ ($r = 0.581$).

Pearson's correlation coefficients between mineral elements and yield parameters in 15 genotypes of dry pea planted in LREC in 2012 showed significant positive correlation with Mg ($r = 0.573$) at $P <0.05$ in Table 2.8. Phosphorous showed a significant positive correlations with Fe ($r = 0.705$) at $P <0.01$ whereas P showed a significant positive correlations with Zn ($r = 0.590$) at $P <0.01$.

Table 2.6. Pearson's correlation coefficients between mineral elements and yield parameters in 15 genotypes of dry pea planted in CREC in 2012.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	0.149	-0.288	-0.690**	0.339	0.134	-0.348	-0.374	0.392	-0.191	0.363
Cu		1	-0.044	0.089	0.083	0.026	0.364	-0.121	0.137	-0.111	-0.137
Fe			1	0.575*	-0.004	0.375	0.080	0.141	-0.265	-0.419	-0.082
K				1	-0.199	0.187	0.501	0.328	-0.421	-0.133	-0.370
Mg					1	0.251	-0.315	-0.584*	-0.081	-0.279	-0.273
Mn						1	0.001	-0.059	-0.006	-0.392	-0.220
P							1	0.699**	-0.369	0.115	-0.401
Zn								1	-0.317	0.066	-0.007
SY									1	0.189	0.613*
TW										1	-0.224
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 2.7. Pearson's correlation coefficients between mineral elements and yield parameters in 15 genotypes of dry pea planted in HREC in 2012.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	-0.375	0.148	-0.701**	0.692**	0.609*	-0.208	0.232	0.040	-0.166	0.060
Cu		1	0.391	0.496	0.061	-0.244	0.364	-0.006	0.090	0.214	-0.334
Fe			1	0.032	0.217	0.256	-0.162	-0.071	-0.090	-0.027	-0.273
K				1	-0.324	-0.397	0.581*	0.096	-0.138	0.012	0.133
Mg					1	0.369	-0.117	0.057	-0.004	-0.025	-0.252
Mn						1	0.052	0.548*	-0.413	-0.138	0.021
P							1	0.602*	-0.009	0.236	0.341
Zn								1	-0.104	0.101	0.624*
SY									1	0.422	0.199
TW										1	0.272
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 2.8. Pearson's correlation coefficients between mineral elements and yield parameters in 15 genotypes of dry pea planted in LREC in 2012.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	-0.083	-0.286	0.131	0.573*	0.334	-0.229	-0.200	0.247	-0.046	0.231
Cu		1	0.431	0.278	0.061	0.115	0.311	0.308	-0.323	-0.504	-0.286
Fe			1	0.292	-0.158	0.080	0.705**	0.457	-0.216	-0.061	0.071
K				1	0.062	0.491	0.312	0.168	-0.258	-0.385	-0.069
Mg					1	0.248	-0.370	-0.276	-0.013	-0.265	-0.205
Mn						1	0.394	-0.126	-0.206	-0.432	-0.366
P							1	0.590*	0.054	-0.023	0.146
Zn								1	-0.020	0.095	0.324
SY									1	0.510	0.448
TW										1	0.405
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Pearson's correlation coefficients between/among mineral elements and yield (SY) parameters i.e. seed yield (SY), test weight (TW) and thousand seed yield (TSW) in 15 common genotypes of dry pea planted in NCREC research location in 2012 showed that (Table 2.9) only test weight and Zn (r = -0.556) had a significant negative correlation at P <0.05. Calcium showed a significant negative correlation with K (r = -0.651), a significant positive correlation with Mg (r = 0.678) at P<0.01 whereas significant positive correlation with Mn (r = 0.611) at P <0.05. Significant positive correlation of P was found with K (r = 0.659) and Fe (r = 0.652) at P <0.01. Iron showed a significant positive correlation with Cu (r = 0.665) at P <0.05.

Pearson's correlation coefficients between/among mineral elements and yield (SY) parameters i.e. seed yield (SY), test weight (TW) and thousand seed yield (TSW) in 15 common genotypes of dry pea planted in Prosper research location in 2012 showed (Table 2.10) no significant correlation between mineral elements and the yield parameter in 15 advanced dry pea cultivar grown at prosper in 2012. However a significant positive correlation was found between K and Cu (r = 0.656) at P <0.01 whereas a significant negative correlation was found between K and Ca (r = 0.629) at P <0.05. Zinc showed a significant positive correlation with Fe (r = 0.523) at P <0.05.

Pearson's correlation coefficients between/among mineral elements and yield (SY) parameters i.e. seed yield (SY), test weight (TW) and thousand seed yield (TSW) in 15 genotypes of dry pea planted in WREC research location showed (Table 2.11) that a strong significant negative correlation was found with the test-weight and P (r = -0.772) and Zn (r = -0.670) concentration at P <0.05 in WREC research location grown dry pea in 2012. Zinc showed a significant positive correlation with P (r= -0.809) at P <0.01 whereas Ca and K (r = -0.636) were found negatively correlated at P <0.05.

Table 2.9. Pearson's correlation coefficients between mineral elements, yield parameters in 15 genotypes of dry pea planted in NCREC in 2012.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	-0.062	-0.048	-0.651**	0.678**	0.611*	-0.206	0.184	0.094	-0.170	0.062
Cu		1	0.665**	0.284	0.149	-0.439	0.280	-0.049	0.179	-0.092	-0.198
Fe			1	0.433	0.071	-0.363	0.652**	0.119	0.393	-0.494	-0.216
K				1	-0.132	-0.410	0.659**	-0.001	-0.030	-0.131	-0.331
Mg					1	0.450	-0.074	0.076	-0.011	0.040	-0.326
Mn						1	-0.277	-0.030	-0.424	0.058	0.000
P							1	0.109	0.267	-0.432	0.020
Zn								1	-0.012	-0.556*	0.320
SY									1	-0.076	0.066
TW										1	-0.230
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 2.10. Pearson's correlation coefficients between mineral elements, yield parameters in 15 genotypes of dry pea planted in Prosper in 2012.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	-0.416	0.005	-0.629*	0.486	0.438	0.101	0.152	0.307	-0.335	0.235
Cu		1	-0.155	0.656**	-0.149	-0.337	0.300	0.029	-0.100	0.120	-0.116
Fe			1	-0.304	0.263	0.123	-0.198	0.523*	0.126	0.065	-0.213
K				1	-0.394	-0.175	0.267	-0.275	-0.036	0.325	-0.392
Mg					1	0.413	-0.198	0.219	0.407	-0.135	0.247
Mn						1	-0.167	-0.174	0.163	-0.118	-0.151
P							1	0.252	-0.107	0.370	0.004
Zn								1	0.170	-0.283	0.422
SY									1	-0.014	0.277
TW										1	-0.216
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 2.11. Pearson's correlation coefficients between mineral elements, yield parameters in 15 genotypes of dry pea planted in WREC in 2012.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	0.243	-0.002	-0.636*	0.352	0.457	-0.026	0.155	-0.207	-0.197	0.268
Cu		1	0.059	-0.124	0.170	0.443	0.047	-0.052	-0.236	0.199	-0.354
Fe			1	-0.270	0.132	-0.144	-0.294	-0.162	0.275	0.455	0.174
K				1	-0.023	-0.135	0.430	0.231	0.316	-0.187	-0.012
Mg					1	0.270	0.145	0.229	-0.080	-0.102	-0.035
Mn						1	-0.214	0.021	0.345	0.222	-0.451
P							1	0.809**	-0.141	-0.772**	0.469
Zn								1	0.105	-0.670**	0.429
SY									1	0.324	-0.061
TW										1	-0.371
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Pearson's correlation coefficients of dry pea planted in CREC research location showed (Table 2.12) that only thousand seed weight and Mn (r= -0.595) had a significant negative correlation at P <0.05. Calcium showed a highly significant negative correlation with K (r = -0.700), a highly significant positive correlation with Mg (r= 0.694) at P <0.001. Magnesium also showed a significant negative correlation with K (r = -0.552) at P<0.01. Significant positive correlation of P was found with Cu (r= 0.409) at P<0.01. Additionally Zn showed a significant positive correlation with Mn (r= 0.455) at P<0.05 and P (r= 0.518) at P<0.01.

Pearson's correlation coefficients between seed yield of 15 genotypes of dry pea planted in CREC in 2013 was found with significant negative correlation with Fe (r= -509) at P<0.01 in HREC research location grown dry pea in 2013 (Table 2.13). Calcium showed a significant positive correlation with Mg (r= -0.615) and Mn (r= -0.530) at P<0.01 however a significant negative correlation was found with K (r= -0.425) at P<0.05. Significant positive correlation was found between Fe and Cu (r= -0.468) at P<0.05 and a highly strong significant positive correlation between Cu and P (r= 0.709) at P<0.001. Manganese also showed a significant positive correlation with Fe (r= 0.503) and Mg (r= 0.447) at P<0.05. Zinc and P (r= 0.447) also was found with significant positive correlation at P<0.05.

In LREC research grown dry pea in 2013 (Table 2.14) Ca showed a significant positive correlation with Mg (r= 0.613) at P<0.01, Mn (r= 0.424) at P<0.05 and test-weight (r= 0.528) at P<0.01 whereas a significant positive correlation with K (r= 0.686) at P<0.001. Cu showed a significant positive correlation with Fe (r= 0.507) and Mn (r= 0.514) at P<0.01. Potassium showed a significant negative correlation with Mg (r= 0.435) and test-weight (r= 0.432) at P<0.05 and a significant positive correlation with Fe (r= 0.556) at P<0.01. Zinc showed a significant positive correlation with P (r= 0.434) at P<0.05 and a significant negative correlation with seed yield (r= -0.584) at P<0.01.

Table 2.12. Pearson's correlation coefficients between mineral elements, yield parameters in 15 genotypes of dry pea planted in CREC in 2013.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	0.048	0.286	-0.700***	0.694***	0.260	-0.110	0.194	-0.309	0.158	0.068
Cu		1	-0.061	0.208	0.201	0.142	0.409*	0.043	-0.111	-0.186	-0.103
Fe			1	-0.007	0.169	0.190	-0.034	0.207	-0.048	0.201	0.029
K				1	-0.552**	-0.038	0.294	-0.006	0.364	-0.276	-0.223
Mg					1	0.251	-0.193	-0.183	-0.340	0.161	-0.093
Mn						1	0.336	0.455*	-0.154	0.249	-0.595**
P							1	0.518**	0.085	-0.342	-0.082
Zn								1	0.177	0.087	-0.037
SY									1	0.056	0.036
TW										1	-0.175
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 2.13. Pearson's correlation coefficients between mineral elements, yield parameters in 15 genotypes of dry pea planted in HREC in 2013.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	0.017	0.012	-0.425*	0.615**	0.530**	-0.015	-0.205	-0.204	-0.053	-0.246
Cu		1	0.468*	0.209	0.177	0.028	0.709***	0.346	-0.249	0.067	-0.096
Fe			1	0.135	0.045	0.503*	0.338	0.353	-0.509**	0.255	-0.119
K				1	-0.150	-0.104	0.209	0.044	-0.245	0.176	-0.287
Mg					1	0.447*	0.063	-0.185	-0.072	-0.180	-0.250
Mn						1	0.224	0.156	-0.258	0.271	-0.329
P							1	0.447*	-0.043	0.053	-0.054
Zn								1	0.269	0.318	0.225
SY									1	0.109	0.110
TW										1	-0.152
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 2.14. Pearson's correlation coefficients between mineral elements, yield parameters in 15 genotypes of dry pea planted in LREC in 2013.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	0.185	-0.326	-0.686***	0.613**	0.424*	-0.218	-0.185	-0.206	0.528**	0.082
Cu		1	0.507**	0.133	0.194	0.249	0.514**	0.240	-0.357	-0.008	0.222
Fe			1	0.365	-0.134	0.261	0.324	0.287	-0.008	-0.270	-0.072
K				1	-0.435*	-0.142	0.556**	0.274	-0.072	-0.432*	-0.119
Mg					1	0.382	-0.051	-0.273	0.032	0.169	-0.101
Mn						1	0.213	0.114	-0.130	0.312	-0.314
P							1	0.434*	-0.584**	-0.269	0.097
Zn								1	-0.139	-0.139	0.158
SY									1	-0.139	-0.075
TW										1	-0.121
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Pearson's correlation coefficients between/among mineral elements and yield (SY) parameters i.e. seed yield (SY), test weight (TW) and thousand seed yield (TSW) in 15 common genotypes of dry pea planted in NCREC research location in the year 2013 showed (Table 2.15) that seed yield and P (r= 0.410) had a significant negative correlation and test-weight and Fe (r= 0.446) had a significant negative correlation at P<0.05. Calcium showed a significant positive correlation with Mg (r= 0.549) and Mn (r= 0.532) at P<0.01 whereas a significant negative correlation with K (r= -0.649) at P<0.001. Iron and Cu (r = 0.404) were also found with significant positive correlation at P<0.05. Potassium with Fe (r= 0.404) and P with Mn (r= 0.489) showed a significant positive correlation at P<0.05. Magnesium and K were found with a significant negative correlation with K (r= -0.491) at P<0.05.

Significant negative correlation was found dry pea planted in Prosper in 2013 with Mn (r= -0.420) at P<0.05 and P (r= -0.637) at P<0.001 in seed yield at Prosper location grown dry pea in 2013 (Table 2.16). Calcium showed a significant negative correlation with K (r= -0.760) at P<0.001 however, a significant positive correlation of Ca was found with Mg (r= -0.767) at P<0.001. Copper showed a significant positive correlation with Fe (r= 0.439) at P<0.05, P (r= 0.548) at P<0.01 and Zn (r= 0.513) at P<0.05. Potassium was found with a significant negative correlation with Mg (r= -0.625) at P<0.001. Manganese showed a significant positive correlation with P (r= 0.439) at P<0.05. Zinc showed a significant positive correlation with P (r= 0.627) at P<0.001.

Pearson's correlation coefficients between/among mineral elements and yield (SY) parameters i.e. seed yield (SY), test weight (TW) and thousand seed yield (TSW) in 15 common genotypes of dry pea grown at WREC research location showed that (Table 2.17) Zn had a positive correlation with seed yield (r= 0.444) and thousand seed weight (r= 0.415). Test weight showed a significant positive correlation with Mn (r= 0.400) and SY (r= 0.398) at P<0.05. Calcium showed a significant negative correlation with K (r= -0.472) at P<0.05

however a significant negative correlation with Mg (r= 0.556) at P<0.01 and Mn (r= 0.417) at P<0.05. Cupper showed a significant positive correlation with Fe (r= 0.502) and P (r= 0.421) at P<0.05. Iron showed a significant positive correlation with P (r= 0.620) and Zn (r= 0.512) at P<0.001. Significant positive correlation was also found among P and K (r= 0.605) at P<0.01 and P and Mn (r= 0.417) at P<0.05 and Mg and Mn at P<0.05 (r= 0.657). Zinc and P (r= 0.521) showed a significant positive correlation at P<0.01.

Table 2.15. Pearson's correlation coefficients between mineral elements, yield parameters in 15 genotypes of dry pea planted in NCREC in 2013.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	0.137	-0.240	-0.649***	0.549**	0.532**	0.017	-0.083	-0.123	0.152	0.105
Cu		1	0.404*	0.247	-0.067	0.122	0.250	0.014	-0.313	0.018	-0.127
Fe			1	0.446*	-0.052	-0.085	0.223	0.368	-0.161	-0.446*	0.053
K				1	-0.491*	-0.073	0.366	0.088	-0.041	0.041	-0.238
Mg					1	0.215	0.056	-0.111	-0.243	-0.230	-0.054
Mn						1	0.489*	-0.385	-0.026	0.275	-0.044
P							1	0.042	-0.410*	0.022	-0.238
Zn								1	-0.381	-0.294	0.060
SY									1	0.158	0.253
TW										1	-0.142
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 2.16. Pearson's correlation coefficients between mineral elements, yield parameters in 15 genotypes of dry pea planted in Prosper in 2013.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	-0.020	0.023	-0.760***	0.767***	0.360	-0.121	0.136	-0.073	-0.146	0.094
Cu		1	0.439*	0.390	-0.151	0.298	0.548**	0.513*	-0.178	0.046	-0.360
Fe			1	0.054	0.141	0.286	0.063	0.171	-0.012	0.025	-0.329
K				1	-0.625**	-0.074	0.395	0.199	0.061	0.186	-0.033
Mg					1	0.240	-0.177	-0.083	0.021	0.233	0.201
Mn						1	0.439*	0.370	-0.420*	-0.142	-0.336
P							1	0.627***	-0.637***	-0.078	-0.333
Zn								1	-0.393	-0.104	-0.252
SY									1	0.353	0.200
TW										1	-0.048
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 2.17. Pearson's correlation coefficients between mineral elements, yield parameters in 15 genotypes of dry pea planted in WREC in 2013.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	-0.130	-0.196	-0.472*	0.556**	0.417*	-0.239	-0.294	0.129	0.175	-0.277
Cu		1	0.502*	0.286	-0.151	0.149	0.421*	0.303	-0.349	-0.225	-0.021
Fe			1	0.362	0.089	0.302	0.620***	0.512***	-0.002	0.126	0.113
K				1	-0.319	-0.122	0.605**	0.193	-0.148	-0.219	0.070
Mg					1	0.281	-0.184	-0.309	0.107	0.265	-0.269
Mn						1	0.417*	0.352	0.309	0.400*	-0.310
P							1	0.521**	0.003	0.175	0.031
Zn								1	0.444*	0.095	0.415*
SY									1	0.398*	0.362
TW										1	-0.042
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

2.4. Discussion

Dry pea is rich in carbohydrate, protein, vitamins dietary fiber and minerals (Gawalko et al., 2009). Research by Ray et al. (2014) reported varying mineral element concentrations in Canada grown pea and lentil and reported that Fe at a range of 47.7 to 58.1mg/kg, Zn at a range of 27.4 to 34mg/kg, Cu at a range of 5.2 to 6.3mg/kg, and Mn at a range of 9.0 to 15.6mg/kg. The range of Fe in our study is similar, but concentrations of Zn, Cu and Mn were greater in the North Dakota samples (Fe was 49.4 to 56.8µg/g DW, Zn was 33.6 to 44.3µg/g DW, Cu was 6.6 to 7.5µg/g DW, Mn was 12.9 to 15.7µg/g DW). However, the range for Fe (49.4 to 56.8µg/g DW) and Mg (1260 to 1453.9mg/kg DW) concentration was greater than the values reported in Amarakoon et al. (2012). A study by Gawalko et al. (2009) reported 45 to 49mg/kg of Fe, 32 to 35mg/kg of Zn, 786 to 802mg/kg of Ca and 1210 to 1270mg/kg of Mg in western Canadian dry pea. The range of mineral concentration among genotypes across two years and six locations was 49.4 to 56.1µg/g DW for Fe, 33.6 to 44.3µg/g DW for Zn, 550 to 1041mg/kg DW for Ca and 1260 to 1454mg/kg DW for Mg, which clearly shows that the concentration range of these four elements tested are higher in our germplasm. This might be due to the differences in genetic potential and environmental conditions like rain fall, soil health and nutrient availability, temperature, soil moisture, soil temperature, and photoperiod. We found a significant three way interaction (genotype x location x year) interaction for Ca, Cu, Fe, K, Mg, and Mn concentration, but not for Zn concentration in dry pea genotypes (Table 2.3) planted in six locations for two consecutive years (2012-2013). In a report by HarvestPlus, Ashutosh Sarker reports Zn being stable across environment compared to Fe in lentil. A significant genotype x environment interaction was reported by Amarakoon et al. (2012) for Ca, Mg, Fe, and Zn while testing for a single year. Ray et al. (2014) reported that a significant variety x location x year interaction was present for Cu, Fe, K, Mg, Mn, and Zn while testing for two years in south and central Saskatchewan, Canada.

DS Admiral (1041 mg/kg DW) was the highest Ca accumulator across both years and locations. Amarakoon et al. (2012) also reported that DS Admiral had the highest Ca accumulation in their study. Highest Cu concentration was found in breeding line PS07ND0190 across both years and locations. Iron was found in high concentration in breeding line PS07100716 across both years and locations. Breeding line PS07ND0163 had high K concentration in the seed and breeding line PS07ND0110 had high Mg concentration in the seed when planted across both years and locations. Commercial lines (check variety) Aragorn was highest Mn and P accumulator in seed whereas Cooper had the highest Zn concentration in the seed when planted across 6 locations and 2 years.

Soil analysis also shows that NCREC, CREC and WREC had the highest plant available Ca and Mg in the soil (Table 2.5). Dry pea grown at NCREC in 2013 had the highest Ca, Cu, Fe, Mg, Mn and P concentration in the seed whereas the dry pea grown in CREC had the highest K concentration in the seed (Table 2.5). Field pea grown in Prosper had the highest Zn concentration in 2013. Langdon (7.8) was one of the locations with high pH and followed by Prosper (7.5). Soil tests from the NCREC location showed the mineral elements to be low to moderately high compared to the other locations. Soil tests from Prosper had the highest Zn concentration among the research sites in 2013 (Table 2.5). Amarakoon et al. (2012) found that field pea grown at Minot had the highest Zn concentration. Further research is warranted to understand the soil-seed mineral concentration relationships at these locations.

Average rainfall and mean air temperature during the growing season at these locations varied between years and among locations (Table A1). The mean air temperature among six locations in 2012 ranged from 16.3°C to 19.0°C. CREC had a mean air temperature of 17.2°C, HREC was 18.1°C, LREC 16.3°C was, NCPEC was 18.3°C, Prosper was 19.0°C, and WREC was 18.7°C (NDAWN, 2014). Average rainfall during the growing season (May to September) ranged from 33.0mm to 55.9mm in 2012 where, CREC had 48.3mm HREC had 53.3mm,

LREC had 55.9mm, NCREC had 33.0mm, Prosper had 33.0mm and WREC had 35.6mm. In 2013 the mean air temperature range was 16.0°C to 18.9°C where, CREC was 16.9°C, HREC was 17.2°C, LREC 16.0°C was, NCREC was 17.2°C, Prosper was 18.9°C, and WREC was 17.9°C (NDAWN). Average rainfall during the growing season (May to September) ranged from 43.2mm to 121.9mm in 2013 where, CREC received 43.2mm of rain, HREC received 96.5mm of rain, LREC received 58.4mm of rain, NCREC received 121.9mm of rain, Prosper received 91.4mm of rain and WREC received 81.3mm of rain. This clearly shows that 2013 was a wet and cool year compared to 2012.

Pearson's correlation coefficient analysis showed numerous significantly positive and negative correlations among/within mineral elements and yield parameters. Some significant correlations between mineral elements were repeated across locations and between years. Particularly, the significant negative correlation between Ca and K was found in all six locations of 2013 and 2012 except LREC in 2012. The significant positive correlation between Zn and P was also detected in all the locations except NCREC in both years and Prosper in 2012. However, the correlation was still positive in NCREC and Prosper, which suggests that selection and breeding for Zn will also improve P content. Significant positive correlation between Ca and Mg was also detected in multiple locations and between years (r = 0.34). Additionally, a positive correlation was detected between Ca and Mn across all locations and years tested. Correlation between yield parameters and the mineral element varied among locations and years, but were low in all cases. A significant correlation was detected between seed yield and one thousand seed weight.

2.5. Conclusions

This research supports the global effort of reducing nutritional deficiency and disorder caused by low mineral nutrients in food. As the current food supply is expected to be insufficient to support growing populations both in quantity and nutritional quality

(HarvestPlus, 2014), the necessity of providing an economic and efficient access to a nutrient dense crop is in high demand. Biofortification through plant breeding is one of the best measures to meet this nutrient crisis and biofortification in pulse crops is an ideal approach to reach out to the malnourished and mineral deficient, needy people in many developing nations where these crops are mainly consumed. Information on environmental factors, genetic ability and the response of the genotype on the environment being tested will help in breeding for the nutrient test in future. As this research found a significant interaction between genotype and the environment, these factors needs to be of major concern while planning for any similar analysis in future. Different genotypes are found to be the highest accumulator of different mineral elements, based on this information we can categories genotypes as higher accumulator of specific element. This information will be useful in improving the concentrations of these elements within the genotype and across different dry pea genotypes.

CHAPTER 3. GENETIC AND ENVIRONMENTAL VARIATION ON MINERAL NUTRIENT CONCENTRATION IN LENTIL SEED

3.1. Introduction

Lentil (*Lens culinaris* Medik.) is a self-pollinated, diploid ($2n = 2x = 14$) species characterized by lens shaped seed and is one of the oldest domesticated pulse crops (Erskine et al., 2009; Harlan, 1992). There are many hypotheses regarding the area and center of origin of this crop. Most biologists believe that it originated from the eastern border of southwest Asia (Barulina, 1930). According to Duke (1981), it spread from the Near East to the Mediterranean region then east to India and north to Europe. Domestication started from Neolithic agricultural time (Harlan, 1992) and was first cultivated in the Fertile Crescent which later moved to Greece, Central and Western Europe along the Danube, to the Nile Delta and eastward to India (Erskine et al., 2009).

Lentil is a cool season annual crop that is grown as a rain fed, summer crop in the Northern Plains of the U.S. and Canada. It is an annual herbaceous, softly pubescent plant that attains 15-75 cm in height (Duke, 1981) and has an indeterminate growth habit. "It is a good source of many nutrients (K, P, Fe, Zn, Fe) and vitamins" (Bhatty, 1986). Lysine and tryptophan are present at relatively high levels in lentil and when consumed with cereal crops they provide a complementary amino acid profile for human diets (Erskine et al., 2009). Lentil is tolerant of different soil types and grows well in limited rainfall and production regions that are considered marginal for other crops (Erskine et al., 2009). Young plants are tolerant of spring frosts which allows for early spring planting dates in frost prone areas (Saxena and Hawtin, 1981). It is mostly cultivated in warm temperate, subtropical, and tropical regions. In the Mediterranean region of west Asia and North Africa, plants complete the vegetative and reproductive growth phase and reach maturity in 75 to 100 days after sowing, however, the season might extend to 120 to160, and even 180 days for winter sown crops due to sub-optimal

ambient temperature (Saxena and Hawtin, 1981). “Lentils are quantitative long day flowering plants which are suitable especially for arid and semi-arid regions; however, they are successfully grown in all soil types and climates” (Muehlbauer et al., 2009).

Lentil are high in Fe, protein, dietary fiber, folate, manganese, phosphorous, thiamin and tannins in the lentils have antimicrobial properties which act as an antioxidant and reduce blood pressure, lower cholesterol, and help regulate the immune response (USA Dry Pea and Lentil Council, 2014). Lentil seeds provide many health benefits and are a rich source of protein and minerals including K, P, Fe, and Zn (Bhatty, 1986). Grusak (2009) estimated that whole dry lentil seeds contain approximately 1638 KJ energy, 28.3 g protein, 67.1 g carbohydrate, 2.5 g fat, 12.2 g total fiber, and 2.2 g ash, 42-165 mg Ca, 13-167 mg, 240-1287 mg P, 38-1360 mg K, 3.1-13.1 mg Fe, 2.3-10.3 mg Zn, 0.6-1.0 mg Mn, 0.4-9.9 mg Cu, 0.4-79 mg Na, 0.6-1.0 mg Se per 100g dry matter. Lentil is a rich source of protein carbohydrate, vitamins, dietary fiber oligosaccharide, resistant starch and a wide range of micronutrients (Johnson et al., 2013) particularly, Fe, Zn, Mg, Mn. It has a primary role in supplying these essential elements in the daily diet of populations in developing countries and also in vegetarian diets worldwide. Primarily, lentil was consumed and grown in developing countries, especially in India and Turkey (Erskine et al., 1990). Lentils soup is popular across North and South America and Europe. In India and elsewhere, lentils are often combined with rice and consumed as a soup or joined with vegetables and boiled to a stew-like also known as Indian dhal. Lately this crop gained popularity among health conscious people because of its health benefits, and recently this crop has been marketed as a snack, baking flour, and is included in many health recipes. This wide range of health benefits has contributed to lentil being an important component of diets worldwide.

In general, the lentil crop can be classified into several market classes based upon the size and the cotyledon color. Lentil can be divided in to six different market classes; the small

red also known as Turkish red, small green, medium green, large green, Pardina and zero tannin types. The Pardina lentil is also known as Spanish brown, is favored for its excellent taste (slightly nutty) and its cooking characteristics. However, USA Dry Pea and Lentil Council classified lentil on 9 different classes based on its availability in U.S. They are 1) USA Pardina lentils: These lentils has a good cooking quality as it maintains their shape and texture very well when cooked and do not fall apart even if slightly overcooked. Over 90 percent of the world's production is now grown in Washington and Idaho. 2) Large green lentil: These are mostly favored among many South American countries. 3) USA Richlea Lentils: these are of medium-sized lentils having greenish-tan and have a similar size and color that of Regular lentil but lack mottling. 4) USA Red Chief Lentils: these red lentils are quick cooking because the brown outer skin is typically removed. Simply boil for six to eight minutes for a great visual and nutritional addition to any meal. Try adding to soups as a thickener or to salads for something different. 5) USA Regular Lentils: these are greenish-tan lentil and are most commonly available in the U.S which is also known as the Brewer lentil. Its mottled appearance was inherited from its Chilean lentil parents. 6)USA Crimson Lentils: these are thought to have originally derive from Turkish red lentils and are colorful, small-sized lentils having pinkish brown skin that covers a red seed. 7) USA Beluga Lentils: these are small, black lentils which are name at its black appearance or similar appearance to Beluga caviar. 8) USA French Green Lentils or du Puy lentil: when du Puy lentil grown in U.S. they are called USA French Green Lentils. They have appealing visuals and nutritious lentils. 9) USA Eston Lentils: these are small green or tanned lentils and are most often exported as the U.S. grown Eston lentil have good cooking quality unlike Eston lentil from outside

Among plant-based foods lentil has the highest level of protein after couscous, barley, and beans and is also one of the best sources of Fe (USA Dry Pea and Lentil Council, 2014). Their consumption is high in the diet of many parts of the world, especially on the Indian

subcontinent (USA Dry Pea and Lentil Council, 2014) where mineral deficiency is also high. Therefore increasing the mineral concentration in crops grown and consumed among nutrition deficient population can be more effective. Therefor this research was aimed to support the global move against malnutrition and mineral deficiency. Additionally, the research also aimed to categorize factors interfering nutrients concentration in lentil seed and quantify the amount of mineral nutrients present in the seeds. Information will be crucial in recommending this crop for human benefit as well as further breeding and selection of this crop in U.S.

3.2. Materials and Methods

3.2.1. Lentil Genotypes

Twenty-five Turkish red lentil breeding lines and check varieties were evaluated (Table 3.1) in the Advanced Red Lentil Yield Trial across 5 site-years in North Dakota, 2013-CREC, 2012-HREC, 2012-NREC, and 2012-WREC and 2013-WREC (Table 3.3). Eleven breeding lines and check varieties that were common across five site-years (location and year combined) were used for analysis of variance and Pearson's correlation coefficient analysis.

Twenty-three small green lentils advanced breeding lines and check varieties (Table 3.1) were evaluated in the Advanced Small Green Lentil Yield Trial across six site-years in North Dakota, 2012-HREC, 2012-NCREC, 2012-WREC, 2013-CREC, 2013-HREC, and 2013-WREC (Table 3.2). Nine small green lentil breeding lines and check varieties common across all six-site years were used for analysis of variance and Pearson's correlation coefficient analysis.

Table 3.1. Entries in the Advanced Turkish Red and Small Green Lentil Yield Trials planted in 2012 and 2013 in North Dakota.

Red/Turkish red	Cotyledon color	Small green	Cotyledon color
CDC REDBERRY¥	Turkish red	CDC VICEROY¥	small green
CDC RED RIDER¥	Turkish red	ESSEX¥	small green
CDC ROBIN¥	Turkish red	LC07ND055E¥	small green
CDC ROSETOWN¥	Turkish red	LC07ND057E¥	small green
LC06601950T	Turkish red	LC07ND059E	small green
LC07ND134T	Turkish red	LC07ND063E¥	small green
LC07ND139T	Turkish red	LC07ND066E	small green
LC07ND142T	Turkish red	LC07ND068E¥	small green
LC07ND148T¥	Turkish red	LC07ND070E	small green
LC07ND162T¥	Turkish red	LC07ND074E	small green
LC07ND165T¥	Turkish red	LC07ND082E¥	small green
LC07ND172T¥	Turkish red	LC07ND087E¥	small green
LC07ND173T¥	Turkish red	LC07ND090E	small green
LC07ND183T¥	Turkish red	LC07ND098E	small green
LC07ND185T	Turkish red	LC07ND102E¥	small green
LC07ND202T¥	Turkish red	NDL080527E	small green
NDL090347T	Turkish red	NDL090185E	small green
NDL090353T	Turkish red	NDL090203E	small green
NDL090368T	Turkish red	NDL090204E	small green
NDL090389T	Turkish red	NDL090215E	small green
NDL090413T	Turkish red	NDL090277E	small green
NDL090541T	Turkish red	NDL090282E	small green
NDL090542T	Turkish red	NDL090288E	small green
NDL090578T	Turkish red		
NDL090580T	Turkish red		

¥ indicates common genotypes in the 2012 and 2013 trials

3.2.2. Field Experiments

Statewide small green (Table 3.2) and red (Table 3.3) lentil trials were conducted in North Dakota in 2012 and 2013 at the HREC, WREC, NCREC, and CREC. Trials at each research location were grown under rain fed conditions and established using a randomized complete block design with four replications. Plot size ranged from 6.9-11.6 square meters per plot and 6-8 rows per plot depending on the equipment available at each location. Row spacing was 18 cm and the targeted seed density was 16 lentil plants per square meter. Grassy and broad leaf weeds were controlled at each location according to local management practices while in crop weeds were controlled manually by hand weeding when necessary.

3.2.3. Mineral Nutrient Analysis in Seed

Fifty grams of whole seed from each replicate plot was thoroughly cleaned removing off-types, debris and broken seeds. The cleaned sample was milled using a UDY mill to pass through a ~0.5mm sieve. The finely ground samples were analyzed for total mineral nutrient concentration in the USDA-ARS Children's Nutrition Research Laboratory, Houston, Texas (Farnham et al., 2011).

3.2.4. Soil Sampling and Mineral Analysis

Four representative soil samples were collected from each trial site in North Dakota. Soil cores representing the 0-15 cm and 16-30 cm were taken and stored separately until they were analyzed. The mean concentration of each mineral nutrient in the 0-15 cm and 16-30 cm soil samples were recorded. Soil samples were analyzed for eight mineral elements (Ca, Cu, K, Fe, Mg, Mn, P, and Zn) at the North Dakota State University soil testing lab following the mineral extraction method explained in the "Recommended Chemical Soil Test Procedures for the North Central Region" (Brown, 1998).

3.2.5. Statistical Analysis

Analysis of variance (ANOVA) using PROC-MIXED of SAS (release 9.3, SAS Institute, Inc., Cary, NC) was performed to partition the environmental, genotype, and genotype x environment variance components for Ca, Cu, Fe, K, Mg, Mn, and Zn concentration in the seed across environments and years. Location, environment and year were considered as random effects and genotype as a fixed effect, whereas Pearson's Correlation Coefficient was used to identify the relationship between and among mineral nutrients. Pearson's correlation coefficient was also calculated between/among mineral elements and the yield parameters. The yield parameters used for this study were seed yield (SY), test weight (TW) and one thousand seed weight (TSW).

3.3. Results

3.3.1. Advanced Red Lentil Yield Trial

Soil pH ranged from 6.3 to 7.7 with CREC having the highest pH at 7.7 and Hettinger had the lowest pH at 6.3 (Table 9). Calcium was highest at Carrington (6930 ppm), Cu was highest in Hettinger (4210 ppm), Fe was highest at NCREC (57.5 ppm), K was highest at Hettinger (455 ppm), Mg was highest at NCREC (770 ppm), P was highest in Carrington (22 ppm) and Zn was highest in Carrington (1.54 ppm). Based on the soil analysis all locations had adequate mineral content for optimum plant growth (Table 3.2).

Table 3.2. Plant available soil mineral nutrients analyzed from top 0-30 cm of the 2013 Advanced Red and Small Green Lentil Yield Trials grown at four locations in North Dakota.

Mineral nutrients in soil	Ca	Cu	Fe	K	Mg	Mn	P	Zn	pH
CREC-2013	6930	0.60	10.30	230	490	4.50	22	1.50	7.70
HREC-2013	4210	1.20	46.80	455	410	17.90	19	0.80	6.30
WREC-2013 green	4020	1.10	33.30	272.50	440	15.90	12	0.40	6.50
WREC-2013 red	4090	1.00	31	240	480	14.30	10	0.60	6.50
NCREC-2013	6400	0.96	57.50	272.50	770	14.20	4.50	0.40	7.10

Seed mineral nutrient concentration of Ca, Cu, K, Mg, Mn, P, and Zn for the 2013 Advanced Red Lentil Yield Trial was greater at WREC compared to seed from CREC; however, seed from CREC had a greater concentration of Fe. Seed concentration of Ca, Cu, Mn and P were greatest at WREC while K and Zn were greatest at HREC and Fe and Mg were greatest at CREC (Table 3.3).

Table 3.3. Location estimates for seed mineral concentration of the 2013 Advanced Red Lentil Yield Trials locations in North Dakota.

Location	Ca	Cu	Fe	K	Mg	Mn	P	Zn
CREC-2013	-1.65	-2.22	1.37	-18.11	-14.11	-1.60	-381.97	-2.00
WREC-2013	1.65	2.22	-1.37	18.11	14.11	1.60	381.97	2.00
y-intercept	711.86	6.83	80.45	8419.19	1025.20	17.91	4115.29	46.04

Note: y-intercept is an estimate of the experimental mean derived from PROC Mixed.

Analysis of variance for the Advanced Red Lentil Yield Trial included data from HREC, WREC, and NCREC in 2012 and CREC and WREC in 2013. Due to only one common location between years location and year were combined and represented as 5 site-years for the

purpose of analysis. Variation among varieties was significant for all mineral nutrients except P (Table 3.4). Environment main effects (Location x Year) were highly significant for all the mineral elements. The variety x environment interaction was also moderately to highly significant for all mineral nutrients (moderately= $P<0.01$, highly =$P<0.001$) but not for Mg.

Table 3.4. Analysis of variance of 11 Advanced Red Lentil Yield Trial grown at five site-years in North Dakota in 2012 and 2013.

Sources of Variance	Ca (mg/g DW)	Cu (µg/g DW)	Fe (µg/g DW)	K (mg/g DW)	Mg (mg/g DW)	Mn (µg/g DW)	P (mg/g DW)	Zn (µg/g DW)
Variety	<.0001***	0.0033**	<.0001***	<.0001***	<.0001***	<.0001***	0.0505ns	<.0001***
Environment	<.0001***	<.0001***	<.0001***	<.0001***	0.0003***	0.0003***	0.0002***	<.0001***
Block (Environment)	0.0123*	<.0001***	<.0001***	<.0001***	0.0002***	<.0001***	<.0001***	<.0001***
Environment*Variety	0.0010**	<.0001***	<.0001***	<.0001***	0.2449ns	<.0001***	0.0041**	0.0002**

Note: * indicates significance at $P<0.05$, ** indicates significance at $P<0.01$, and *** indicates significance at $P<0.001$.

LS means for all genotypes across locations and years showed that variety had a wide range of variation for each mineral nutrient. Ca concentration ranged from 600.67 to 921.99mg/g DW, Cu ranged from 8.4 to 9.7µg/g DW, Fe ranged from 57.9 to 73µg/g DW, K ranged from 7457.4 to 8438.6mg/kg DW, Mg ranged from 983.3 to 1126mg/kg DW, Mn ranged from 16 to 21.6µg/g DW, P ranged from 4013.6 to 4408.1mg/kg DW, and Zn ranged from 41.3 to 47.8µg/g DW.

Among the eleven common red lentil genotypes CDC Red rider (921.99mg/kg DW) had the highest Ca concentration across environments (Table 3.5) and years followed by LC07ND183T (885.83mg/kg DW) and CDC Redberry (791.50mg/kg DW). Breeding line LC07ND173T (9.68µg/g DW) had the highest Cu concentration (Table 3.5) followed by LC07ND148T (9.61µg/g DW) and CDC Rosetown (9.58µg/g DW). CDC Rosetown (72.96µg/g DW) had the highest Fe concentration (Table 3.5) in the seed across environments and years followed by LC07ND183T (70.43µg/g DW) and CDC Robin (68.94µg/g DW). Breeding line LC07ND183T (8438.59mg/kg DW) showed the highest K concentration (Table 3.5) in the seed across environments and years followed by CDC Rosetown (8341.22 mg/kg DW) and LC07ND165T (8218.12mg/kg DW). CDC Red Rider (1126.0 mg/kg DW) had the highest Mg concentration (Table 3.5) in the seed across years and environments followed by LC07ND202T (1118.34 mg/kg DW) and CDC Robin (1081.67 mg/kg DW). CDC Red Rider (21.58 µg/g DM) had the highest Mn concentration (table 14) across environments and years followed by LC07ND183T (20.96µg/g DW) and CDC Robin (20.88µg/g DW). Breeding line LC07ND165T (4408.05mg/kg DW) showed the highest P concentration (Table 3.5) in the seed across environments followed by CDC Robin (4167.17mg/kg DW) and LC07ND148T (4146.8mg/kgDW). LC07ND165T (47.80µg/g DW) had the highest Zn concentration (Table 3.5) across environments followed by CDC Rosetown (46.71µg/g DW) and CDC Red Rider (45.89µg/g DW).

Table 3.5. LS mean estimates for mineral nutrient concentration for eleven red lentil genotypes that were common across site years.

Red lentil genotypes	Ca (mg/kg DW)	Cu (µg/g DW)	Fe (µg/g DW)	K (mg/kg DW)	Mg (mg/kg DW)	Mn (µg/g DW)	P (mg/kg DW)	Zn (µg/g DW)
CDC Redberry	791.50	8.40	68.23	7970.14	983.28	19.46	4106.68	45.35
CDC RedRider	921.99	8.57	60.77	8156.84	1126.00	21.58	4038.28	45.89
CDC Robin	630.77	9.52	68.94	7941.21	1081.67	20.88	4167.17	44.69
CDC Rosetown	600.67	9.58	72.96	8341.22	1021.37	19.97	4043.31	46.71
LC07ND148T	717.41	9.61	61.58	7920.85	1043.83	17.11	4146.76	42.54
LC07ND162T	640.90	9.52	59.03	7836.40	1014.98	15.97	4013.59	41.23
LC07ND165T	658.22	9.48	64.11	8218.11	1050.16	17.09	4408.05	47.80
LC07ND172T	658.38	9.34	57.87	8027.85	1023.67	15.90	4097.94	41.63
LC07ND173T	772.88	9.68	63.26	7863.86	1056.59	17.78	4105.75	44.86
LC07ND183T	885.83	8.87	70.43	8438.59	1080.12	20.96	4073.37	44.60
LC07ND202T	762.89	8.41	62.94	7457.39	1118.34	17.44	4049.85	41.31

Mineral concentration in the seed of the red lentil genotypes planted in 5 different site-years (Table 3.6) shows that there is variation across site-years. PROC-MIXED calculated estimates of the mineral concentration in different locations shows that CREC-2013 had the highest estimated Ca and Fe concentration. WREC-2013 had high K and P concentration whereas, Cu, Mg and Zn concentration were highest in at HREC in 2013.

Table 3.6. Estimates of mean seed mineral nutrient concentration for the Advanced Red Lentil Yield Trials in 2012 and 2013.

Site-years	Ca	Cu	Fe	K	Mg	Mn	P	Zn
HREC-2012	-59.67	1.99	1.26	138.41	52.43	-0.27	74.17	7.26
NCREC-2012	-65.99	0.44	-8.41	122.87	11.89	2.56	-9.27	-6.35
WREC-2012	-74.78	1.89	-5.11	-493.33	-70.12	0.09	-361.74	1.11
CREC-2013	114.53	-4.34	7.01	70.73	9.57	-2.93	-162.84	-3.00
WREC-2013	85.91	0.02	5.25	161.31	-3.77	0.55	459.69	0.98
y-intercept	600.67	9.58	72.95	8341.22	1021.37	19.96	4043.31	46.71

Note: y-intercept is an estimate of the experimental mean derived from PROC Mixed.

Pearson's correlation coefficients shows that Ca concentration in the red lentil trial at HREC in 2012 had a significant positive correlation with Mg (r=0.672, P<0.05) (Table 3.7) and a significant negative correlation with Cu (r=0.699, P<0.05) and test-weight (r=0.637, P<0.05). Magnesium showed a significant positive correlation at P<0.05 with Mn (r= 0.657).

Zinc showed a significant positive correlation with K (r=0.610) at P<0.05 where as a significant positive correlation with P (r=0.780) at P<0.01.

Pearson's correlation coefficient between/among mineral elements and yield parameters for the red lentil advanced yield trial planted at NCREC in 2012 showed that none of the 9 mineral elements tested had a significant positive or negative correlation with seed yield and one thousand seed weight except Ca which showed a significant negative correlation with test weight at P<0.05 (r= -0.623) (Table 3.8). Zinc showed a significant positive correlation at P<0.05 with Cu (r= 0.617), Fe (r= 0.725), K (r= 0.623) and P (r= 0.672). Copper showed a significant positive correlation with P (r= 0.776, P<0.01).

Pearson's correlation coefficient between/among mineral elements and yield parameters from the 11 red lentil advanced cultivar planted in WREC-2012 showed (Table 3.9) that none of the 8 mineral elements tested had a significant positive and negative correlation with seed yield and thousand seed weight except Fe (r= 0.716), Zn (P= 0.606) and Mn (r= 0.685) which showed a significant positive correlation with seed yield at P<0.05. Calcium showed a significant negative correlation with Cu (r= -0.679) at P<0.05. A significant negative correlation was also seen between Mn and P (r= -0.673) at P<0.05. Zinc showed a significant positive correlation with K (r= 0.646) at P<0.05.

Table 3.7. Pearson's correlation coefficients between mineral elements and yield parameters for the red lentil trial planted at HREC in 2012.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	-0.699*	-0.028	0.194	0.672*	0.540	0.032	0.031	-0.381	-0.637*	0.400
Cu		1	0.219	0.260	-0.332	-0.263	0.267	0.332	0.516	0.481	-0.201
Fe			1	0.429	0.125	0.533	0.339	0.544	-0.043	-0.087	-0.546
K				1	0.107	0.313	0.471	0.610*	0.048	-0.497	0.306
Mg					1	0.657*	0.153	0.139	0.020	-0.252	0.059
Mn						1	0.042	0.073	-0.366	-0.516	-0.319
P							1	0.780**	-0.113	0.147	0.115
Zn								1	0.030	0.036	0.248
SY									1	0.494	-0.015
TW										1	-0.312
TSW											1

Note: $P<0.05$ = *, $P<0.01$, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 3.8. Pearson's correlation coefficients between mineral elements and yield parameters in 11 red lentil genotypes planted at NCREC in 2012.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	-0.551	-0.532	0.051	0.401	0.077	-0.254	-0.238	-0.023	-0.623*	0.543
Cu		1	0.328	0.377	0.084	-0.186	0.776**	0.617*	-0.393	0.276	-0.088
Fe			1	0.451	-0.100	0.259	0.309	0.725*	-0.459	-0.197	-0.384
K				1	0.036	0.367	0.500	0.716*	-0.409	-0.591	0.306
Mg					1	-0.350	0.400	0.246	-0.242	-0.277	-0.006
Mn						1	-0.233	0.254	-0.036	-0.165	-0.195
P							1	0.672*	-0.338	-0.022	0.079
Zn								1	-0.476	-0.230	-0.086
SY									1	0.486	0.251
TW										1	-0.417
TSW											1

Note: $P<0.05$ = *, $P<0.01$, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 3.9. Pearson's correlation coefficients between mineral elements and yield parameters in 11 red lentil genotypes planted in WREC in 2012.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	-0.679*	-0.138	-0.120	0.573	0.601	-0.528	0.028	0.095	-0.345	0.329
Cu		1	0.149	0.429	-0.317	-0.298	0.202	0.252	-0.179	0.274	-0.248
Fe			1	0.318	-0.045	0.329	-0.342	0.389	0.716*	-0.039	-0.462
K				1	-0.197	0.357	-0.262	0.646*	0.271	-0.565	0.294
Mg					1	0.537	-0.089	-0.029	0.245	0.352	-0.268
Mn						1	-0.673*	0.425	0.685*	-0.421	0.091
P							1	-0.198	-0.380	0.588	-0.150
Zn								1	0.606*	-0.234	0.424
SY									1	-0.122	-0.110
TW										1	-0.600
TSW											1

Note: $P<0.05$ = *, $P<0.01$, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Pearson's correlation coefficient between/among mineral elements and yield parameters from the 11 red lentil advanced cultivars planted in CREC in 2013 (Table 3.10) showed more correlations with yield than in any locations. Potassium showed a significant positive correlation with seed yield (r= 0.656) at P<0.05. Test weight showed a significant negative correlations with Ca (r= -0.652), Mn (r= -0.641) at P<0.05 and a significant negative correlation with seed yield (r= -0.826) at P<0.01. Thousand seed yield also showed a significant negative correlation with seed yield (r= -0.767) at P<0.01, and K (r= -0.685) at P<0.05 where as a significant positive correlation with test-weight (r= 0.720) at P<0.05. Significant positive correlations of Zn was observed with Fe (r= 0.819), K (r= 0.756), and P (r= 0.740) at P<0.05 whereas significant positive correlation at P<0.01 was observed with Mn (r= 0.705). Significant positive correlations of Mn was observed with Fe (r= 0.768) P<0.01 and with K (r= 0.621) at P<0.05. Magnesium showed a positive correlations with Cu (r= 0.602) P<0.05.

Pearson's correlation coefficient of mineral nutrient and yield parameter on eleven advanced red lentil cultivar planted in WREC-2013 (Table 3.11) showed a significant positive correlation of test-weight with seed yield (r= 0.628) whereas a significant negative correlation of test-weight with Cu (r= -0.722) at p<0.05.

Table 3.10. Pearson's correlation coefficients between mineral elements and yield parameters in 11 red lentil genotypes planted in CREC in 2013.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	0.022	-0.006	0.046	0.194	0.500	-0.069	0.000	0.325	-0.652*	-0.244
Cu		1	0.009	-0.602	0.602*	-0.099	-0.060	-0.291	-0.251	-0.002	0.292
Fe			1	0.524	0.121	0.768**	0.546	0.819**	0.395	-0.371	-0.255
K				1	-0.207	0.621*	0.320	0.756**	0.656*	-0.508	-0.685*
Mg					1	0.312	0.469	0.137	0.017	-0.162	0.343
Mn						1	0.454	0.705*	0.494	-0.641*	-0.293
P							1	0.740**	0.425	-0.351	-0.048
Zn								1	0.586	-0.479	-0.485
SY									1	-0.826**	-0.767**
TW										1	0.720*
TSW											1

Note: $P<0.05$ = *, $P<0.01$, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 3.11. Pearson's correlation coefficients between mineral elements and yield parameters in 11 red lentil planted genotypes planted in WREC, 2013.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	-0.297	0.306	-0.001	0.516	0.588	-0.219	0.256	0.313	-0.120	-0.549
Cu		1	-0.092	0.331	-0.252	-0.359	0.280	-0.371	-0.662*	-0.722*	-0.226
Fe			1	0.528	-0.106	0.692*	-0.043	0.490	-0.356	-0.013	0.030
K				1	-0.153	0.124	0.416	0.286	-0.442	-0.079	-0.334
Mg					1	0.358	-0.353	-0.034	0.173	0.087	-0.063
Mn						1	-0.308	0.479	0.068	0.122	0.149
P							1	0.433	-0.363	-0.104	-0.054
Zn								1	0.132	0.376	0.184
SY									1	0.628*	-0.158
TW										1	0.247
TSW											1

Note: $P<0.05$ = *, $P<0.01$, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

3.3.2. Small Green Lentil

Analysis of variance was performed for mineral nutrient concentration of nine common genotypes in the Advanced Small Green Lentil Yield Trial grown at three locations (HREC, WREC, and NCREC) in 2012 and three locations (HREC, WREC and CREC) in 2013. Due to the unbalanced data across years and locations the locations and years were combined to represent six environments.

The analysis of variance showed that variety main effect was significant for all the mineral nutrient concentrations with the exception of Mn (Table 3.12). The environment (Location*Year) main effect was significant for all the mineral elements analyzed with the exception of Mg and Mn. The variety x environment interaction was highly significant for Mg, (P<0.0001), moderately significant for Ca, Cu, K, and Zn (P<0.01, and Fe, Mn and P were significant at P<0.05.

Table 3.12. Analysis of variance of 9 Advanced Small Green Lentil genotypes grown at 5 site-years in North Dakota in 2012 and 2013.

Sources of Variation	Ca (mg/kg DW)	Cu (µg/g DW)	Fe (µg/g DW)	K (mg/kg DW)	Mg (mg/kg DW)	Mn (µg/g DW)	P (mg/kg DW)	Zn (µg/g DW)
Variety	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	0.5586ns	0.0009***	0.0003***
Environment	0.0005***	<.0001***	0.0003***	<.0001***	0.0517ns	0.6116ns	0.0136*	0.0214**
Block (Env)	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***
Env*Variety	0.0062**	0.0178**	0.0416*	0.0040**	<.0001***	0.0449*	0.0354*	0.0013**

Note: $P<0.05$ = *, $P<0.01$, and $P<0.001$ = ***

LS means for variety across locations and years (Table 3.13) indicated a wide range of variation for each mineral nutrient. Ca ranged from 506 to 681mg/g DW, Cu ranged from 7.8 to 9.5µg/g DW, Fe ranged from 59.5 to 70.8µg/g DW, K ranged from 7950 to 8551mg/g DW, Mg ranged from 996.7 to 1087.3mg/g DW, Mn ranged from 18 to 19.8µg/g DW, P ranged from 3578.6 to 3960mg/g DW, and Zn ranged from 39.5 to 42.2µg/g DW.

Table 3.13. LS means estimates for mineral nutrient concentrations for small green lentil genotypes grown at six site years in 2012 and 2013.

Small Green Lentil Genotypes	Ca (mg/kg DW)	Cu (µg/g DW)	Fe (µg/g DW)	K (mg/kg DW)	Mg (mg/kg DW)	Mn (µg/g DW)	P (mg/kg DW)	Zn (µg/g DW)
CDC Viceroy	665.14	9.42	69.17	8163.39	1067.17	19.71	3959.92	41.59
Essex	642.05	8.01	59.44	8440.27	1069.78	19.65	3601.32	39.80
LC07ND055E	506.01	8.23	60.67	8550.91	1087.28	18.85	3702.48	39.67
LC07ND057E	537.47	8.57	64.31	8326.27	1060.97	19.54	3578.58	39.46
LC07ND063E	569.37	8.72	66.13	8061.43	1049.29	17.97	3767.05	40.35
LC07ND068E	548.03	8.20	64.19	8075.11	996.65	18.60	3612.80	39.98
LC07ND082E	680.94	8.45	67.57	7949.67	1028.86	19.23	3691.10	41.05
LC07ND087E	578.25	7.87	69.25	8332.45	1039.37	19.16	3599.10	42.23
LC07ND102E	587.02	7.70	70.73	8471.55	1043.60	18.19	3743.18	42.11

Among small green lentil genotypes (Table 3.13) LC07ND082E had the highest Ca concentration (680.94 mg/kg DW) across environments followed by CDC Viceroy (665.14mg/kg DW) and Essex (642.05mg/kg DW). CDC Viceroy had the highest Cu concentration (9.42µg/g DW) followed by LC07ND063E (8.72µg/g DW) and LC07ND057E (8.57µg/g DW). LC07ND102E had the highest Fe concentration (70.73µg g^{-1} DW) across environments followed by LC07ND087E (69.25µg/g DW) and CDC Viceroy (69.17µg/g DW). LC07ND055E (8550.91mg/kg DW) showed the highest K concentration in the seed across environments followed by LC07ND102E (8471.55mg/kg DW) and Essex (8440.27mg/kg DW). Breeding line LC07ND055E (1087.28mg/kg DW) showed the highest Mg concentration in the seed across environments followed by Essex (1069.78mg/kg DW) and CDC Viceroy (1067.17mg/kg DW). CDC Viceroy (19.71µg/g DW) had the highest Mn concentration across environments followed by Essex (19.61µg/g DW) and LC07ND057E (19.54µg/g DW). CDC Viceroy had the highest P concentration (3959.92mg/kg DW) in the seed across environments

followed by LC07ND163E (3767.05mg/kg DW) and LC07ND102E (3743.18mg/kg DW). LC07ND087E (42.23µg/g DW) had the highest Zn concentration in the seed across environments followed by LC07ND102E (42.11µg/g DW) and CDC Viceroy (41.59µg/g DW).

Table 3.14. ICP-EMS analyzed seed mineral nutrient of the 2012-2013 Small Green lentil Advanced Yield Trials at six environment in North Dakota.

Environment estimates	Ca	Cu	Fe	K	Mg	Mn	P	Zn
HREC-2012	-7.24	1.46	-1.24	201.46	11.69	-1.89	-90.30	2.70
NCREC-2012	-15.35	-0.48	-7.72	385.38	8.97	13.00	34.00	-3.92
WREC-2012	-91.22	1.78	-1.47	-312.58	-20.89	-1.22	-79.88	3.48
CREC-2013	38.66	-2.50	7.17	-325.51	-2.37	-4.07	-5.58	-2.28
HREC-2013	3.09	-0.49	-0.49	411.33	5.37	-4.87	-92.37	0.50
WREC-2013	72.06	0.23	3.74	-360.08	-2.79	-0.96	234.13	-0.48
y-intercept	665.14	9.42	69.17	8163.39	1067.17	19.71	3959.92	41.59

The estimates of mineral element concentration in the small green lentil trials planted at 6 site-years (Table 3.14) showed that Ca and P concentrations were the highest at WREC-2013 compared to the other sites. Potassium estimates was high in HREC-2013 whereas Fe estimates were high at CREC-2013. Copper and Zn estimates were high at WREC-2012, Mn estimates was high at NCREC-2012, whereas, Mg estimates were high at HREC-2012.

Estimates of soil mineral element concentrations for the 2013 small green lentil trial sites showed that the WREC-2013 had the highest Ca, Cu, Mn and P. Potassium and Zn were high at HREC-2013, whereas Fe and Mg were high at CREC-2013.

Table 3.15. Seed mineral nutrient concentration for the 2013 Small Green Lentil Advanced Yield Trials at three locations in North Dakota.

Location	Ca	Cu	Fe	K	Mg	Mn	P	Zn
CREC-2013	-3.52	-1.43	1.43	-181.29	53.31	-1.20	-54.78	-0.23
HREC-2013	-2.62	0.45	-2.31	509.10	-33.76	-1.11	-134.43	0.16
WREC-2013	6.14	0.98	0.88	-327.82	-19.56	2.31	189.21	0.07
y-intercept	726.16	8.16	73.34	8051.82	1081.76	16.59	3990.95	41.17

Pearson's correlation coefficient between/among mineral elements and yield parameters from the 9 small green lentil advanced cultivar planted in HREC research location in 2012 showed that Cu had significant positive correlation with test-weight (r= 0.763) at P<0.05 and a significant negative correlation with thousand seed weight (r= -0.678) at

P<0.05(Table 3.16). Additionally K showed a significant negative correlation with seed yield (*r*= -0.696) at *P*<0.05. Significant positive correlation was observed between Ca and P (*r*= 0.725) at *P*<0.05 whereas a significant positive correlation was observed between Mg and K (*r*= 0.772) at *P*<0.05.

Pearson's correlation coefficient between/among mineral elements and yield parameters from the 9 small green lentil advanced cultivar planted in NCREC research location in 2012 showed (Table 3.17) that Ca had a significant negative correlation with K (*r*= -0.672) at *P*<0.05. Cupper showed a significant positive correlation with P (*r*= 0.672) and test-weight (*r*= 0.691) at *P*<0.05 whereas a significant negative correlation with thousand seed weight (*r*= -0.838) at *P*<0.01. Iron showed a significant positive correlation with P (*r*= 0.692) at *P*<0.05 and Zn (*r*= 0.871) at *P*<0.01 where as a significant negative correlation with Mn (*r*= -0.895) at *P*<0.01. However, Mn showed a significant positive correlation with Mg (*r*= 0.673) at *P*<0.05 and Mg showed a significant positive correlation with Mg (*r*= 0.691) at *P*<0.05. Zinc showed a significant negative correlation with Mn (*r*= -0.830) at *P*<0.01.

Pearson's correlation coefficient between/among mineral elements and yield parameters from the 9 small green lentil advanced cultivar planted in WREC research location in 2012 showed (Table 3.18) that Cu had a significant negative correlation with thousand seed weight (*r*= -0.671) at *P*<0.05. Manganese showed a significant positive correlation with test-weight (*r*= 0.739) at *P*<0.05. Zinc showed a significant positive correlation with Fe (*r*= 0.723) at *P*<0.05 whereas a significant negative correlation with Cu (*r*= -0.683) at *P*<0.05. Manganese showed a significant positive correlation with Ca (*r*= 0.676) at *P*<0.05 whereas a significant negative correlation with K (*r*= -0.668) at *P*<0.05.

Table 3.16. Pearson's correlation coefficients between mineral elements and yield parameters in 9 green lentil genotypes planted in HREC-2012.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	0.354	0.386	-0.145	0.167	0.439	0.725*	0.432	0.380	0.082	0.147
Cu		1	0.110	-0.216	0.332	0.408	0.532	-0.065	0.472	0.763*	-0.678*
Fe			1	-0.015	0.003	0.420	0.423	0.579	0.545	-0.218	0.033
K				1	0.772*	-0.035	0.167	0.554	-0.696*	-0.592	0.004
Mg					1	0.162	0.524	0.570	-0.336	-0.079	-0.278
Mn						1	0.097	0.083	0.252	0.337	0.009
P							1	0.642	0.377	0.019	-0.273
Zn								1	-0.068	-0.468	0.308
SY									1	0.455	-0.309
TW										1	-0.379
TSW											1

Note: $P<0.05$ = *, $P<0.01$, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 3.17. Pearson's correlation coefficient between mineral elements and yield parameters in 11 green lentil genotypes planted in NCREC-2012.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	0.171	0.344	-0.672*	-0.541	-0.457	0.226	0.378	-0.179	0.387	0.059
Cu		1	0.121	-0.458	0.162	0.111	0.689*	-0.252	0.217	0.691*	-0.838**
Fe			1	-0.401	-0.433	-0.895**	0.692*	0.871**	0.430	0.178	-0.291
K				1	0.691*	0.569	-0.280	-0.148	0.191	-0.738*	0.300
Mg					1	0.673*	0.070	-0.311	-0.041	-0.426	-0.160
Mn						1	-0.363	-0.830**	-0.126	-0.137	0.042
P							1	0.434	0.520	0.444	-0.682*
Zn								1	0.203	-0.159	0.127
SY									1	0.008	-0.372
TW										1	-0.437
TSW											1

Note: $P<0.05$ = *, $P<0.01$, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 3.18. Pearson's correlation coefficients between mineral elements and yield parameters in 9 green lentil genotypes planted in WREC-2012.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	0.041	0.184	-0.382	-0.354	0.676*	0.070	-0.300	0.193	0.549	0.335
Cu		1	-0.421	-0.042	-0.159	0.269	0.160	-0.683*	0.163	0.448	-0.671*
Fe			1	-0.271	-0.114	0.362	0.359	0.723*	-0.214	0.008	0.069
K				1	0.445	-0.668*	0.466	0.101	0.454	-0.244	-0.427
Mg					1	-0.640	0.199	-0.071	-0.352	-0.591	-0.283
Mn						1	-0.155	-0.187	0.051	0.739*	0.122
P							1	0.210	0.193	-0.152	-0.634
Zn								1	-0.154	-0.357	0.231
SY									1	0.591	-0.138
TW										1	-0.013
TSW											1

Note: $P<0.05$ = *, $P<0.01$, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Pearson's correlation coefficient between/among mineral elements and yield parameters from the 9 advanced green lentil cultivar planted in CREC in 2013 showed (Table 3.19) that none of the 8 mineral elements tested had a significant positive and negative correlation with seed yield, thousand seed weight and test weight. Zinc showed a significant positive correlation with Cu (r= 0.661) and P (r= 0.770) at P<0.05. Calcium showed a significant negative correlation with K (r= 0.664) at P<0.05.

Pearson's correlation coefficient between/among mineral elements and yield parameters from the 9 advanced green lentil cultivar planted in WREC in 2013 showed that (Table 3.20) Ca had a significant positive correlation with Mn (r= 0.827) at P<0.01. Cupper showed a significant positive correlation with Mg (r= 0.671) and P (r= 0.712) at P<0.05. And Zn showed a significant positive correlation with Fe (r= 0.873) at P<0.01.

Table 3.19. Pearson's correlation coefficients between mineral elements and yield parameters in 9 green lentil genotypes planted in CREC-2013.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	-0.065	0.032	-0.664*	-0.319	0.465	-0.371	-0.436	0.578	0.405	0.407
Cu		1	0.584	0.251	0.174	0.270	0.624	0.661*	0.241	0.585	-0.533
Fe			1	0.103	0.404	0.066	0.621	0.431	0.144	0.402	-0.626
K				1	0.547	-0.476	0.566	0.340	-0.297	-0.078	-0.201
Mg					1	-0.614	0.287	-0.149	-0.081	0.192	-0.619
Mn						1	-0.215	0.117	-0.024	0.174	0.389
P							1	0.770*	-0.049	0.155	-0.550
Zn								1	-0.187	0.245	-0.337
SY									1	0.304	-0.092
TW										1	-0.148
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

Table 3.20. Pearson's correlation coefficients between mineral elements and yield parameters in 9 green lentil genotypes planted in WREC-2013.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn	SY	TW	TSW
Ca	1	0.158	0.438	-0.652	0.152	0.827**	0.250	0.577	0.028	0.245	-0.096
Cu		1	0.274	-0.402	0.671*	-0.051	0.712*	0.083	-0.572	0.380	-0.379
Fe			1	-0.376	-0.132	0.529	0.380	0.873**	-0.226	-0.242	0.171
K				1	-0.033	-0.493	-0.223	-0.243	-0.180	-0.530	0.293
Mg					1	0.142	0.326	-0.203	-0.236	0.562	-0.409
Mn						1	-0.072	0.579	0.296	0.317	-0.033
P							1	0.477	-0.716	-0.112	-0.137
Zn								1	-0.312	-0.364	0.209
SY									1	0.214	-0.222
TW										1	-0.367
TSW											1

Note: $P<0.05$ = *, $P<0.01$ = **, $P<0.001$ = ***, SY= seed yield, TW= test weight, and TSW= thousand seed yield.

3.4. Discussion

The nutritional status of people who are at high risk of malnutrition can be improved through increasing the mineral concentration in seed through biofortification (Bouis and Welch, 2010). Among many nutritionally valued crops, legumes are an excellent source of complex carbohydrate, protein, dietary fiber, vitamins and minerals like Fe and Zn (Gawalko et al., 2009; Wang and Daun, 2004; Wang et al., 2008). Dry pea and lentil are good sources of mineral elements including Fe, Zn, and Mg (Amarakoon et al., 2012; Thavarajah et al., 2012). Iron, Zn, K, and Ca deficiencies are the most widespread nutrient deficiencies affecting more than half the world population and Mn and Mg deficiencies are also present on a more limited basis (Ray et al., 2014). The opportunity for enrichment through plant breeding holds the highest possibility to resolve the world nutritional crisis effectively and economically. Knowing the concentration and diversity of these mineral elements in existing germplasm, their heritability, and the understanding of their physiological pathways of uptake and translocation is crucial to breeding for these traits. Past research has reported adequate variation in pea and lentil germplasm for selection of increased mineral nutrient concentration. However, micronutrient enrichment through conventional breeding is influenced and often limited by genetic as well as environmental factors (Amarakoon et al., 2012). Therefore, existing germplasm needs to be tested across multiple locations and years to develop cultivars with improved mineral element concentrations in the seed (Frimpong et al., 2009).

Panadian et al. (2011) also reported a significant genotype x location interaction for Cu, Fe, Mn and Zn in a rice kernel planted in a single year at three different research stations in India. Ray et al. (2014) reported a significant variety x location x year interaction only in Fe and Zn. Other elements like Ca, Cu, K, Mg and Mn were not significant while testing for two years in south and central Saskatchewan, Canada.

Although, the variation in mineral concentration among the small green lentil genotypes and the small red lentil genotypes are similar, red lentil had higher concentrations of Ca, Cu, Fe, K, and P in the seed. A two year study by Ray et al. (2014) using a mixture of lentil genotypes at two locations (Saskatoon and Kyle, Saskatchewan) reported K at a range of 8802 to 10,024 mg/kg, Mg at a range of 938 to 1071 mg/kg, Ca at a range of 268 to 430 mg/kg, Fe at a range of 75.6 to 100.0 mg/kg, Zn at a range of 36.7 to 50.6 mg/kg, Mn at a range of 12.2 to 14.8 mg/kg, and Cu at a range of 7.0 to 9.2 mg/kg. In general, the mineral nutrient concentrations in both the green and red lentils fall within the range of Canadian lentils. Zinc, K and Fe concentrations were lower in the current research and Ca, Cu, Mn and Mg were higher compared to Ray et al. (2014).

3.4.1. Red Lentil

Soil analysis for the 2013 red lentil trial sites showed that NCREC (6400ppm) had the second highest plant available Ca after CREC (6930ppm). Plant available Mg was high at NCREC compared to CREC (Table 3.6). Red lentil genotypes grown at CREC had the highest Fe concentration in the seed and red lentil grown at HREC had the highest concentration of Ca, Cu, K, Mg, Mn, P, and Zn in 2013 (Table 3.6). Further research of the actual soil-seed mineral relationship in these locations is necessary.

Variation in average rainfall and mean air temperature during the growing season at 5 site-years for the red lentil yield trial is summarized in Table A1. The mean air temperature ranged from 16.0 to 18.7 °C. HREC-2012 had a mean air temperature of 17.1°C, NCREC-2012 was 18.2° WREC-2012 was 18.7°C, CREC was 16.9°C, and WREC was 17.9°C. Average rainfall during growing season (May to September) ranged from 33.0 mm to 81.3 mm and HREC-2012 had 53.3 mm, NCREC, WREC had 35.6 mm CREC-2013 had 43.2 mm, and WREC-2013 had 81.3 mm. This clearly shows the difference in climatic conditions at each location (NDAWN, 2014).

Selection for increased concentration of mineral elements and higher seed yield is complicated with the correlation among mineral elements. Karakoy et al. (2012) found many micronutrient and macronutrient correlation in lentil landraces collected from South-Eastern Turkey. He found that P content in seed was positively correlated with K, Mg, Ca, Cu, and Zn at $P<0.01$, and with Fe at $P<0.05$. Phosphorous showed a weak to strong positive correlation with Cu and K in the Turkish red germplasm evaluated in this study. Positive correlations of K with Cu and Zn at $P<0.01$ was reported by Karakoy et al. (2012), and significant positive correlations of K with Zn was observed in all site-years except WREC-2013 in this research. Karakoy et al. (2012) reported positive correlation of Mg with Cu and Zn ($P<0.01$); however, a weak to strong positive correlation between Ca and Mg was detected in this research. Very strong to moderate positive correlation was detected between Fe and Zn ($r= 0.38$ to $r=0.81$). Karakoy et al. (2012) reported negative correlation of Zn with seed size and seed yield. The current study detected a positive correlation of seed yield with Zn and Mg concentration at all site-years tested for red lentil genotypes. In addition, seed yield had a negative correlation with Cu concentration at all site-year except HREC-2012. Test weight showed a negative to significant negative correlation with Ca concentration in red lentil variety tested at multiple site-years.

3.4.2. Small Green Lentil

Soil analysis for the 2013 Small Green Lentil Advanced Yield Trial locations showed that NCREC (6400ppm) had the second highest plant available Ca and that CREC (6930ppm) had the highest. Mg was high at NCREC compared to CREC (Table 3.15), but the seed produced at WREC had the highest Ca, Cu, Mn and P concentration seed grown at CREC had the highest Fe and Mg concentration. Seed grown at HREC had the highest K and Zn concentration in their seed in 2013. Therefore, the soil-seed mineral relationship is important when developing breeding methodology to alter the mineral nutrient concentration in lentil.

Differences in mean air temperature and average rainfall during growing season were observed in all 6 site-years (Table A1). Mean air temperature ranged from 16.0 to 18.7°C and HREC-2012 had a mean temperature of 17.1°C, NCREC-2012 was 18.2°C WREC-2012 was 18.7°C, CREC was 16.9°C, HREC-2013 was 17.2°C and WREC was 17.9°C. Average rainfall during the growing season (May to September) ranged from 33.0mm to 96.5mm and HREC-2012 had 53.3mm, NCREC, WREC had 35.6mm CREC-2013 had 43.2mm, HREC-2013 had 96.5mm and WREC-2013 had 81.3mm. This clearly shows that there were differences in climatic conditions at each site-year (NDAWN, 2014).

Pearson's correlation coefficient analysis detected numerous significantly positive and negative correlations among/within mineral elements and yield parameters in this research with small green lentil. Karakoy et al. (2012) characterized the micro- and macronutrient concentrations of lentil landraces collected from South-Eastern Turkey and showed that P content in seed was positively correlated with K, Mg, Ca, Cu, Zn at $P<0.01$, and with Fe at $P<0.05$. P concentrations of small green lentils were positively correlated with Ca, Mg, Cu, Zn and Fe in the current study and corroborate the results of Karakoy et al. (2012). The correlation between P and Zn ($r= 0.21$ to 0.77) and P and Cu ($r= 0.16$ to 0.71) and P and Fe ($r= 0.35$ to 0.69) was detected in all site-years. These positive correlations suggest that it is possible to breed for multiple elements simultaneously. Karakoy et al. (2012) showed that Fe had a strong positive correlation with Mn and Zn ($P<0.01$) and the results from the current study also detected strong positive correlation between Fe and Zn. Small green lentil in this research showed that Ca concentration was positively correlated with seed yield in all the site-years with the exception of NCREC-2012. In addition, Cu was also found with a negative correlation with thousand seed weight at all site-years (range $r= 0.38$ to 0.84). However, Cu showed a positive correlation with test weight at all the site-years ($r= 0.38$ to 0.76) for small green lentils tested in this research.

3.5. Conclusions

Significant genetic variation was detected among the lentil genotypes in the current study suggesting that there is potential to improve mineral nutrient concentration in both small green and small red lentil genotype. Environmental influence was identified as an important factor controlling mineral nutrient concentration and was important for both red and green lentils. This information will be crucial in further breeding for these traits. A report released by Harvest Plus on "Biofortification Progress Brief", Ashutosh Sarkar (ICARDA) also highlighted that multi-location testing for mineral nutrient quantification is very important and has a significant genotype x environment interaction for most of the mineral nutrients tested. The information generated from this study will be useful in improving the lentil germplasm and will be useful in future breeding for these traits. Categorical differentiation of genotypes based on their mineral concentration will be helpful in selection and breeding for each mineral nutrient separately. Therefore; this research supports an approach towards breeding for mineral nutrient dense pulse crops to fight against global malnutrition and provides useful information for future breeding.

CHAPTER 4. OVER ALL CONCLUSION

North Dakota has emerged as one of the leading pulse crop producing states in the U.S. This research showed strong environmental influences on mineral nutrient accumulation and highlights the challenges in breeding for higher mineral concentration. Both lentil and dry pea genotypes showed significant potential in breeding for increased mineral concentration or biofortification through conventional breeding. Select genotypes can be further used in breeding and genetic research purposes. However, the significant genotype and environmental interaction that prevailed in all of the locations and minerals tested limits the breeding efforts.

Further research to understand the soil-seed relation is necessary. Research to understand the complex physiology of the mineral nutrient uptake, translocation and partition into seeds and different parts of the plants might shed more light on understanding this complex process. As most of the essential mineral nutrient accumulations are controlled by the plant correlation among mineral nutrients might also be helpful in breeding for these elements. This emphasizes the fact that finding an ideal genotype with high mineral concentration with high stability across wide environment is challenging and demands collaborative and multi-dimensional research. In conclusion, this research has set a foundation for further research on soil mineral availability to the plant, pathway analysis of different mineral nutrients in the plant system and breeding for higher mineral concentrations in the seed.

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APPENDIX. MEAN AIR TEMPERATURE AND AVERAGE RAINFALL IN PEA AND LENTIL GROWING SEASON

Research Locations	2012- Air Temperature °C	2013-Air Temperature °C	2012- Avg. Rainfall (mm)	2013-Avg. Rainfall (mm)
CREC	17.2	16.9	48.3	43.2
HREC	18.1	17.2	53.3	96.5
LREC	16.3	16.0	55.9	58.4
NCREC	18.3	17.2	33.0	121.9
Prosper	19.0	18.9	33.0	91.4
WREC	18.7	17.9	35.6	81.3