Increasing Nutrient Density of Food Crops through Soil Fertility Management and Cultivar Selection

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Increasing Nutrient Density of Food Crops through Soil Fertility Management and Cultivar Selection

A Dissertation Presented

by

MD J. MEAGY

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University of Massachusetts in partial fulfillment
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Plant and Soil Sciences

Increasing Nutrient Density of Food Crops through Soil Fertility Management and Cultivar Selection

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DEDICATION

To my mother, Mrs. Saleha Begam, for her prayer for this achievement, to the memory of my father, Md Sirazul Islam, who did not live to see this accomplishment and passed away three months before of the achievement, to my great wife, Sumaiya Sharmin, whose love, vision, sacrifice, understanding, encouragement, and commitment have made this accomplishment possible, and to my sister, Mosammat Taslima Akter and Banosree Biswas, who truly supports and feels proud for this accomplishment.

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ABSTRACT

INCREASING NUTRIENT DENSITY OF FOOD CROPS THROUGH SOIL FERTILITY MANAGEMENT AND CULTIVAR SELECTION

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The mineral nutrient density of vegetables has fallen in the past fifty years. As a result, some people are suffering chronic diseases due to shortage of mineral elements in these foods. The causes of this decline in nutritive value of vegetables have been attributed to a depletion of soil fertility and to a decrease in nutrient concentration in modern cultivars of vegetables. Lettuce (*Lactuca sativa* L.) is the most widely used leafy vegetable around the world. Research is needed to develop a nutrient content of lettuce that was help to supply adequate mineral nutrition to people. The objectives of the study are to determine if the mineral nutrient densities of lettuce can be increased through cultivar selection and soil fertility practices and to assess genetic diversity of the selected cultivars with use of molecular markers. This experiment was conducted with lettuce grown in organic and conventional fertility management practices in a greenhouse and in field sites. Butterhead, Romaine, and Loose-leaf phenotypes were selected in Heritage and Modern groups of lettuce. Eighteen lettuce cultivars were used from among three phenotypic groups with half of the varieties being Heritage and half being Modern cultivars. Commercial organic and water-soluble nutrient solutions (including Hoagland No. 1 Solution and 20-10-20 Peat Lite) were used in the greenhouse experiments. In the field, compost, organic, and conventional fertility regimes were used. Molecular diversity tested phenotypes and cultivars of lettuce through use of EST-SSRs markers. Growth parameters of height and fresh and dry weights were reported for the experiment. Elemental analysis of P, K, Ca, Mg, S, Mn, Fe, Cu, and Zn were conducted by ICP spectrophotometry. Generally, no differences or only small differences in nutrient accumulation were noted between Heritage and Modern cultivars in the greenhouse or field. Differences among phenotypes were small with somewhat higher accumulations of nutrients occurring with the Loose-leaf and Romaine cultivars than with the Butterhead cultivars. However, large differences in nutrient accumulation occurred among cultivars. ‘Red Deer Tongue’, ‘Forellenschluss’, ‘Winter Density’, ‘Coastal Star’, ‘Simpson Black-Seeded’, and ‘Tom Thumb’ were high in P, K, Ca, Mg, and Zn contents. ‘Two Star, ‘Tropicana’, ‘Red Rosie’, ‘Simpson Elite’, ‘Focea’, and ‘Claremont’ were low in these elements. Overall, in the field, the conventional fertility regime showed higher produce yield than compost or organic fertility regimes. Modern and Loose-leaf types of lettuce showed higher weight yields than Heritage or Butterhead varieties. In the greenhouse, higher elemental accumulation occurred in conventional organic and Hoagland no. 1 fertility regimes than with a conventional fertilizer (20-10-20 Peat Lite), and accumulation was higher in Loose-leaf and Romaine lettuce than in Butterhead cultivars. In molecular assessment, higher heterozygosity was observed in Loose-leaf than in Romaine or Butterhead types. These studies allowed assessments of cultivars and

management of conventional fertilizers on the accumulation of nutrients in lettuce and determination of genetic diversity. It is clear that varietal differences occur among cultivars of lettuce and that accumulation of nutrients can be controlled by management of the fertility regimes. Organic and conventional management might be equally effective as long as adequate plant nutrition is provided by each regime.

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CHAPTER 1

GENERAL INTRODUCTION

Background

Malnutrition is a primary factor limiting human productivity in modern times, and deficiencies of certain elements including calcium, magnesium, potassium, phosphorus, zinc, copper, and others known as mineral nutrients, in diets of humans are a substantial nutritional problem throughout the World (Darnton-Hill et al., 2005; Kataki and Babu, 2002; Schaetzel and Sankar, 2000). Davis (2009) reports 5% to 40% declines in mineral contents of vegetables and fruits in the past 50 to 70 years in the United States. Reports from the United Kingdom indicated that foods were depleted by about 20% during this time (Lobstein, 2004; Mayer, 1997; Thomas, 2006). Research on the diets of subjects in Philadelphia warranted a downward revision in the intakes of iron, magnesium, and vitamins (Guenther et al., 1994). The result of a study concluded that many Americans are not meeting the current recommendations for calcium intake through diet alone or with supplements (Ma et al., 2007).

Davis (2009) suggests that the decline in mineral and protein content in fruits and vegetables is due partly to a dilution effect of high yields. Side-by-side comparisons of low- and high-yielding vegetables and grains showed negative correlations between yield of produce and concentrations of minerals. However, White et al. (2009) reported that with potato (*Solanum* spp. L.), a dilution effect of high yields on nutrient concentration was not observed universally and that soil fertility affected the mineral nutrients more than the dilution effect.

Soil fertility problems associated with nutrient depletion by crop production are worldwide (Tan et al., 2005). In the United States, potassium and phosphorus contents are being drawn down in soils at an increasing rate every year, and the depletion occurring for 40 years for potassium and for nearly 30 years for phosphorus (Stewart, 2004). Similar nutrient mining of essential elements occurs throughout the World (Ayoub, 1998; Dobermann et al., 1996; Lal and Singh, 1998; Nandwa and Bekunda, 1998). Elemental depletion of soils must be compensated for by fertilization for sustainable production of nutrient-sufficient foods (Buol, 1995).

Organically grown fruits and vegetables might differ from conventionally grown produce due to differences in the types of fertilizers used in the two practices. Low availability of nutrients in organic fertilizers could limit mineral accumulation in plants relative to fertilization with chemical fertilizers with high nutrient availability, thereby making the chemical fertilizers a superior nutrient source. On the other hand, high availability of certain nutrients or failure to provide elements in chemical fertilizers could lead to nutrient imbalances in foods (Lundegaardh and Maartensson, 2003). A study noted that compost increased nutrient concentrations in soils but not always in plants (Roe, 1998). The result of another study reported no consistent differences in nitrogen, phosphorus, and potassium concentrations in several vegetables in crops fertilized with composts and crop residues or with synthetic mineral fertilizers (Herencia et al., 2007). A recently published study (Benbrook et al., 2008) reported that organically grown foods derived from plants were superior to those grown conventionally with respect to phosphorus, potassium, nitrates, several antioxidants, and vitamin C. An experiment suggested that the high yields achieved through farming systems with high nitrogen fertilizer inputs led to a dilution of nutrient density in vegetables relative to organic systems with low nitrogen inputs (Benbrook, 2009). High nitrate concentrations in foods are considered as a factor adversely affecting human health (Maynard et al., 1976). Organically grown vegetables may have less nitrate accumulation than conventionally fertilized vegetables (Benbrook et al., 2008). The result of a study reported that farm manure had a more favorable effect on certain plant constituents (nitrate and oxalic acid) than potassium nitrate (Turan and Sevimli, 2005). However, Maynard et al. (1976) noted that the amount of nitrogen fertilization regardless of source was the principal factor leading to nitrate accumulation in vegetables. A study noted that with fertilization for optimum yield, nitrate concentrations of vegetables were not different between organically and chemically fertilized vegetables (Barker, 1975).

Regardless of soil conditions, fruits and vegetables, although high in vitamins, are typically low in mineral nutrients (Elless et al., 2000). Except for potassium, the fruit-vegetable food group contributes less than 30% of the total dietary intake of mineral nutrients (Levander, 1990). To counter this problem, several attempts have been made to increase the mineral nutrient content of fruits and vegetables. These actions have included enrichment of foods in processing, but not much attention has been directed toward improvement of foods through enhanced soil fertility and cultivar selection, although these two factors appear to be principal agents affecting the nutrient density of fruits and vegetables.

Methods of increasing the nutrient content of foods must be developed through improved practices in fertilization and in the development and selection of crop varieties that accumulate the nutrients in amounts that are adequate for intake in normal diets of humans. This research involved studies with heritage and modern cultivars of several types of lettuce crops grown under differing regimes of fertilization.

Literature Review

Impact on Health

All varieties of lettuce are near zero in calories and cholesterol but are excellent source of dietary fiber, beta-carotene, and folic acid. Lettuce contains alkaloids which are responsible for its therapeutic effect. Most lettuce varieties are an excellent source of vitamins A, B_1, B_2, and C, folic acid, manganese, chromium, and chlorine. Lettuce contains appreciable amounts of water-soluble antioxidants such as vitamin C and various phenolic compounds (phenolic acids, anthocyanidins), as well as lipid-soluble antioxidants such as lutein or tocopherols (Rice-Evans et al., 1995; Rice-Evans et al., 1996; Szeto et al., 2002). Lettuce appears to exert a diversity of interesting effects on risk factors of cardiovascular diseases by its fiber content, antioxidant supply, and possibly by various other micronutrients such as phytosterols or folic acid. A recent results strongly suggest that a regular intake of antioxidant compounds from lettuce is useful to improve the lipid status and to prevent lipid peroxidation in tissues (Nicolle et al., 2004).

Furthermore, Nicolle et al. (2004) stated in a study on rats that feeding rats a 20% lettuce diet for 3 weeks resulted in a decrease cholesterol LDL/HDL ratio and a marked decrease of liver cholesterol levels (-41%). Concurrently, fecal total steroid excretion increased (+44%) and apparent absorption of dietary cholesterol was depressed significantly (-37%) by the lettuce diet. Lettuce diet also displayed an improvement of vitamin E/TG (triglyceride) ratio in plasma and limited lipid peroxidation in the heart as evidenced by TBARS (Thiobarbituric Acid-Reactive Substances).

High fiber intakes are associated with lower serum cholesterol concentrations, lower risk of coronary heart disease, reduced blood pressure, enhanced weight control, better glycemic control, reduced risk of certain forms of cancer, and improved gastrointestinal function (Anderson et al., 1994). Dietary fiber can be categorized into water-soluble and water-insoluble components. Current guidelines advise a doubling of dietary fiber intake for Americans. Servings of lettuce as salads along with vegetables, whole grains, and dried beans and peas will help individuals meet these guidelines. Diabetes is more prevalent in populations with low fiber intakes than in those with high fiber intakes (Trowell, 1975). Recent studies further support the concept that individuals whose diets are high in fiber are less likely to develop diabetes than those whose diets are low in fiber (Anderson and Bryant, 1986; Vinik and Jenkins, 1988).

Effect of Nutrients

Nutrient sufficiency is the main stream of healthy, productive lives and longevity for everyone (Welch, 2002). The deficiency of micronutrients such as iron can cause nutritional anemia, problem pregnancies, stunted growth, lower resistance to infections, long-term impairment in mental function, decreased productivity and food-energy conversion and impaired neural motor development. And *zinc* deficiency can cause growth retardation, delayed skeletal and sexual maturity, dermatitis, diarrhea, alopecia and defects in immune function with resulting increase in susceptibility to infection (Welch and Graham, 1999).

On other hand, excessive N-fertilizers can adversely affect the accumulation of vitamin C in various vegetable crops such as lettuce and beets (*Beta vulgaris cicla* L.) by as much as 26% (Welch, 2002). Macronutrient fertilizers containing N, P, K and S and certain micronutrient fertilizers containing, for example Zn, Ni, and Se, can have significant effects on the accumulation of micronutrients in edible plant products (Allaway, 1986). The study indicated that increasing the supply certain essential micronutrient elements to food crops (e.g., Zn, Ni, and Se) can result in significant increases in micronutrient concentrations in edible plant products. For example, increasing the supply of Zn to pea (*Pisum sativum* L.) plants at levels in excess of that required for maximum yield has been shown to increase the concentration of bioavailable Zn in pea seeds (Peck et al., 1980).

Plant shows respective deficiency symptoms in absence of certain elements. A research finding suggested that heading Chinese cabbage (*Brassica pekinensis* Rupr.) grown with low calcium or boron developed dark tan lesion near the leaf margins. The results also concluded that plants grown under humid conditions were free of tipburn but less humidity condition developed tipburn. The chemical analysis of this study showed 7 times more water-soluble calcium in the outer than in the inner leaves (Kuo et al., 1981).

Effect of Fertility Practices

Organic farmers claim that foods grown organically contained a better arrangement of nutrients as a result of the superior soil management and fertilization practices. Simultaneously, food grown with chemical fertilizers is causing deteriorating health in animals and humans. A study revealed that organic crops contained significantly more vitamin C (+ 27%), iron (+21.1%), magnesium (+29.3%), phosphorus (+13.6%) and significantly low nitrates than conventional crops (Worthington, 2001). The results further revealed that lower amounts of some heavy metals found in organic crops compared to conventional ones.

In recent years, organic farming has grown rapidly in its potential of producing healthful foods as well as meeting consumer demands. In this regards, the perception among consumers is that organically produced crops possess higher nutritional quality than conventionally produced crops. In support of these ideas, a long-term experiment was conducted comparing food quality of crops with organic versus mineral fertilization. The result supported that nitrate concentration in the edible parts was significantly lower and that phosphorus content was higher in organically fertilized crops (Herencia et al., 2011).

A similar study revealed that the plants grown under organic agricultural conditions had a higher micronutrient content than conventionally grown plants (Hunter et al., 2011). In this regard, organic plant foods had a 5.7% higher content of vitamins than their conventionally grown counterparts. Also, higher amounts of micronutrients occurred in organic carrots (+6.1%), potatoes (+12.2%), and peas (+7.4%).

Organic and conventional farming mainly differs in tillage methods, crop rotations, fertilizer applications, and pest control methods. Conventional farming practices have greatly increased crop production and labor efficiency, whereas organic farming practices may reduce some of the negative effect of conventional agriculture on the environment (Vereijken, 1986). In this respect, the data showed that organically farmed soil had significantly higher organic matter and less soil erosion than conventionally farmed soil. Furthermore, in the long term, the organic fertility practice was more effective than conventional in reducing soil erosion and in maintaining soil productivity (Reganold et al., 1987).

The results of another experiment showed that inorganic fertilizer (2:3:2) plus 0.5 % zinc and limestone ammonium nitrate (LAN 28 %) were less suitable in lettuce production in river sand than organic fertilizers (e,g, chicken manure, cattle manure, and bounce back compost (composted chicken manure) (Masarirambi et al., 2010). Organic fertilizers of chicken manure (40 tons/ha) gave numbers of leaves, plant height, marketable yield, and leaf dry mass than conventional fertilizer. Lettuce grown by bounce back compost were higher in calcium (7.7 mg/100g), iron (4.91 mg/100g) and zinc (3.18 mg/100g) content compared to cattle manure, inorganic fertilizers, and chicken manure respectively.

Studies suggested that the accumulation of nitrates in lettuce has been affected by the soil texture and the source of fertilizer-N (Gianquinto and Borin, 1992; Gunes et al., 1995), the NH_4-N and NO_3-N fertilizer-N ratio (Demsar and Osvald, 2003), the timing of fertilizer-N release (Tesi and Lenzi, 1998), and type of lettuce cultivars (Escobar-Gutierrez et al., 2002). The data also suggested that the romaine type of lettuce had the highest concentration of nitrates in the external leaves than in internal leaves (Abu-Rayyan et al., 2004).

Similarly, a study on lettuce showed that the highest levels of accumulation of nitrate were with inorganic fertilization treatments having 572- 664 mg N/kg compared with organic fertilization treatments having 253-435 mg N/kg. The data also showed that the lettuce biomass yield was significantly higher with high amounts of inorganic fertilization treatment than low amounts of fertilization. However, different amounts of organic fertilization did not differ in lettuce yields (Pavlou et al., 2007).

An experiment conducted to evaluate the effect of nitrogen fertilizer on growth and yield of lettuce. The results showed that fertilizers ENTEC 26% N [a nitrogen fertilizer with a nitrification inhibitor, NH_4NO_3+ $(NH_4)_2SO_4$+0.8% DMPP (3,4-dimethylpyrazole phosphate)] and ammonium nitrate 34% N (NH_4NO_3] obtained significantly higher yields compared to calcium nitrate 15.5% N [$Ca(NO_3)_2$ $xH_2O+NH_4NO_3$], or ammonium sulfate 20.0% N [$(NH_4)_2SO_4$]. However, the treatment ENTEC 26 contains a nitrification inhibitor, 3, 4-dimethyl pyrazole phosphate, which acted to stabilize the ammonium form of nitrogen by blocking the activity of nitrifying bacteria. The data further suggested the recommended concentration of nitrogen in the soil for leaf lettuce should not exceed 100 mg $N.dm^{-3}$ (Chohura and Kolota, 2009).

A soilless experiment was carried out to study the effect of biogas fertilizer (residue from a biogas plant) on the yield and quality of lettuce. The results showed that 100, 200, or 300 g of biogas fertilizer increased the yield of lettuce by 59%, 108%, and 99%, the reducing sugar by 12%, 10%, and 74%, the total sugar by 4%, 65%, and 73%, vitamin C by 107%, 95%, and 48%, and decreased the nitrate by 85%, 83%, and 88% respectively compared with chemical fertilizer. The treatment was not only increased the yield of lettuce but also remarkably improved the quality that was very crucial for greenhouse agriculture (Su et al., 2008).

Research studied the effect of molybdenum application in nutrient solutions of lettuce. The data showed that the N, P, or K contents of head lettuce significantly increased with increasing doses of Mo [0, 0.25, 0.50 mg/kg $(NH_4)_6Mo_7O_2$. $4H_2O$] application. However, Fe, Zn, or Mn contents of lettuce significantly decreased with increasing of Mo application, whereas Cu content of lettuce was not affected (Adiloglu et al., 2011).

Effect of Cultivars on Nutrients Uptake

Nutrient uptake of lettuce depends on cultivars selection as well as fertility practices. In this regard, nutrients were significantly higher in some selected cultivars of lettuce. An experiment was conducted on five selected leaf lettuce (Bergamo, Dubacek, Frisby, Lollo Rossa, and Redin) cultivars to evaluate the nutrient contents and yield. The data showed the best performing cultivars for nutrient accumulation in spring cultivation were Fisby (278 mg/kg Vit C and 156 mg/kg Mn), Lollo Rossa (363 mg/kg Ca, 6.74 g/kg fiber, and 3817 mg/kg NO_3^-), and Redin (4165 mg/kg K, 76 mg/kg Na, 79 g/kg dry matter, and 347 g wt of leaf rosette What's that?). However, the nutrient content of the selected cultivars differed in different growing seasons (Koudela and Petrikova, 2008).

As lettuce is a very popular leafy vegetable and plays an important role in daily diet and nutrition. A study result revealed that crisphead lettuce has much lower nutrient content than leaf or romaine types of lettuce. The low level of nutrient in crisphead is due to its leaves and morphology of head giving a compact structure. The nutrients concentration of romaine lettuce were double (0.97 mg Fe, 14 mg Mg, 30 mg P, 247 mg K, 0.23 mg Zn, and 33 mg Ca per 100 g) in compared with crisphead lettuce (Mou, 2009).

A similar study was conducted to evaluate the yield of Chinese cabbage (e.g. Optiko, Manoko, Nikita, Nerva, Elliot, Aten, Spectrum, and Sapporo) cultivars in different growing seasons. The result showed that the highest marketable yield in spring was produced by Elliot (66.17 t/ha) and in the autumn by Nerva (72.46 t/ha). However, the cultivars harvested in spring showed a tendency to bolt, especially Nerva and Spectrum. The cultivars Asten and Optiko appeared resistant to bolting (Krezel and Kolota, 2003).

Research

Justification

The need for this project was raised by the food consumers and producers as well as by the scientific community. Literature on food composition demonstrates that the mineral nutrient density of vegetables has fallen in the past 50 years. This decline is associated with declines in soil fertility and with the genetics of plant cultivars that increase yield at higher rates than mineral nutrients increases. Research is needed to develop systems of food crop production that supply adequate mineral nutrition to people directly. Use of nutrient-dense crops provide an opportunity for vegetable producers to diversify production and market, and to increase income and profitability as there appear to be a ready market for these crops.

Objectives

The following objectives were tested in the five different experiments described below;

1. To determine if the mineral nutrient densities of lettuce can be increased through cultivar selection.
2. To determine if the nutrient densities of lettuce can be increased by elevating nutrient contents in the medium in which the crop grows.

3. To determine if the nutrient densities of lettuce can be increased through soil fertility practices (e.g., organic vs. conventional fertilizers; different fertilizer regimes), and
4. To assess genetic diversities among selected cultivars of lettuce and genetic purity using EST-SSR molecular markers.

Experiments

Experiment 1: Assessment of Mineral Nutrient Density of Lettuce in Response to Cultivar Selection and Nutritional Regimes

This experiment was designed to determine the mineral nutrient contents of lettuce through cultivar selection using different fertilizer regimes. The experiment was conducted in the greenhouse using different fertilizer regimes and cultivars. The same cultivars suggested for the field evaluation were used in this experiment. The greenhouse experiment was help to evaluate selected cultivars before and after application in the field. The selected cultivars were grown in peat-based media (Canadian Growing Mix 1-PV, Conrad Fafard Inc, Agawam, MA) (Boodley et al., 1996). Three different fertilizer regimes were tested for this experiment. The elemental nutrient compositions were determined in edible parts (whole heads) of lettuce. Based on their adaptability to greenhouse, the cultivars were selected from two genetic and three phenotype groups. Each cultivar was evaluated for its growth and nutrient contents. The experiment had a randomized complete block design.

Experiment 2: Assessment of Organic and Conventional Soil Fertility Practices and Cultivar Selection on Mineral Accumulation in Lettuce

Fertilization was provided in the experiment in organic and conventional fertilizer regimes. Two regimes of organic fertilization, individual fertilizers and compost, and one conventional fertilizer were selected to provide nutrients. Organic fertilization with individual nutrient sources includes soybean meal for nitrogen, bone meal for the phosphorus, and mined potassium sulfate for the potassium. The compost was obtained from the University Office of Waste Management, which produces compost from dining commons food waste and yard waste from the Amherst campus. Eighteen cultivars were selected from two genetic and three phenotype groups. Produce was harvested and evaluated for yield and nutrient densities at edible plant parts (whole heads). The experimental had a randomized complete block design.

Experiment 3: Nutrient Density in Lettuce Cultivars Grown with Organic or Conventional Fertilization with Elevated Calcium Concentrations

This experiment was assessed differences in the mineral densities of selected lettuce cultivars through increasing the calcium contents in the medium. The experiment evaluated the calcium accumulation of the cultivars with elevated calcium in the medium. The experiment was conducted in a greenhouse. The cultivars were grown in peat-based medium (Canadian Growing Mix 1-PV, Conrad Fafard Inc, Agawam, MA) (Boodley et al., 1996). The fertilizer regimes were chemical fertilizer and organic fertilizer with increasing calcium contents. The nutrient contents were measured in the edible parts (whole heads) of the plants. The cultivars were evaluated for growth and calcium accumulation in produce. The experimental design of the experiment was a randomized complete block design.

Experiment 4: Zinc Accumulation in Lettuce Cultivars Grown with Organic or Chemical Based Nutritional Regimes

This experiment was assessed differences in the mineral densities of selected cultivars through increasing the nutrient contents in the medium. The experiment was conducted in greenhouse and determined the response of lettuce using the increasing zinc supply. The selected cultivars were grown in peat-based medium (Canadian Growing Mix 1-PV, Conrad Fafard Inc, Agawam, MA) (Boodley et al., 1996). The fertilizer regimes were Hoagland # 1 solution and organic fertilizer with increasing zinc nutrient contents. The nutrient contents were measured in the edible parts (whole heads) of the produce. The cultivars were evaluated for growth and zinc accumulation at harvest. The experimental design was a randomized complete block design.

Experiment 5: Assessment of the Genetic Diversity in Selected Lettuce Cultivars with Simple Sequence Repeats Markers

This experiment determined the genetic diversity among selected cultivars using EST-SSR molecular markers. The experiment was conducted in the greenhouse using the same cultivars used in the previous experiments. Standard fertilizer 20-10-20 was used to grow the cultivars as normal. Fresh tissues were sampled after 4 weeks of growth for genomic analysis. Genomic DNA was extracted from plant tissues according to the procedures used by (Curley and Jung, 2004; Sim et al., 2009; Simko, 2009). EST-SSR markers of lettuce were selected and assessed from the most recent papers (Cavagnaro et al., 2009; Kong et al., 2006; Liu et al., 2007a; Liu et al., 2007b; Richards et al., 2004;

Simko, 2009; Yi et al., 2006) against selected cultivars for genetic diversities. PCRs were conducted in conditions as described in the respective papers (Simko, 2009). DNA diversities among selected cultivars within each of species were estimated.

Statistical Analyses

The data of plant heights, fresh weights, and dry weights were processed as for analysis of variance (Steel and Torrie, 1980) with statistical software (PROC GLM, SAS 9.1.3, SAS Institute Inc., SAS Campus Drive, Cary, NC). Mean separations were conducted by Duncan's New Multiple Range Test and Least Significant Difference. Trends were assessed by polynomial regression analysis (Steel and Torrie, 1980).

CHAPTER 2

ASSESSMENT OF MINERAL NUTRIENT DENSITY OF LETTUCE IN RESPONSE TO CULTIVAR SELECTION AND NUTRITIONAL REGIMES

Abstract

Mineral nutrient contents in vegetable-based foods are a substantial concern in human diets, but depleted soil fertility and high-yielding cultivars have been associated with reports of low nutrient contents in vegetables. This study explored if mineral nutrient densities of lettuce (*Lactuca sativa* L.) can be increased though selection of cultivars and nutritional regimes. Eighteen cultivars including butterhead, romaine, and loose-leaf phenotypes of heritage and modern genetics were studied in a greenhouse. Hoagland solution no.1, a commercial inorganic fertilizer (20–10–20 peat-lite special), and a commercial organic fertilizer (3–1.5–4) were the nutrient regimes. Elements in whole heads were determined by plasma spectrometry. Heritage cultivars had about 10% higher P, K, Ca, Mg, and Zn concentrations than modern cultivars. Differences in elemental concentrations occurred among phenotypes and nutritional regimes but with no consistent trend among elements for phenotypes or regimes. Differences among individual cultivars for each element were large with some cultivars having twice the concentrations of nutrients of others and with considerable uniformity in cultivar rankings among the elements. This work suggests that cultivars can be selected for production of mineral nutrient-rich lettuce.

Key words: Macronutrients; micronutrients; nutrient accumulation; fertilizer regimes; Lactuca sativa.

Introduction

Deficiency of certain mineral nutrients like calcium, potassium, magnesium, phosphorus, zinc, iron, copper, and manganese, and other elements is a substantial problem in diets of humans worldwide (Banuelos and Lin, 2008; Darnton-Hill et al., 2005; Kataki and Babu, 2002). Nutrient content in vegetables and fruits have shown declines of 5% to 40% or more in approximately the past 50 in the United States (Davis, 2009). Reports indicated that in the United Kingdom nutrient contents decreased by about 20% in various foods during this time (Lobstein, 2004; Mayer, 1997; Thomas, 2006). A study of subjects in Philadelphia, Pennsylvania, suggested that a downward revision in the dietary intakes of iron, magnesium, and vitamins should be made (Guenther et al., 1994). Another study concluded that many Americans, particularly men, socially disadvantaged groups, and ethnic minorities are not meeting the current recommendations for calcium intake through diet alone or with supplements (Ma et al., 2007). Micronutrient malnutrition is a growing concern all over the developing world (Cakmak, 2009). Nutrient-dense (high in mineral nutrients) foods derived from vegetable crops may help to solve this problem.

Davis (2009) suggests that the decline in mineral content in fruits and vegetables is due partly to a dilution effect of high yields, noting that side-by-side comparisons of low- and high-yielding vegetables and grains showed negative correlations between concentrations of minerals and yields of produce. However, a dilution effect of high yield on mineral nutrient concentration was not observed universally on potato (*Solanum* spp. L.), and soil fertility affected the nutrients more than the dilution effect (White et al., 2009). Soil fertility problems associated with nutrient depletion by crop production are worldwide (Tan et al., 2005). In United States, potassium and phosphorus are being drawn down in soils on a national basis at an increasing rate every year, and the depletion has occurred for 40 years for potassium and for nearly 30 years for phosphorus (Stewart, 2003).

For well-balanced diets, increasing consumption of vegetable and fruits is advised (Kawashima and Valente Soares, 2003). A study compared the nutrient status of leafy vegetables of butterhead lettuce, rucola, watercress, kale, chicory, cabbage, Chinese cabbage, and spinach. Among them, kale offered the highest amounts of calcium (283 mg Ca/100g), and Chinese cabbage, cabbage, and butterhead lettuce had the lowest, with values from 33 to 58 mg/100g. However, the highest amounts of magnesium were in kale and in spinach at 52 and 55 mg/100g, respectively.

The low status of calcium in lettuce suggested that this food might be improved by cultivar selection and management of plant nutrition. The objectives of the current study were to determine if the mineral nutrient densities of lettuce can be increased through cultivar selection and nutrient management.

Materials and Methods

Materials

Eighteen lettuce cultivars of heritage and modern genetics with butterhead, romaine, and loose-leaf phenotypes were studied in a greenhouse at the University of Massachusetts, Amherst (N42° 23´, W72° 31´). Seeds were from Seeds of Change (Rancho Dominguez, CA) and from Johnny's Selected Seeds (Waterville, ME). The vendors identified the heritage varieties, which were verified against a data base (http://compositdb.ucdavis.edu/), when possible. All of the cultivars are readily available from seed vendors. All seeds were planted in peat-moss-based medium (Fafard Growing Mix 1-PV; Agawam, MA) for raising seedlings without fertilization. At the two-true-leaf stage, seedlings were transplanted to 15-cm diameter x 15-cm deep round pots filled with the peat moss medium used to start the seedlings. Temperature ranged from 23 to 30°C during the day and 18 to 24°C at night. Light conditions in the greenhouse were from sunlight of about 8 hr/day during December and January 2009.

Methods

Three regimes of fertilizer were solutions of Hoagland no.1 solution, organic fertilizer, and chemical fertilizer, each being supplied in amounts to give about 200 mg N L^{-1} with supply of other elements based on the composition of the fertilizer. The Hoagland no.1 treatment, contained in mg L^{-1}: 210 N, 31 P, 234 K, 200 Ca, 48 Mg, 64 S, and a micronutrient composite and Fe EDDHA (Hoagland and Arnon, 1950). The organic fertilizer (Pure Blend Pro Grow, Botanicare, 3-0.7-3.3 NPK, Tempe, AZ) formulation contained in mg L^{-1}: 200 N, 47 P, 220 K, 67 Ca, and 0.81 Mg, and 64 S, with micronutrients included but not listed, and with this fertilizer 4 ml 0.5 M $MgSO_4$ L^{-1} were added. The third fertilizer was chemical, peat-lite professional fertilizer (Peters Fertilizer Products Inc., 20-4.4-16.6 NPK, Allentown, PA) formulated to contain in mg L^{-1}: 200 N, 44P, 166 K, 15 Mg, with micronutrients as included on the label; 1 g $CaSO_4$/pot was mixed in media, and 4 ml 0.5 M $MgSO_4$ L^{-1} were added to the solution. The fertilizer regimes were applied daily at 100 ml/plant for first 10 days after transplanting and then daily at 200 ml/plant for 21 days until harvest. The nutrient solution drained through the medium during application to avoid nutrient accumulation in the medium and to provide a constant daily supply of nutrients.

At harvest, plant height was measured, fresh weights were recorded, and the samples were washed once in tap water and twice in deionized water, oven-dried at 70°C with dry weights being recorded. For analysis of mineral nutrients, 0.5 g of ground sample was burned at 500°C for 8 h in a furnace, and the ash was dissolved in 10% (v/v) HCl prepared with distilled H_2O and concentrated HCl. The solutions were analyzed for mineral elements by inductively coupled plasma spectrophotometry (Jones Jr. et al., 1991; Kalra, 1998) by the UMass Soil and Plant Tissue Testing Laboratory. Sodium was supplied by the medium and was included in the chemical analyses as it is a nutrient for humans.

Statistical Analyses

Statistical analyses of data were performed by analysis of variance or by regression analysis (Steel and Torrie, 1980) with data processing by statistics software (SAS, v. 9.2, Cary, NC). Means were separated by least significant difference.

Results and Discussion

Results

Plant growth

Heritage and modern cultivars differed in plant heights but did not differ in head fresh or dry weights (Table 2.1). Loose-leaf cultivars had higher head fresh and dry weights than butterhead or romaine cultivars (Table 2.1). No differences occurred in plant height among the fertilizer regimes (Table 2.1). Organic fertilizers yielded higher head fresh weights than Chemical or Hoagland solutions, but dry weights did not differ among the fertilizer treatments. Cultivars differed in plant height and head fresh and dry weights (Table 2.2). As a group, Two Star, Tropicana, Simpson Elite, Buttercrunch, and Cosmo

Savoy had higher fresh weights averaging 127 g/head than Tom Thumb, Red Rosie, Claremont, and Australe, which as a group averaged 67 g/head.

Elemental analysis

The concentration (% dry wt) of macronutrients, P, K, Ca, Mg, and S were higher in heritage cultivars than in modern cultivars (Table 2.1). However, only total accumulation (mg/head) of K in lettuce heads was higher in the heritage cultivars. The concentration or total accumulation of Na did not differ between modern and heritage groups. Among the micronutrients, higher concentration (mg/kg) of Zn, B, and Cu occurred with heritage cultivars, but concentrations of Mn and Fe did not differ between heritage and modern cultivars (Table 2.3). Total accumulation of micronutrients did not vary between heritage and modern cultivars.

Butterhead phenotypes had higher concentrations of P, Ca, Mg, and S; but higher concentration of K occurred in romaine phenotypes, and higher concentration of Na occurred in loose-leaf phenotypes (Table 2.1). Higher accumulation of total P, K, Ca, Mg, S, and Na occurred with loose-leaf phenotypes than with butterhead or romaine phenotypes. Highest concentration of Zn and B occurred with butterhead, and highest concentration of Mn and Cu occurred with loose-leaf phenotype (Table 2.3). Highest accumulation of total Zn, B, Mn, Cu, and Fe occurred with loose-leaf phenotypes (Table 2.3).

Organic fertilizer gave higher concentration of P in plant tissue than the other treatments (Table 2.1). However, highest concentration of K, Ca, S, and Na occurred with the chemical treatment, and highest concentration of Mg occurred with the Hoagland and chemical treatment. Highest accumulation of total P and K occurred in lettuce with organic fertilizer. Concentration of Ca, Mg, S, and Na was highest with the chemical treatment. Highest accumulation of total Ca, Mg, S, and Na also occurred with chemical fertilizer. Total Mg accumulation did not differ among treatments. Highest concentrations of Zn and B, and Fe occurred with chemical fertilizer (Table 2.3). However, highest concentration of Mn and Cu were with organic fertilizer. Highest accumulation of total Zn, B, and Fe occurred with the chemical regime, and highest accumulation of total Mn and Cu was with organic fertilizer.

Accumulation of mineral nutrients in the cultivars

Phosphorus

Concentrations of P differed significantly among the cultivars and ranged from 1.58% to 2.40% (Table 2.4). High concentration of P were in Tom Thumb (2.40%), Forellenschluss (2.34%), Focea (2.32%), Winter Density (2.27%), and Red Deer Tongue (2.40%), which as a group averaged 2.35% P. Moderate accumulators of P were cultivars Australe (2.06%), Simpson Elite (1.97%), and Coastal Star (1.92%), which as a group averaged 1.98% P. Lower concentrations of P were in Two Star (1.58%), Tropicana (1.62%), and Red Rosie (1.67%), which averaged 1.62% P as a group. Total accumulation of total P ranged from 51 mg/head to 110 mg/head. Simpson Elite (110 mg per head), Two Star (107 mg per head) Buttercrunch, and Tropicana (100 mg per head) were high accumulators of P. Red Rosie (51 mg/head), Tom Thumb (63 mg/head), and Australe (70 mg/head) were low accumulators of P.

Potassium

Concentrations of K ranged significantly from 11.86% to 16.16% among the cultivars (Table 2.4). Forellenschluss (16.16%), Coastal Star (15.38%), Red Deer Tongue

(15.43%), Black Seeded Simpson (15.08%), and Tom Thumb (14.62%) averaging 15.33% K were the top-ranking cultivars, and Simpson Elite (12.57%) and Winter Density (12.78%) averaging 12.68% K were moderately performing cultivars. Two Star (11.86%), Tropicana (11.91%), and Australe (11.91%) averaging 11.89% K were low-accumulating cultivars. Total K accumulation was higher in Two Star (794mg/head), Cosmo Savoy (762 mg), Tropicana (734 mg), and Buttercrunch (707 mg) than Tom Thumb (382 mg) and Red Rosie (422 mg).

Calcium

Concentration of Ca ranged significantly from 1.78% to 2.76% (Table 2.4). High accumulators, averaging 2.55% Ca were Red Deer Tongue, (2.76%), Winter Density (2.74%), Tom Thumb (2.46%), Focea (2.41%), and Adriana (2.38%). Low accumulators averaged 1.83% Ca and included Tropicana (1.78%), Red Rosie (1.85%), and Two Star (1.86%), whereas Australe (1.98%), Bronze Mignonette (2.4%), and Black Seeded Simpson (2.05%) accumulated moderate concentrations averaging 2.14% Ca. Total Ca ranged from 57 mg to 125mg per head. Cultivars with high total accumulation were Two Star (125 mg), Simpson Elite (124 mg), Cosmo Savoy (121 mg), Buttercrunch (119 mg), Red Deer Tongue and Tropicana (each 110 mg), and Bronze Mignonette (103 mg). Red Rosie (57 mg) and Tom Thumb (65 mg) accumulated only an average of 61 mg Ca/head.

Magnesium

Magnesium concentrations varied significantly from 0.88% to 1.49% (Table 2.4). Highest concentrations of Mg averaging 1.35% occurred in Red Deer Tongue (1.49%), Winter Density (1.43%), Cosmo Savoy (1.30%), Adriana (1.29%), and Focea (1.24%), whereas Red Rosie (0.88%), Tropicana (0.95%), and Simpson Elite (0.97%) had the lowest concentrations, which averaged 0.93%. Two Star (1%), Salad Bowl (1.02%), Forellenschluss (1.06%), Black Seeded Simpson (1.06%), and Australe (1.07%) were moderate accumulators with 1.04% average Mg. Accumulation of total Mg ranged from 27 mg to 69 mg per head. Highest accumulation of total Mg averaging 64 mg/head was in Cosmo Savoy (69 mg), Two Star (68 mg), Buttercrunch (64 mg), and Red Deer Tongue and Tropicana (each 59 mg), whereas Red Rosie (27 mg), Tom Thumb (32 mg), Australe (36 mg), Claremont (36 mg), Black Seeded Simpson (40 mg), and Forellenschluss (41 mg) were low accumulators averaging 35 mg per head.

Sulfur

Concentration of S varied significantly from 0.32% to 0.57% (Table 2.4). Bronze Mignonette (0.57%), Salad Bowl (0.53%), Winter Density (0.51%), Tom Thumb (0.51%), and Coastal Star (0.50%) averaged 0.52% S, whereas Red Rosie (0.32%), Australe (0.41%), and Tropicana (0.44%) averaged 0.39% S as low accumulators. Adriana, (0.47%), Buttercrunch (0.48%), and Forellenschluss (0.49%) were intermediate accumulators with 0.48% S. Accumulation of total S varied from 10 mg to 30 mg per head. Highest total S was in Two Star (30 mg), Tropicana (27 mg), Simpson Elite (27 mg), Cosmo Savoy (26), Buttercrunch (25 mg) averaging at 26 mg per head, and lower accumulation of 13 mg per head in average was in Tom Thumb, Claremont, Australe, and Red Rosie.

Sodium

Concentration of Na ranged significantly from 0.66% to 1.13% (Table 2.4). Highest concentrations of Na averaging 1.01% were in Two Star (1.13%), Tropicana (1.08%), Cosmo Savoy (0.97%), Coastal Star (0.95%), and Buttercrunch (0.90%), and lowest concentrations averaging 0.69% were in the Red Rosie (0.66%), Australe (0.70%), and Focea (0.71%). Moderate accumulators of Na were Forellenschluss (0.84%), Simpson Elite (0.84%), Bronze Mignonette (0.84%), and Winter Density (0.82%), which averaged 0.84% Na as a group. Accumulation of total Na ranged from 19 mg to 77 mg/head. Highest accumulation of total Na averaging 59 mg/head was in Two Star (77 mg), Tropicana (68 mg), Cosmo Savoy (52 mg), Buttercrunch (50 mg), and Simpson Elite (48 mg). Lowest total Na averaging 22 mg/head was in Tom Thumb, Focea, Claremont, Australe, and Red Rosie. However, moderate accumulation of total Na was in Winter Density, Red Deer Tongue, Forellenschluss, and Simpson Black Seeded at average 33 mg per head, and Coastal Star, Adriana, Salad Bowl, and Bronze Mignonette at average 40 mg per head.

Zinc

Concentrations of Zn ranged significantly from 105 mg/kg to 238 mg/kg (Table 2.5). With an average concentration of 206 mg Zn/kg, Tom Thumb (238 mg/kg), Focea (213 mg/kg), Black Seeded Simpson (204 mg/kg), Winter Density (192 mg/kg), and Bronze Mignonette (184 mg/kg) had the highest concentrations, whereas cultivars with low Zn accumulation included Australe (105 mg/kg), Coastal Star (123 mg/kg), Two Star (129 mg/kg), Red Deer Tongue (134 mg/kg), and Claremont and Tropicana (each 135 mg/kg) and averaged 126 mg Zn/kg. Buttercrunch (157 mg/kg), Simpson Elite (158 mg/kg), Forellenschluss (174 mg/kg), and Salad Bowl (174 mg/kg) were moderate accumulators with an average of 165 mg Zn/kg. Total Zn accumulation varied significantly from364 μg/head to 887 μg/head. Cultivars with the highest accumulation of Zn, averaging 847 μg/head were in Simpson Elite (887 μg), Two Star (864 μg), Buttercrunch (847 µg), Tropicana (831 µg), and Salad Bowl (808 µg). Averaging 399 µg/head, Australe (364 µg), Claremont (396 µg), and Red Rosie (437 µg) were low accumulators of Zn.

Boron

Concentration of B ranged significantly from 48 mg/kg to 76 mg/kg (Table 2.5). Highest concentrations of B were in Focea (76 mg/kg), Claremont (74 mg/kg), Red Deer Tongue (73 mg/kg), Bronze Mignonette (72 mg/kg) and Adriana (70 mg/kg) with an average 73 mg B/kg. Lowest and lower concentrations were in Tropicana (48 mg/kg), Simpson Elite (49 mg/kg), and Two Star (51 mg/kg). Moderate concentrations were in Cosmo Savoy (63 mg/kg), Winter Density (66 mg/kg), and Tom Thumb (69 mg/kg). Accumulation of total B ranged from 183 µg to 340 µg per head. Two Star (340 µg), Cosmo Savoy (323 µg), Bronze Mignonette (313 µg), Buttercrunch (311 µg), and Tropicana (295 µg) had high accumulation of total B with an average 316 µg B/head, and Tom Thumb (183 µg), Red Rosie (193 µg), Australe (197 µg), and Coastal Star (222 µg) had low accumulations at an average 114 µg B/head.

Manganese

Concentration of Mn varied significantly from 131 mg/kg to 214 mg/kg (Table 2.5). Black Seeded Simpson (214 mg/kg), Simpson Elite (211 mg/kg), Red Deer Tongue (200 mg/kg), Salad Bowl (199 mg/kg), and Coastal Star (198 mg/kg) had the highest mean concentration of 204 mg Mn/kg, and cultivars Red Rosie (131 mg/kg), Forellenschluss (142 mg/kg), and Australe (164 mg/kg) had the lowest mean concentrations of 145 mg Mn/kg. Claremont (180 mg/kg), Tom Thumb (181 mg/kg), Tropicana (182 mg/kg), and Cosmo Savoy (184 mg/kg) were intermediate in accumulating Mn. Accumulation of total Mn varied from 399 µg to 1256 µg per head. Two Star (1256 µg), Simpson Elite (1191 µg), Tropicana (1121 µg), Buttercrunch (986 µg), and Cosmo Savoy (970 µg) were top ranking cultivars for accumulation, whereas Red Rosie (399 µg), Tom Thumb (479 µg), and Claremont (530 µg) were low performing cultivars for accumulation of total Mn per head.

Copper

Concentration of Cu ranged from 10 mg/kg to 18 mg/kg (Table 2.5). High concentration of Cu occurred in Black Seeded Simpson (18 mg/kg), Salad Bowl (16 mg/kg), Forellenschluss, Bronze Mignonette, and Winter Density (each 14 mg/kg) with a mean concentration of 15 mg Cu/kg. Red Rosie and Claremont (each 10 mg/kg), Tropicana (11 and Adriana (each 11 mg/kg) had low concentrations averaging 11 mg Cu/kg. Simpson Elite (12 mg/kg), Focea, Tom Thumb, and Cosmo Savoy (each 13 mg/kg) were intermediate in Cu concentrations. Total accumulation of total Cu ranged significantly from 30 µg to 82 µg per head. Two Star (82 µg), Salad Bowl (72 µg), Simpson Elite (70 µg), Cosmo Savoy (68 µg), Black Seeded Simpson and Tropicana (each 66 µg), and Buttercrunch (63 µg) had higher per head accumulation of total Cu than Red Rosie and Claremont (each 30 µg), Tom Thumb (34 µg), and Australe (41 µg).

Iron

Concentration of Fe ranged significantly from 211 mg/kg to 317 mg/kg (Table 2.5). Coastal Star (317 mg/kg), Tom Thumb (289 mg/kg), Black Seeded Simpson (281 mg/kg), Winter Density (273 mg/kg), and Focea (263 mg/kg) averaged 285 mg Fe/kg and exceeded the mean concentrations of 211 mg/kg in Red Rosie and Claremont (each 211 mg/kg), and Cosmo Savoy (215 mg/kg). Total Fe varied from 620 µg to 1651 µg per head. Two Star (1651 µg), Simpson Elite (1364 µg), Tropicana (1328 µg), Coastal Star (1325 µg), and Buttercrunch (1256 µg) had higher accumulation of total Fe per head than Claremont (620 µg), Red Rosie (626 µg), and Tom Thumb (773 µg).

Interactions in elemental accumulation

Several interactions occurred among treatments (Table 2.6). None of the interactions are tabulated or discussed as the effects of the interactions were small. The significant interactions will be noted however. The interaction between fertilizers and genotypes (heritage, modern) was significant for the concentrations of Ca, Zn, and Cu. The interaction of fertilizer and phenotypes was significant for P, S, Na, and Fe. The interaction of genetics and phenotypes was significant for P, K, Ca, Mg, Na, B, Cu, and Fe. The interaction between fertilizers and cultivars were significant for the concentrations of P, K, Mg, Na, S, B, and Cu. The interactions among fertilizers, genetics, and phenotypes were significant for the elemental concentration of K, Na, and B.

Nutrient accumulation as a function of head weight

Nutrients concentrations were affected significantly as a function of dry weight of lettuce for P, K, Ca, Mg, Na, B, and Mn (Fig. 2.1 and 2.2). The trend was for a linear decrease in elemental concentration as dry weight of heads increased. However, the decreases in concentrations were small with low coefficients of determination for the linear regressions. Concentrations of S, Zn, Cu, and Fe did not vary with dry weight of heads. On the other hand, total accumulation of each of the elements showed highly significant increases as dry weights of heads increased (Figure 2.3 and 2.4). The increases were substantial with high coefficients of determination for the linear regressions.

Discussion

The concentrations of all plant nutrients were at the sufficiency level for optimum growth of lettuce (Hochmuth, 2003; Mills and Jones Jr., 1996). Hence, no growth limitations due to limitations in nutrient concentrations were apparent.

Modern and heritage cultivars did not differ in average head size. However, loose-leaf phenotypes had higher plant height and fresh and dry weights than romaine or butterhead. Larger head sizes occurred with chemical fertilization than with organic or Hoagland treatments. Head size differed among cultivars. Coastal Star, Black Seeded Simpson, Red Rosie, and Two Star had higher plant heights than other cultivars. Two Star, Tropicana, Simpson Elite, Buttercrunch, and Cosmo Savoy had higher plant fresh and dry weights. Concentrations (% dry wt) of P, K, Ca, Mg, and S were higher in the heritage cultivars than in the modern cultivars. This effect has been perceived as being due to smaller head size in heritage cultivars than in modern cultivars (Mou, 2005; 2009); however, in this experiment no significant differences in head weights occurred between heritage and modern cultivars. No significant differences occurred in total accumulation (mg/head) for P, Ca, Mg, S, and Na with heritage or modern cultivars, but accumulation of total K with higher K was higher in the heritage cultivars. Zinc, B, and Cu concentrations were higher in the heritage cultivars, but other micronutrients did not vary between heritage and modern cultivars. Also, total Zn, B, Mn, Cu, and Fe accumulation did not differ between modern and heritage cultivars. The nutrient density differed with phenotypes with butterhead phenotypes containing higher P, Ca, Mg, S, Zn, B, and Fe than romaine or loose-leaf phenotypes. However, romaine phenotypes contained higher K, and loose-leaf phenotype contained higher Na, Mn, and Cu than butterhead phenotypes. Mou (2005) and Mou and Ryder (2004) also reported significant differences in mineral concentration with lettuce head morphology, noting that crisphead lettuce with leaves hand-opened had higher nutrient contents than romaine lettuce with heads tied shut.

Nutrient density varied with fertilizers regimes but showed no consistent trend among regimes, perhaps because of the effects on individual cultivars on accumulation of individual nutrients. Total nutrient accumulation varied because of differences in head dry weight. Cultivars with higher dry weight had higher total nutrients sometimes in spite of lower nutrient concentration in plant tissues.

A wide variation occurred for individual nutrient accumulation among lettuce cultivars. High concentrations of P were in Tom Thumb and Focea of the butterhead group, in Forellenschluss and Winter Density of the romaine group, and Red Deer Tongue and Black Seeded Simpson of the loose-leaf phenotypes. Low concentrations of P were in loose-leaf cultivars Two Star and Tropicana, romaine cultivar Red Rosie, and butterhead cultivar Bronze Mignonette in. An overall higher total accumulation of P occurred in Simpson Elite, Two Star, Buttercrunch, and Tropicana, and low total accumulation of P occurred in Red Rosie, Tom Thumb, Claremont, and Australe. This variation in accumulation occurred in response to larger head growth with the high accumulators.

Potassium concentration was high in Forellenschluss, Coastal Star, Red Deer Tongue, Black Seeded Simpson, and Tom Thumb. Lower K concentration in some cultivars such as Two Star and Tropicana appears due to their large head size contributing to a dilution effect. These cultivars, however, accumulated high total K because of the larger head size. High Ca concentrations occurred in Red Deer Tongue, Winter Density, and Tom Thumb. Perhaps, these loose-leaf cultivars had more transpiration than close-leaf cultivars, thereby leading to enhanced Ca accumulation (Mou and Ryder, 2004). The lower Ca concentration in Two Star and Tropicana was attributed to a dilution effect of the larger dry mass, but again these large headed cultivars had higher total Ca than cultivars with lower weights. Overall, larger dry mass of heads did not dilute the concentrations of elements in the produce.

High Mg concentrations occurred in Red Deer Tongue, Winter Density, Cosmo Savoy, and Adriana perhaps due to high uptake by these cultivars. However, all cultivars had above optimum levels for plant growth (0.3 to 0.8 %) (Hochmuth, 2003; Smith, 1962). Accumulation of total Mg was significantly higher in Cosmo Savoy, Two Star, and Buttercrunch because of higher total dry weight than in other cultivars

Overall highest concentrations of S were in Bronze Mignonette, Salad Bowl, Winter Density, and Tom Thumb. Red Rosie and Australe had lower levels of S. The concentration in all cultivars was higher than optimal range of the sulfur in the plant tissues (Hochmuth, 2003; Smith, 1962). Accumulation of total S was higher in Two Star, Tropicana, Salad Bowl, Cosmo Savoy, and Simpson Elite because these cultivars were loose-leaf phenotypes with high dry weights per head.

The most highly performing cultivars Two Star, Tropicana, Cosmo Savoy, and Coastal Star had high total Na because of high total dry weight.

A wide difference occurred with micronutrients Zn, B, Mn, Cu, and Fe concentration among cultivars. Tom Thumb, Focea, Simpson Black Seeded, Winter Density, Bronze Mignonette, and Salad Bowl were nutrient rich perhaps because of being loose leaf or semi-loose leaf phenotypes. Most micronutrients are relatively immobile and are transported through water movement in the xylem. Loose leaf or semi-loose-leaf cultivars perhaps have enhanced and even distribution of micronutrients in the heads (Marschner, 1986). The average micronutrients concentration was higher than adequate range for plant normal growth (Hochmuth, 2003; Smith, 1962) in all cases. An overall higher accumulation of total Zn, B, Mn, Cu, and Fe occurred in cultivars with higher head weight. Loose-leaf cultivars Two Star, Tropicana, Simpson Elite, and Salad Bowl were notable in this regard.

Ranking of the top five accumulators of macronutrients and micronutrients showed consistency in performance among cultivars (Table 2.7). For example, among elements selected for comparison, Red Deer Tongue ranked in the top five for P, K, Ca, and Mg. However, Red Deer Tongue was not in the top five for accumulation of micronutrients. Winter Density was in the top five for accumulation of macronutrients P, Ca, and Mg and of micronutrients Zn, Mg, Cu, and Fe. Other cultivars showing consistent potentials for high accumulations of macronutrients and micronutrients were Black Seeded Simpson, Focea, Forellenschluss, and Tom Thumb.

Conclusions

On average, heritage cultivars had higher concentration of P, K, Ca, Mg, and Zn than modern cultivars. Total accumulation of nutrients did not differ among heritage and modern cultivars. The variations in nutrient contents with phenotype appeared to be due to head size and morphology with loose-leaf and romaine phenotypes being higher accumulators of nutrients than the butterhead structure. Loose-leaf cultivars had highest head weights and highest total nutrient accumulation. Fertilizer regime did not appear to be a major factor in nutrient accumulation, and each regime provided accumulation of nutrients at amounts for optimum of yields. Individual cultivars differed widely in nutrient density with a wide range of variability in mineral nutrient concentrations occurring among different cultivars within phenotypes and genetics. Therefore, the potential for mineral nutritional improvement of different types of cultivated lettuce through breeding and selection is apparent. Improving the mineral nutrition levels of lettuce will enhance nutrient intake in diets without requiring an increase in consumption, as nutrient density was unrelated to head sizes.

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Table 2.1. Head heights, weights, concentration, and total accumulation of macronutrients in heritage and moder lettuce grown under different regimes of fertilization in a greenhouse

Factor	Height (cm/plant)	Head weight, (g/plant)		Macronutrient concentration						Total macro	
				P	K	Ca	Mg	S	Na	P	K
		Fresh	Dry	(% dry wt)							
-----Genetics-----											
Heritage	21	96	4.17	2.08	14.45	2.34	1.21	0.51	0.85	84	596
Modern	22	98	4.40	1.91	13.13	2.09	1.10	0.45	0.85	82	569
LSD (0.05)	0.5	5	0.22	0.07	0.52	0.07	0.03	0.02	0.03	6	45
-----Phenotypes-----											
Butterhead	18	86	3.86	2.07	13.61	2.26	1.20	0.48	0.80	80	520
Romaine	23	91	3.84	2.02	14.29	2.24	1.19	0.48	0.83	80	550
Loose-leaf	23	114	5.16	1.89	13.47	2.15	1.08	0.47	0.93	90	670
LSD (0.05)	0.7	9	0.38	0.05	0.14	0.04	0.04	0.01	0.05	10	50
-----Fertilizers[†]-----											
Hoagland	21	90	4.11	1.92	14.77	2.25	1.18	0.44	0.77	76	601
Organic	21	102	4.33	2.15	14.72	2.10	1.11	0.39	0.79	92	627
Chemical	21	100	4.42	1.90	11.88	2.29	1.18	0.60	0.99	81	518
LSD (0.05)	0.8	8	0.36	0.06	0.33	0.05	0.02	0.02	0.06	7	52

[†] Hoagland = Hoagland no. 1, Chemical = 20-10-20 Peatlite Special, Organic = Pure Blend Pro

Table 2.2. Plant heights and head weights of eighteen cultivars of loose-leaf, romaine, and butterhead lettuce

Cultivar	Cultivar type		Head height	Head weight, g/plant	
	Genetics	Phenotype	(cm/plant)	Fresh	Dry
Coastal Star	Modern	Romaine	26	108	4.1
Black-Seeded Simpson	Heritage	Loose-leaf	25	92	3.7
Red Rosie	Modern	Romaine	25	64	3.1
Two Star	Modern	Loose-leaf	24	139	6.8
Forellenschluss	Heritage	Romaine	24	89	3.9
Tropicana	Modern	Loose-leaf	23	136	6.2
Simpson Elite	Modern	Loose-leaf	23	125	5.6
Cosmo-Savoy	Heritage	Romaine	23	114	5.3
Buttercrunch	Heritage	Butterhead	22	120	5.3
Salad Bowl	Heritage	Loose-leaf	22	97	4.7
Adriana	Modern	Butterhead	22	89	4.1
Red Deer Tongue	Heritage	Loose-leaf	21	96	3.9
Winter Density	Heritage	Romaine	20	105	3.8
Claremont	Modern	Romaine	19	68	2.9
Bronze Mignonette	Heritage	Butterhead	18	91	4.3
Focea	Modern	Butterhead	18	78	3.5
Australe	Modern	Butterhead	16	75	3.4
Tom Thumb	Heritage	Butterhead	12	61	2.6
LSD (0.05)			1	15	0.7

Table 2.3. Concentration and total accumulation of micronutrients in heritage and modern phenotypes o
grown under different regimes of fertilization in a greenhouse

Factor	Micronutrient concentration					Total micronutrient accumu			
	Zn	B	Mn	Cu	Fe	Zn	B	Mn	C
	(mg/kg dry wt)					(µg/head)			
	Genetics								
Heritage	178	64	184	14	255	729	265	772	5
Modern	142	60	180	11	248	725	255	809	5
LSD (0.05)	3.1	2.2	12	1.2	43	42	14	73	7
	Phenotypes								
Butterhead	172	67	178	12	261	660	260	686	4
Romaine	151	63	171	12	245	593	238	670	4
Loose-leaf	156	57	199	13	248	779	282	1016	6
LSD (0.05)	5.5	1.5	15	1	22	64	26	112	
	Fertilizers[†]								
Hoagland	107	46	193	11	269	427	182	808	4
Organic	167	65	201	15	199	703	273	872	6
Chemical	206	75	153	12	286	902	325	692	5
LSD (0.05)	8	2	17	1	24	71	22	127	

[†] Hoagland = Hoagland no. 1, Chemical = 20-10-20 Peatlite Special, Organic = Pure Blend Pro

Table 2.4. Concentration and total accumulation of macronutrients in eighteen cultivars of lettuce listed in order
concentrations

Cultivar	Macronutrient concentration						Total nutrien		
	P	K	Ca	Mg	S	Na	P	K	Ca
	----------------(% dry wt)------------------						--------------(mg		
Tom Thumb	2.40	14.62	2.46	1.20	0.51	0.73	63	382	65
Forellenschluss	2.34	16.16	2.24	1.06	0.49	0.84	89	605	85
Focea	2.32	13.52	2.41	1.24	0.45	0.71	80	467	84
Winter Density	2.27	12.78	2.74	1.43	0.51	0.82	87	490	105
Red Deer Tongue	2.24	15.34	2.76	1.49	0.49	0.87	88	606	110
Simpson Black-Seeded	2.19	15.08	2.05	1.06	0.50	0.88	81	558	76
Claremont	2.13	13.41	2.20	1.23	0.49	0.72	63	397	65
Australe	2.06	11.91	1.98	1.07	0.41	0.70	70	401	67
Buttercrunch	1.97	13.61	2.30	1.20	0.48	0.90	102	707	119
Simpson Elite	1.97	12.57	2.22	0.97	0.50	0.84	110	701	124
Coastal Star	1.92	15.38	2.10	1.22	0.50	0.95	78	631	87
Adriana	1.91	14.11	2.38	1.29	0.47	0.89	77	570	96
Cosmo-Savoy	1.81	14.46	2.30	1.30	0.50	0.97	94	762	121
Salad Bowl	1.74	13.89	2.04	1.17	0.57	0.84	75	594	88
Bronze Mignonette	1.74	14.07	2.20	1.02	0.53	0.79	80	656	103
Red Rosie	1.67	13.52	1.85	0.88	0.32	0.66	51	422	57
Tropicana	1.62	11.91	1.78	0.95	0.44	1.08	100	734	110
Two Star	1.58	11.86	1.86	1.00	0.45	1.13	107	794	125
LSD (0.05)	0.13	0.82	0.13	0.07	0.04	0.08	11	85	1

Table 2.5. Concentration and total accumulation of micronutrients in eighteen cultivars of lettuce listed in order of descending Zn concentrations

	Micronutrient concentration					Total nutrient accumulation				
Cultivar	Zn	B	Mn	Cu	Fe	Zn	B	Mn	Cu	Fe
	-------(mg/kg dry wt)-----					--------------(µg/head)----------				
Tom Thumb	238	69	181	13	289	632	183	479	34	773
Focea	213	76	198	13	263	746	265	691	45	924
Simpson Black-Seeded	204	64	214	18	281	746	234	802	66	1082
Winter Density	192	66	187	14	273	744	254	718	53	1046
Bronze Mignonette	184	72	166	14	259	802	313	710	60	1134
Forellenschluss	174	57	142	14	245	686	218	561	53	935
Salad Bowl	174	55	199	16	249	808	257	926	72	1187
Simpson Elite	158	49	211	12	242	887	276	1191	70	1364
Buttercrunch	157	58	186	12	238	847	311	986	63	1256
Cosmo-Savoy	146	63	184	13	215	760	323	970	68	1136
Red Rosie	138	62	131	10	211	437	193	399	30	626
Adriana	137	70	174	11	254	567	290	705	46	1054
Claremont	135	74	180	10	211	396	219	530	30	620
Tropicana	135	48	182	11	220	831	295	1121	66	1328
Red Deer Tongue	134	73	200	12	248	539	290	800	48	989
Two Star	129	51	185	12	247	864	340	1256	82	1651
Coastal Star	123	54	198	11	317	534	222	841	48	1325
Australe	105	57	164	12	262	364	197	546	41	942
LSD (0.05)	21	3	21	2	64	141	40	164	11	349

Table 2.6. Interaction of genetics (heritage or modern), phenotypes (butterhead, romaine, loose leaf), fertility reg organic, compost), and cultivar of lettuce for growth and elemental concentrations.

Interaction	Plant growth			Macronutrient concentration						Micro
	(cm)	(gm/head)		P	K	Ca	Mg	S	Na	Zn
	Height	Fresh wt	Dry wt	--------------(% dry wt)----------						-------
T × G[†]	*	NS	NS	NS	NS	*	NS	NS	*	*
T × F	NS	NS	NS	*	NS	NS	NS	*	*	NS
G × F	NS	*	*	*	*	*	*	NS	*	NS
T × C	*	NS	NS	*	*	NS	*	*	*	NS
T × G × F	NS	NS	NS	NS	*	NS	NS	NS	*	NS

[NS] Non-significant ($P > 0.05$), *significant ($P \leq 0.05$) by F-test.

[†] G, Genotypes of lettuce; F, Phenotypic form of lettuce; C, Cultivar of lettuce; T, Fertility regimes.

Table 2.7. Top-ranking lettuce cultivars for concentrations (micronutrients, % dry wt; micronutrients, mg/kg drywt) of nutrients

Macronutrients			
Phosphorus	**Potassium**	**Calcium**	**Magnesium**
Tom Thumb	Forellenschluss	Red Deer Tongue	Red Deer Tongue
Forellenschluss	Coastal Star	Winter Density	Winter Density
Focea	Red Deer Tongue	Tom Thumb	Cosmo-Savoy
Winter Density	Simpson Black-Seeded	Focea	Adriana
Red Deer Tongue	Tom Thumb	Adriana	Focea
Micronutrients			
Zinc	**Manganese**	**Copper**	**Iron**
Tom Thumb	Simpson Black-Seeded	Simpson Black-Seeded	Coastal Star
Focea	Simpson Elite	Salad Bowl	Tom Thumb
Simpson Black-Seeded	Red Deer Tongue	Winter Density, Bronze Mignonette, Forellenschluss tied	Simpson Black-Seeded
Winter Density	Salad Bowl	Focea, Cosmo-Savoy, Tom Thumb tied	Winter Density
Bronze Mignonette	Focea, Coastal Star tied	Simpson Elite	Focea

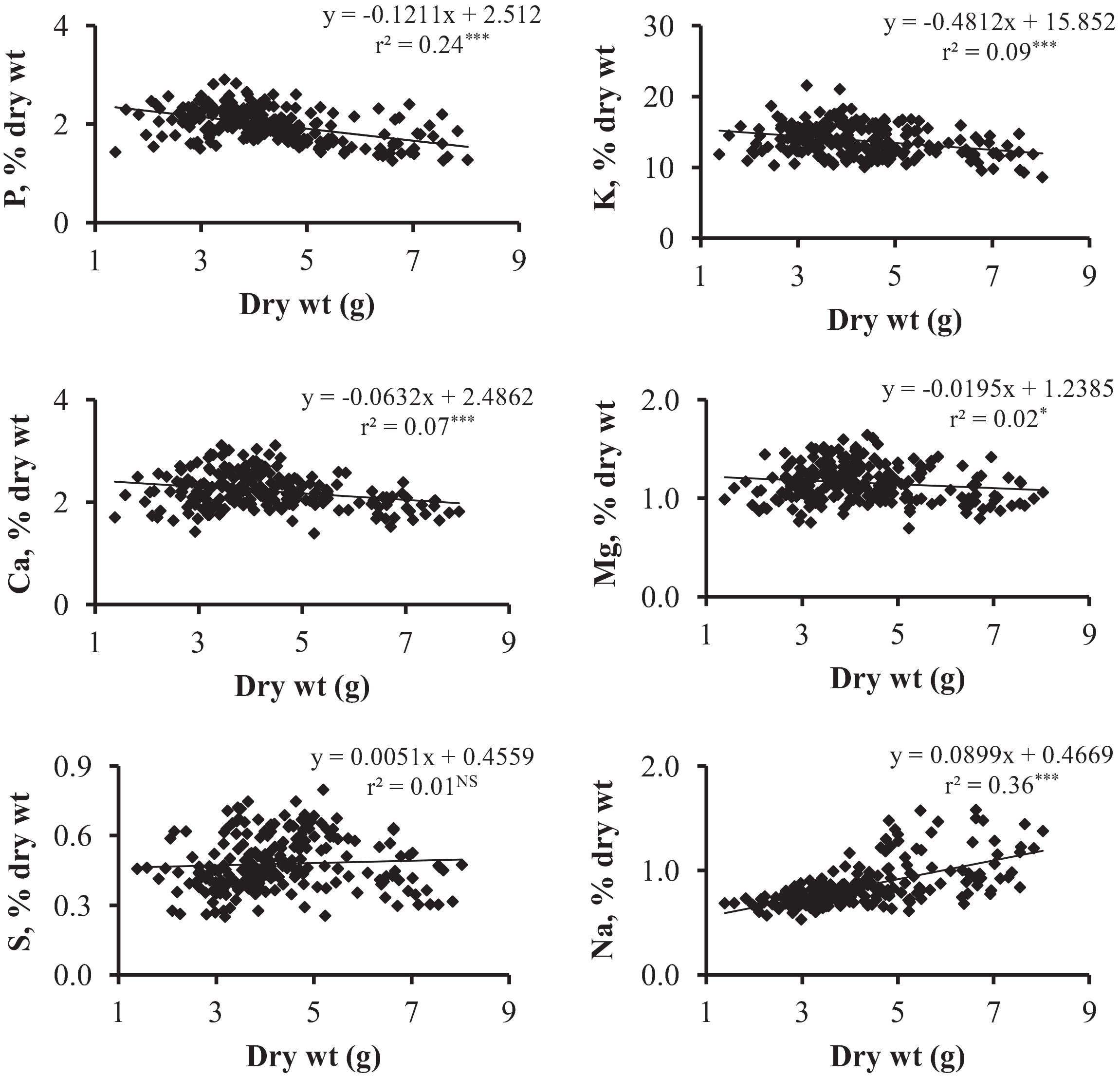

Fig. 2.1. Elemental concentration in percent dry weight as a function dry weight per head of lettuce. Element include: P, K, Ca, Mg, S, and Na.

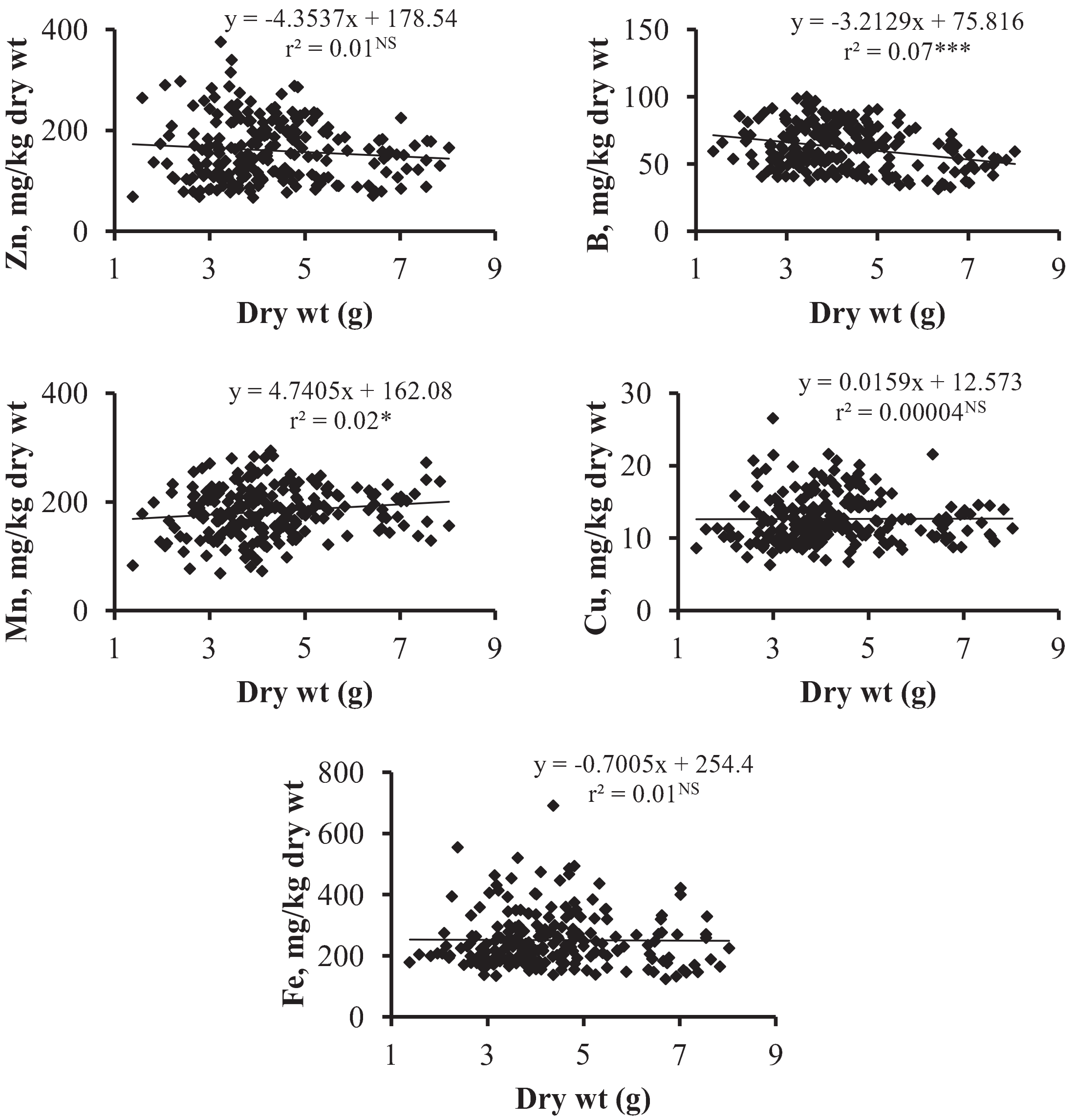

Fig. 2.2. Elemental concentration in mg/kg dry weight as a function dry weight per head of lettuce. Element include: Zn, B, Mn, Cu, and Fe.

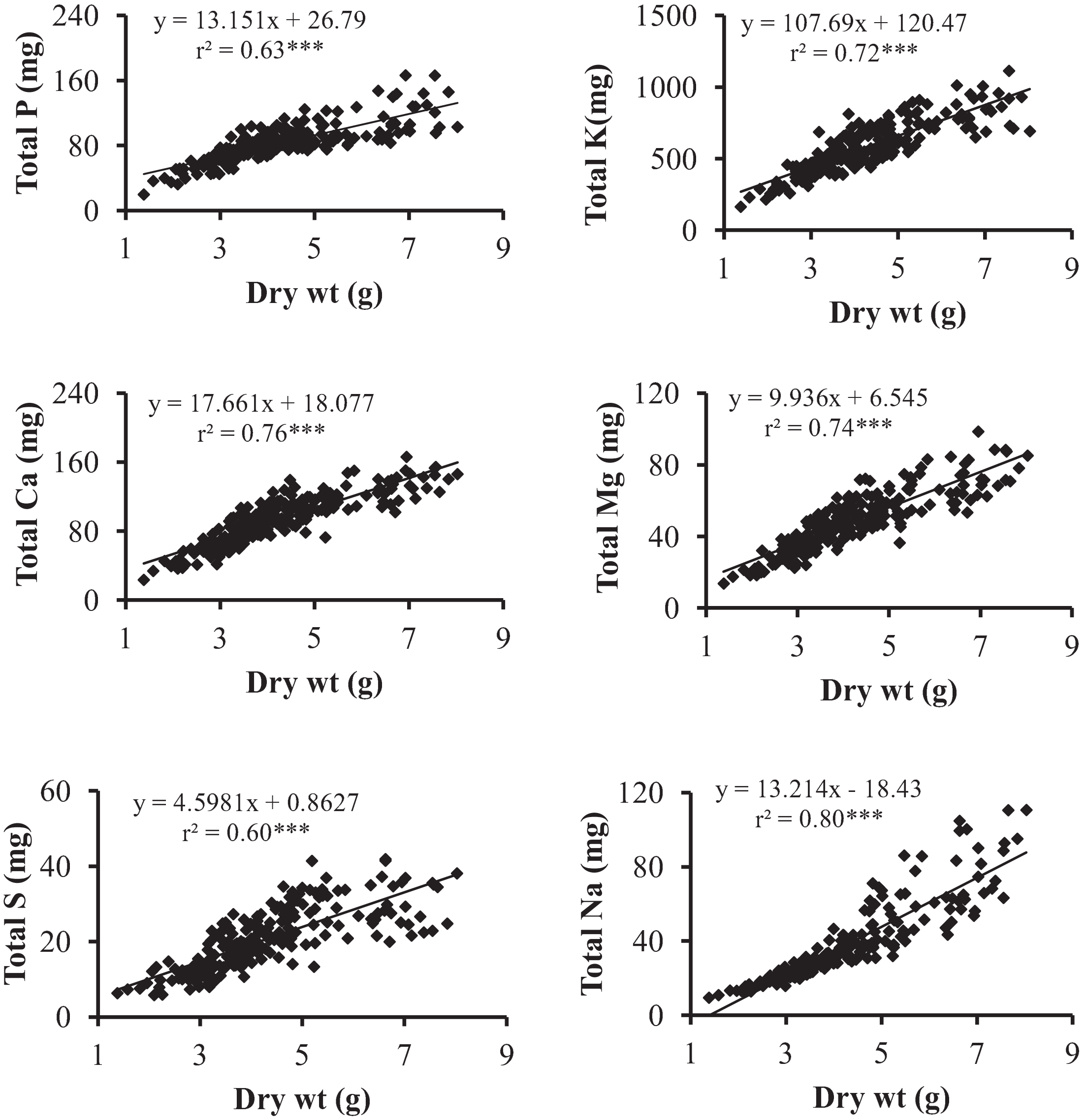

Fig. 2.3. Total elemental accumulation in milligrams per head as a function dry weight per head of lettuce. Element include: total P, K, Ca, Mg, S, and Na.

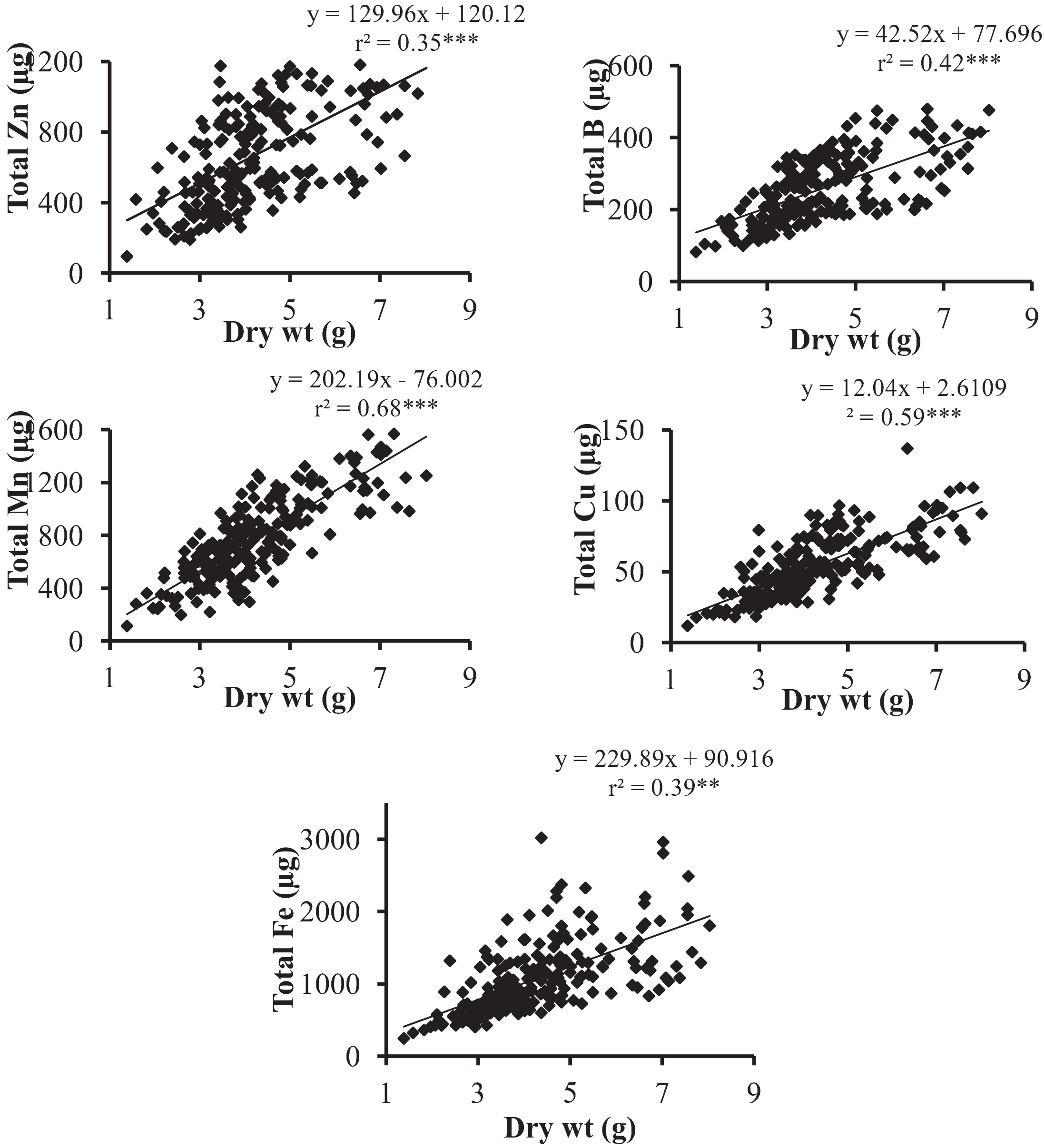

Fig. 2.4. Total elemental accumulation in micrograms per head as a function dry weight per head of lettuce. Elements include: total Zn, B, Mn, Cu, and Fe.

CHAPTER 3

ASSESSMENT OF ORGANIC AND CONVENTIONAL SOIL FERTILITY PRACTICES AND CULTIVAR SELECTION ON MINERAL ACCUMULATION IN LETTUCE

Abstract

Mineral nutrient deficiency in vegetable-based foods is a substantial concern in human diets. Depleted soil fertility and high yielding cultivars have been associated with low nutrient contents in vegetables. In response to these difficulties, research is needed to develop systems that introduce nutrient-dense crops to the markets. This study explored if mineral nutrient densities of lettuce (*Lactuca sativa* L.) can be increased though selection of cultivars and soil fertility regimes. Eighteen cultivars including butterhead, romaine, and loose-leaf phenotypes of heritage and modern origins were studied in field experiments. Conventional (complete fertilizer), compost, and an organic (soybean meal, bone meal, and potassium sulfate) regimes were assessed. Compost was applied at 20 Mg/acre, and chemical or organic fertilizers provided 84 kg N–37 kg P–70 kg K/ha. Elements in whole heads were determined by plasma spectrophotometry. Heritage cultivars had about 6 % higher Zn, B, Mn, Cu, and Fe concentrations than modern cultivars with no differences occurring for P, K, Ca, Mg, Na, and S. Differences for each element occurred among phenotypes and nutritional regimes but with no consistent trends for phenotypes or regimes. Differences among individual cultivars for each element were large with some cultivars having nearly twice the concentrations of nutrients of others and with considerable uniformity in cultivar rankings among the elements. This work suggests that cultivars can be selected for production of nutrient-dense lettuce.

Key words: Chemical fertilizer; compost; macronutrients; micronutrients; nutrient dense lettuce; organic fertilizer; phenotypes.

Introduction

Deficiencies of mineral nutrients like calcium, potassium, magnesium, phosphorus, zinc, iron, copper, and manganese, and others are substantial nutritional problems in diets of humans worldwide (Banuelos and Lin, 2008; Darnton-Hill et al., 2005; Kataki and Babu, 2002). Consuming nutrient-rich vegetables will provide an array of mineral nutrient substances needed for good human health. However, concentrations of mineral nutrients in vegetables and fruits have shown declines of 5% to 40% or more in the past 50 to 100 years in the United States (Davis, 2009). Reports indicated that in the United Kingdom, nutrient contents decreased by about 20% in various foods during this time (Lobstein, 2004; Mayer, 1997; Thomas, 2006). Micronutrient malnutrition is a growing concern all over the developing world (Cakmak, 2009). Nutrient-dense vegetable crops are needed to help solve this problem.

Davis (2009) suggests that the decline in mineral content in fruits and vegetables is due partly to a dilution effect of high yields. Comparisons of low- and high-yielding vegetables and grains showed negative correlations between yield of produce and concentrations of minerals. However, a dilution effect of high yield on nutrient concentration was not observed universally on potato (*Solanum* spp L.), and soil fertility affected the mineral nutrients more than the dilution effect (White et al., 2009). Soil fertility problems associated with nutrient depletion by crop production are worldwide (Tan et al., 2005). In United States, potassium and phosphorus are being drawn in soils on a national basis at an increasing rate every year, and the depletion has occurred for 40 years for potassium and for nearly 30 years for phosphorus (Stewart, 2003).

Leafy vegetables represent an important source of proteins, vitamins, and minerals for humans. Regular consumption of lettuce is reported to improve protection against cardiovascular diseases (Nicolle et al., 2004). A study was conducted to compare the nutrient status of butterhead lettuce, kale, cabbage, spinach, and other leafy vegetables (Kawashima and Valente Soares, 2003). Among them, kale offered the highest amounts of calcium (283 mg Ca/100g), whereas cabbage and butterhead lettuce had the lowest calcium (33 to 58 mg Ca/100g). The nutritional status of lettuce was low compared to others suggesting that studies are needed to enrich its mineral nutrient content.

Some studies have suggested that organic fertilization gives higher concentrations of some nutrients in soils than chemical fertilization (Adeli et al., 2007; Herencia et al., 2007; Malhi et al., 2007; Tewolde et al., 2011; Yang et al., 2007). However, the increase in soil-borne nutrients did not always result in increased concentrations of nutrients in produce.

The objectives of the current study were to determine if the mineral nutrient densities of selected lettuce can be increased through cultivar selection and nutrient management in fertility regimes.

Materials and Methods

Materials

Eighteen lettuce cultivars of heritage and modern genetics with butterhead, romaine, and loose-leaf phenotypes were studied. Heritage seeds were obtained from Seed of Change Seeds Co., Spicer, MN, and modern type of seeds were obtained from Johnny Seeds Co., Winslow, ME. Heritage varieties commonly are understood as being introduced in or before 1950. All seeds were planted in peat moss-based medium (Fafard Growing Mix 1-PV, Conrad Fafard Inc, Agawam, MA) for raising seedlings. The seedlings were transplanted at the two-true-leaf stage to the 4-cm-square cells filled with the peat moss medium before transplant at five weeks of age to the field in June 2010 and in June 2011 for two experiments with the same varieties planted onto the same plots with three replications in a randomized complete block design with plots split for fertilizer treatments in each year in the field at the University of Massachusetts South Deerfield research farm. The crop was grown for three weeks after transplanting until harvest.

Methods

Three regimes of soil fertility with chemical, organic, or compost fertilization were included. The chemical regime was provided with a complete fertilizer (10-4.4-8.3, Loveland Products Inc., Greeley, CO). The organic regime was provided with soybean meal (7 % N) (Kent Nutrition Group Inc., Muscatine, IA), bone meal (5.3 % P) (The Espoma Co., Millville, NJ), and K_2SO_4 (42.4 % K) (Loveland Products Inc., Greeley, CO). These materials are used commonly by organic growers in the region. The chemical and organic fertilizer regimes were applied at 84-37-60 kg NPK/ha at planting according to the New England Vegetable Management Guide (Anonymous, 2010; Rosen and Eliason, 2005). The third regime was compost obtained from the UMass Amherst Office of Waste Management. The organic compost regime was applied with a manure spreader and at 20 Mg/ha disked at one time during soil preparation at one week before planting and provided about 280 kg N, 60 kg P, and 140 kg K per hectare.

At harvest, fresh weights of plant were recorded, and the samples were washed once in clean tap water and twice in clean deionized water and oven-dried at 70°C with dry weights being recorded. For analysis of phosphorus, potassium, calcium, magnesium, sulfur, sodium, zinc, boron, manganese, copper, and iron, 0.5 g of ground samples was ashed at 500°C for 8 h in a muffle furnace, and the ash was dissolved in 10% (v/v) HCl. The solutions were analyzed by inductive couple plasma spectrophotometry (Jones Jr. et al., 1991; Kalra, 1998) at Micro-Macro Laboratories, Athens, GA.

Statistical Analyses

Statistical analyses of data were performed by analysis of variance or regression analysis (Steel and Torrie, 1980) with data processing by SAS software (SAS v. 9.2, Cary, NC). The two years of investigation were treated as random effects. Soil test results for the initial uncropped, unfertilized soil in 2010 and for the soil after one year of cropping and fertilization in 2011 as of (Morgan, 1941) showed no differences among nutrients, pH, or cation exchange capacity between the years (Table 3.1). Means were separated by least significant difference (Fisher's LSD) for treatments with significant effects in the analysis of variance (Steel and Torrie, 1980).

Results and Discussion

Results

Head weights

Modern cultivars (193 g/head) and heritage cultivars (189 g) but did not differ in plant head wt (Table 3.2). Romaine (210 g/head) and loose-leaf (209 g) phenotypes had higher fresh weights than butterhead (155 g). Higher dry weights (13 g/head) also occurred with romaine and loose-leaf than with butterhead (10 g) phenotypes (Table 3.2).

The chemical regime (226 g/head) produced higher fresh weight than the organic (176 g) or compost (173 g) treatments, and the dry weight of three fertility regimes differed in the same order as the fresh weight (Table 3.2). The differences of plant fresh and dry weights in the cultivar group were significant (Table 3.3). Cultivars Cosmo-Savoy (260 g/head), Tropicana (248 g), Simpson Elite (230 g), Claremont (228 g), and Winter Density (227 g) had the highest fresh weights, and Tom Thumb (98 g), Red Rosie (123 g), and Australe (141 g), and Bronze Mignonette (144 g) had the lowest fresh weights. Tropicana (15.8 g/head), Cosmo-Savoy (15.6 g), Coastal Star (15.6 g), Simpson Elite (14.2 g), and Salad Bowl (14 g) had higher plant dry weight than other cultivars.

Nutrient accumulation in genetic, phenotypic, and fertility regimes

Macronutrients

Concentration (% dry wt) or total accumulation (g/head) for P, K, Ca, and Na did not differ between heritage and modern genetic groups, but Mg and S content differed slightly (Table 3.2). Phenotypic groups differed, however, in concentrations or total accumulation (Table 3.2). The concentrations of P and K were higher in loose-leaf than butterhead or romaine phenotypes. However, the total contents of Ca, Mg, S, or Na were higher than or same in butterhead as in romaine and loose-leaf phenotypes. The total accumulation of P, K, Ca, S, and Na were higher in loose-leaf than in romaine or butterhead phenotypes, but total Mg was higher in romaine. Concentration of P and K were higher in the chemical and compost systems that in the organic regime, but concentrations did not differ for Ca, Mg, and Na among the fertility regimes (Table 3.2). The concentration of S were higher in organic than in compost or chemical regimes. The total P (mg/head), K, Ca, Mg, S, and Na were higher in the chemical than in the organic and compost fertility regimes.

Micronutrients

With the exception for B concentrations, micronutrient concentrations (mg/kg dry wt) or total accumulation (µg/head) did not differ between the modern and heritage genetic groups (Table 3.4). The content or total accumulation of Zn, B, Mn, Cu, and Fe differed with phenotypes (Table 3.4). The concentrations of Zn, B, Mn, Cu, and Fe were higher in butterhead than in loose-leaf or romaine phenotypes. However, the total accumulation of Zn, B, Mn was higher in loose-leaf than in romaine and butterhead phenotypes. Total Cu or Fe were higher in romaine than butterhead or loose-leaf. The concentrations and total accumulation of Zn, Mn, or Cu were higher with chemical fertilization than with the organic or compost regimes (Table 3.4).

Nutrient Accumulation among Cultivars

<u>Macronutrients</u>

Phosphorus

The concentration of P ranged significantly from 1.13 to 0.87 % dry wt (Table 3.5. Cultivars Red Deer Tongue (1.13 % dry wt), Focea (1.06 %), Australe (1.02 %), and Forellenschluss (1.01 %) had the highest accumulation. Bronze Mignonette (0.87 %), Red Rosie (0.91 %), Coastal Star (0.92 %) had the lowest accumulation of P. Two Star, Salad Bowl, Claremont, Cosmo Savoy, and Black Seeded Simpson had accumulated P averaging 0.98 % dry wt. The total accumulation of total P varied ranging from 63 to 155 mg/head. The highest total P was in Cosmo Savoy (155 mg/head), Tropicana (149 mg), and Coastal Star (146 mg), and the lowest total P was in Tom Thumb (63 mg), Forellenschluss (78 mg), and Red Rosie (89 mg).

Potassium

The concentration K ranged significantly from 9.22 to 11.30 % dry wt (Table 3.5). Cultivars Simpson Elite (11.30 % dry wt), Two Star (11.27 %), Red Deer Tongue (11.22 %), Tropicana (11.10 %), and Salad Bowl (10.92 %) had the highest concentrations. Buttercrunch (9.22 %), Coastal Star' (9.41 %), Winter Density (9.52 %), and Cosmo Savoy (9.59 %) had the lowest concentrations of K. The total accumulation of total K also widely differed ranging from 649 to 1724 mg/head. The highest total K was in Tropicana (1724 mg/head), Simpson Elite (1590 mg), and Two Star (1480 mg), and lowest total K was in Tom Thumb (649 mg), Forellenschluss (871 mg), and Australe (944 mg) cultivars.

Calcium

The concentration of Ca content ranged significantly from 1.72 to 2.14 % dry wt (Table 3.5). The highest concentration was in Buttercrunch (2.14 %), Bronze Mignonette (2.13 %), Cosmo Savoy (2.06 %), Red Deer Tongue (2.03 %), and Simpson Elite (2.03 %), and the lowest Ca was in Forellenschluss (1.72 %), Claremont (1.78 %), Focea (1.84 %), and Tom Thumb (1.88 %). Total accumulation of Ca ranged from 119 to 331 mg per head. Cultivars Cosmo Savoy (331 mg), Tropicana (302 mg), and Coastal Star (297 mg) had the highest total Ca, and Tom Thumb (119 mg), Bronze Mignonette (183 mg), and Red Rosie (184 mg) had the lowest total Ca.

Magnesium

The concentration of Mg varied significantly from 0.63 to 0.92 % dry wt (Table 3.5). The highest concentration was Bronze Mignonette (0.92 %), Red Deer Tongue (0.83 %), Cosmo Savoy (0.78 %), Coastal Star (0.75 %), and Australe (0.74 %), and the lower concentration was in Tropicana (0.63 %), Focea (0.64 %), and Simpson Elite (0.66 %). Total accumulation ranged from 41to 128 mg/head. The highest accumulation was in Cosmo Savoy (128 mg), Coastal Star(121 mg), Tropicana (103 mg), and 'Two Star' (98 mg), whereas the lowest accumulation was in Tom Thumb (41 mg), Focea (71 mg), and Red Rosie (72 mg).

Sulfur

Concentration of S ranged significantly from 0.27 to 0.39 % dry wt (Table 3.5). Cultivars Bronze Mignonette (0.39 %), Tom Thumb (0.36 %), Salad Bowl (0.35 %), and Winter Density (0.34 %) accumulated the highest concentrations of S, and Red Rosie (0.27 %), Coastal Star (0.29 %), and Adriana (0.30 %) had the lowest concentrations. Total S varied from 23 to 49 mg/head. The highest total S accumulated was in Cosmo Savoy, Tropicana, and Salad Bowl at about 48 mg/head, and lowest total S was in Tom Thumb, Red Rosie, and Red Deer Tongue at about 26 mg/head.

Sodium

Concentration of Na ranged significantly from 0.16 to 0.21% dry wt. The highest concentrations were in Red Deer Tongue (0.21 %), Australe and 'Focea (each 0.18 %), and Adriana and Tropicana (each 0.17 %) (Table 3.5). Sodium was lowest in Two Star, Forellenschluss, Bronze Mignonette, Red Rosie, and Costal Star (all at 0.16 %). Total Na accumulation ranged from 11 to 27 mg/head. The highest accumulation was in Tropicana

(27 mg/head), Coastal Star (26 mg), and Cosmo Savoy (25 mg), and lower amount was in Tom Thumb (11 mg), Bronze Mignonette (14 mg), and Red Rosie (16 mg).

Micronutrients

Zinc

The concentration of Zn content varied significantly from 60 to 80 mg/kg dry wt (Table 3.6). Cultivars Focea (80 mg/kg), Black Seeded Simpson (79 mg/kg), Salad Bowl (77 mg/kg), Buttercrunch (76 mg/kg), and Tom Thumb (75 mg/kg) had the highest concentrations. Tropicana (60 mg/kg), Coastal Star (61 mg/kg), Cosmo-Savoy (61 mg/kg), Winter Density (62 mg/kg), and Red Deer Tongue (64 mg/kg) had the lowest Zn concentrations. Other varieties accumulated between 66 and 70 mg Zn/kg. Total Zn accumulation varied from 504 to 1149 µg/head. The top ranking cultivars were Salad Bowl (1149 µg/head), Simpson Elite (1077 µg), Black Seeded Simpson (1046 µg), Coastal Star (1025 µg), and Buttercrunch (1021 µg). The lowest accumulation was in Tom Thumb (504 µg), Red Rosie (647 µg), Red Deer Tongue (656 µg), and Bronze Mignonette (673 µg).

Boron

The concentrations of B ranged significantly from 26 to 40 mg/kg dry wt (Table 3.6). Australe (40 mg/kg), Red Deer Tongue (39 mg/kg), Adriana, Claremont, and ‘Focea (each with 38 mg/kg) had the highest accumulation of B. Coastal Star (26 mg/kg), Buttercrunch (30 mg/kg), Forellenschluss (33 mg/kg), and Tropicana (34 mg/kg) had the lowest accumulation. The accumulation of total B also widely differed ranging from 224 to 515 µg/head. The highest total B was in Tropicana (515 µg/head), Cosmo-Savoy’ (506 µg), Simpson Elite (490 µg), and Adriana (460 µg), and the lowest total B was in Tom Thumb (224 µg), Bronze Mignonette (294 µg), and Red Deer Tongue (342 µg).

Manganese

The concentrations of Mn ranged significantly from 97 to 145 mg/kg dry wt (Table 3.6). Cultivars with high concentrations were Australe (145 mg/kg dry wt), Salad Bowl (141 mg/kg), Tom Thumb (140 mg/kg), and Bronze Mignonette (134 mg/mg). Low concentrations occurred in Coastal Star (97 mg/kg), Two Star (98 mg/kg), and Red Rosie (103 mg/kg. Total accumulation of Mn ranged from 960 to 2066 µg per head. Salad Bowl (2066 µg/head), Tropicana (2023 µg), and Simpson Elite (1935 µg) were high accumulators whereas Tom Thumb (960 µg/head), Red Rosie (1006 µg), and Red Deer Tongue (1195 µg) had low Mn.

Copper

The concentration of Cu varied significantly from 25 to 35 mg/kg dry wt (Table 3.6). High contents of Cu occurred in Tom Thumb (35 mg/kg), and Australe, Red Deer Tongue, and 'Bronze Mignonette (each with 34 mg/kg). Low concentrations of Cu occurred in Tropicana (25 mg/kg), Coastal Star and Two Star (each 26 mg/kg), and Winter Density (28 mg/kg). Accumulation of total Cu ranged from 233 to 468 µg/head. Highest accumulation was in Cosmo Savoy (468 µg/per head.), Forellenschluss (451 µg), Simpson Elite (431 µg), and Coastal Star (428 µg), and the lowest total Cu was in Tom Thumb (233 µg), Bronze Mignonette (313 µg), and Red Rosie (318 µg)

Iron

Concentrations of Fe varied significantly from198 to 709 mg/kg dry wt (Table 3.6). Cultivars Tom Thumb (709 mg/kg), Bronze Mignonette (534 mg/kg), Red Deer Tongue (524 mg/kg), and Australe (417 mg/kg) had high concentrations whereas Coastal Star (198 mg/kg dry wt), Cosmo Savoy (232 mg/kg), Claremont (259 mg/kg), and Forellenschluss (268 mg/kg) had low concentrations of Fe. Accumulation of total Fe varied from 1919 to 3903 µg/head. Highest total Fe accumulated in Tropicana (3903 µg/head), Salad Bowl (3630 µg), Tom Thumb(3518 µg), Simpson Elite (3503 µg), and Buttercrunch (3176 µg). Lowest total Fe accumulation was in Coastal Star (1919 µg)', 'Focea', 'Forellenschluss (2237 µg), Focea (2190 µg), Red Rosie (2306 µg), and Claremont (2611 µg).

<u>Interactions of genetics, phenotypes, fertilizers, and cultivars</u>

The interaction (G*T) of genetics (heritage and modern) and fertilizer regime (chemical, organic, and compost) was not significant ($P > 0.05$) for head weight or nutrient concentration (Table 3.7). The interaction (G*F) of genetics with phenotypes had an effect ($P \leq 0.05$) on head weight and accumulation of P, Na, or Cu; however, these interactions had small F-values compared to the main effects that were significant. The interaction (G*F*T) of genetics and phenotypes with fertility regimes had no effect ($P > 0.05$) on plant head weight or accumulation of macronutrients or micronutrients except Mn ($P \leq 0.05$), an effect which also had a small F-value. The interaction [T*V(G*F)]of cultivars with fertilizer had no effect ($P > 0.05$) on dry weight and accumulation of all macronutrients and most micronutrients but had an effect ($P \leq 0.05$) on plant fresh wt and accumulation of Mn. Both of these significant effects had low *F*-values. The results of the interactions are not tabulated because the effects are not considered important given their rarity and low *F*-values.

Relationship of head dry weight to nutrient accumulation

The concentrations of macronutrients (% dry wt., Fig. 3.1) and micronutrients (mg/kg dry wt., Fig. 3.2) were not affected substantially by the size of lettuce heads. Regressions models of nutrient concentration as a function of dry weight of heads had essentially flat slopes and nonsignificant or small coefficients of determination. On the other hand, total accumulation of macronutrients (mg/head, Fig. 3.3) and micronutrients (µg/head, Fig. 3.4) increased substantially with increases in dry weight of heads. Regression models for total accumulation had steep slopes and highly significant coefficients of determination for all nutrients.

Discussion

Plant growth

No differences in head fresh or dry weights occurred among heritage and modern genetic groups of lettuce. This result is contrary to a common perception that modern cultivars due to crop improvement have larger sizes of heads than heritage cultivars (Davis, 2009; Mou, 2009). The phenotypes, however, differed in head weights. Romaine and loose-leaf phenotypes had higher fresh weight than butterhead. Transpiration and transport of nutrients into heads are considered to be higher in heads that are morphologically open structured, as with loose-leaf and romaine phenotypes, than with the tighter headed butterhead, thereby leading to more growth (Mou, 2005; 2009). Head weights were higher with the fertility regime than with organic or compost regime perhaps because the chemical fertilizer provided readily available and balances nutrient supplies relative to the other regimes that provided a slow release of nutrients. However, lettuce in the organic and compost regimes accumulated the same amount of nutrients as ones in the chemical regime suggesting that over the growing season nutrient availability was about equal among the regimes. Plant head weight widely varied among cultivars. This variance was not due to heritage or modern origins but was associated with phenotype with cultivars of the loose-leaf or romaine phenotype having larger head weights than butterhead phenotypes.

Nutrient accumulation

Modern and heritage cultivars

No differences in nutrient concentrations or total accumulation occurred between modern and heritage cultivars. This result is contrary to perceptions that heritage cultivars have smaller head weights and thus have higher nutrient concentrations than modern cultivars (Mou, 2009). No element declined substantially in concentration as head size increased; thus, no dilution effect occurred. This result indicates that diets with modern or heritage will supply the same level of mineral nutrition.

Phenotypes

Macronutrient concentrations did not differ among the phenotypes. However, total accumulation was higher in the romaine and loose-leaf than with the butterhead phenotype because of the larger head sizes of the romaine and loose-leaf phenotypes. On the other hand, micronutrient concentrations tended to be highest in the butterhead phenotype, but total accumulation was higher in the romaine and loose-leaf phenotypes. Mou (2005) suggested that the open-leafed structures of romaine and loose-leaf phenotypes allowed for more growth due to high transport of nutrients to the heads with transpiration.

Fertilization

Very small differences occurred in nutrient concentrations occurred among the three fertility regimes. However, the chemical regime had the highest total accumulation of almost all nutrients, a result that is attributed to the larger head sizes attained with the chemical fertilizers.

Cultivars

Lettuce was a rich accumulator of plant nutrients. All nutrients were at or above levels needed for sufficiency for production of the crop (Hochmuth et al., 2012; Mills and Jones Jr., 1996). Differences among individual cultivars for each element were large. Cultivars with the highest accumulation of nutrients had about 30% to 50% more of each nutrient than cultivars with the lowest concentrations, with the exception of Fe, which was 250% higher in the top cultivar than in the lowest cultivar. Considerable consistency occurred among cultivars that were the top accumulators of nutrients, but no cultivar ranked in the top five positions for all elements (Table 3.8). Red Deer Tongue (heritage, loose-leaf) and Australe (modern, butterhead) were in the top five ranked cultivars for seven of the eleven elements measured. Bronze Mignonette (heritage, butterhead) was in the high grouping for six of the elements, and Focea (modern, butterhead) was in the top group for five of the elements. The rankings were not dominated by heritage or modern origins or by any of the three phenotypic groups, although loose-leaf cultivars accumulated more K than the other phenotypes. Some reports have suggested that an open-leafed morphology of heads allows for high rates of transpiration and flow of nutrients into the leaves (Marchner, 1995; Mou, 2005; 2009; Mou and Ryder, 2004) reported that lettuce genotypes varied in organic nutritional constituents (vitamins, carotene, lutein) and noted that evaluation of assessment of existing genetic variation was a good starting point for improving nutrient density through breeding. Mou and Ryder (2004) noted also that crisphead lettuce had lower mineral nutrient concentrations than loose-leaf or romaine phenotypes.

Assessed over all cultivars, head size had no effect on concentrations of elements. This finding does not support that growth by increases in dry mass dilutes the nutrient content of lettuce heads (Davis, 2009) and means that consumption of larger amounts of high-yielding lettuce is not needed to meet dietary needs. For all nutrients, the larger the head weight, the higher the total accumulation in the produce.

Fertilizer management was a major determinant for elevated yield and mineral content in the lettuce. Chemical fertilization produced the highest head yields. The high yields obtained with chemical fertilization gave heads with the highest total nutrient accumulation and no changes in nutrient concentrations relative to the organic and compost regimes. These results note that chemical fertilization increased yields without any diminishing of the nutritional quality of the produce. The slow-release properties of the organic and compost fertilizers likely nutrient availability for a fast-growing crop like lettuce and restricted yields. (Polat et al., 2008) also reported that conventional growing of lettuce had higher yield than organic growing but no difference in terms of food nutrition value and quality.

Conclusions

Nutrients concentration or total accumulation did not differ between heritage and modern cultivars, thereby the perception that heritage produce is more nutritional than that from modern cultivars is not supported. Phenotype had significant effect with nutrient concentration and total accumulation, but the effect appeared to be due to head size and morphology with loose-leaf and butterhead phenotypes being higher accumulators of nutrients than romaine phenotypes. However, romaine and loose-leaf cultivars had same higher head weights than butterhead and had higher total nutrient accumulation. Head size did not affect the concentrations of nutrients in lettuce; hence, no dilution effect with high-yielding cultivars occurred. Fertilizer regime was a major factor in nutrient accumulation, and each regime provided a nutrient supply for optimum of yields. Chemical regime yields the highest accumulation of total nutrients, also without any dilution effect on nutrient concentration. Individual cultivars differed widely in nutrient density with a wide range of mineral nutrients concentrations and total accumulation. Therefore, the potential for mineral nutritional improvement of different types of cultivated lettuce through breeding and selection is apparent. Improving the mineral nutrition levels of lettuce through selection of cultivars and fertilization will enhance nutrient uptake in diet without requiring an increase in consumption of produce.

Table 3.1. Soil test reports for first year of experiment and for second year of experiment following one year of cropping with fertilized lettuce

Nutrients	First year 2010	Second year 2011 (Fertilizer added in 2010)		
		Chemical	Organic	Compost
	-----------------------------Extractable nutrients, mg/kg-------------------			
Phosphorus	21	21	22	22
Potassium	94	79	71	90
Calcium	893	811	813	900
Magnesium	104	97	99	98
Boron	0.0	0.5	0.5	0.5
Manganese	4.6	8.0	9.3	8.0
Zinc	0.4	1.0	1.0	1.0
Copper	2.9	3.1	3.0	3.2
Iron	1.6	2.2	2.2	2.0
Sulfur	15.6	19.7	20.1	20.9
	-----------------------Other measurements-----------------------------------			
pH	6.9	6.4	6.3	6.5
Cation exchange capacity	4.9 me/100 g	4.9 me/100 g	4.9 me/100 g	5.2 me/100 g

Table 3.2. Head weights and concentration and accumulation of macronutrients in lettuce of genetic, phenotypes regimes

Factor	Head weight		Macronutrient concentration						Total nutrien		
	Fresh	Dry	P	K	Ca	Mg	S	Na	P	K	Ca
	-----g/plant----		--------------------% dry wt------------------------						--------------------mg		
	---Genetics---										
Heritage	191	12	0.99	10.14	1.98	0.75	0.34	0.17	118	1166	2
Modern	191	13	0.97	10.53	1.92	0.70	0.31	0.17	123	1313	2
LSD (0.05)	43	2	0.04	0.52	0.14	0.04	0.01	0.01	24	229	
	---Phenotype--										
Butterhead	155	10.2	0.97	10.11	1.98	0.74	0.34	0.17	100	1009	20
Romaine	210	13.2	0.97	9.87	1.89	0.74	0.31	0.16	130	1280	25
Loose-leaf	209	13.2	1.00	11.03	1.97	0.70	0.32	0.17	132	1430	25
LSD (0.05)	11	0.6	0.03	0.30	0.10	0.04	0.01	0.01	5	81	1
	---Fertilizer--										
Chemical	226	13.4	1.01	11.01	1.99	0.71	0.31	0.17	135	1457	26
Organic	176	11.8	0.92	9.22	1.92	0.74	0.35	0.15	109	1068	22
Compost	173	11.4	1.01	10.79	1.93	0.72	0.30	0.17	118	1194	22
LSD (0.05)	16	1	0.06	0.62	0.14	0.04	0.02	0.02	12	119	2

Chemical, 10-10-10 complete fertilizer; organic, combination of soybean meal, bone meal, and K_2SO_4; co university farm

Table 3.3. Cultivars type and head weights of eighteen cultivars of lettuce

Cultivar	Cultivar type		Head weight, g/plant	
	Genetics	Phenotype	Fresh	Dry
Cosmo-Savoy	Heritage	Romaine	260	15.6
Tropicana	Modern	Loose-leaf	248	15.8
Simpson Elite	Modern	Loose-leaf	230	14.2
Claremont	Modern	Romaine	228	12.3
Winter Density	Heritage	Romaine	227	13.3
Salad Bowl	Heritage	Loose-leaf	223	14.0
Forellenschluss	Heritage	Romaine	216	12.9
Two Star	Modern	Loose-leaf	210	13.4
Buttercrunch	Heritage	Butterhead	208	12.9
Coastal Star	Modern	Romaine	205	15.6
Red Deer Tongue	Heritage	Loose-leaf	178	9.4
Adriana	Modern	Loose-leaf	172	12.5
Simpson Black-Seeded	Heritage	Loose-leaf	166	12.2
Focea	Modern	Butterhead	165	10.6
Bronze Mignonette	Heritage	Butterhead	144	8.6
Australe	Modern	Butterhead	141	10.0
Red Rosie	Modern	Romaine	123	9.7
Tom Thumb	Heritage	Butterhead	98	6.3
LSD (0.05)			35	1.8

Table 3.4. Concentration and accumulation of micronutrients in lettuce of genetic, phenotypes, and fertilizer regimes.

Factor	Micronutrient concentration					Total nutrient accumulation				
	Zn	B	Mn	Cu	Fe	Zn	B	Mn	Cu	Fe
	mg/kg dry wt					µg/head				
	Genetics									
Heritage	71	34	125	32	407	875	386	1532	381	3042
Modern	68	36	116	30	313	897	431	1557	385	2795
LSD (0.05)	19	2	18	2	38	163	71	191	53	771
	Phenotype									
Butterhead	75	36	132	33	436	794	356	1402	348	2949
Romaine	65	34	111	30	260	888	425	1545	407	2452
Loose-leaf	70	35	118	29	383	975	445	1687	394	3356
LSD (0.05)	6	2	7	2	43	79	28	91	23	270
	Fertilizer									
Chemical	73	38	162	32	392	998	473	2192	433	3641
Organic	71	35	109	30	314	866	400	1349	360	2532
Compost	65	32	91	30	373	792	353	1093	355	2583
LSD (0.05)	7	16	10	2	84	118	167	232	41	1064

Chemical, 10-10-10 complete fertilizer; organic, combination of soybean meal, bone meal, and K_2SO_4; compost, generated on university farm.

Table 3.5. Concentration and accumulation of macronutrients in cultivars of lettuce

	Macronutrient concentration						Total nutrient accumulation					
Cultivar	P	K	Ca	Mg	S	Na	P	K	Ca	Mg	S	Na
	---------------------% dry wt-------------------						---------------mg/head---------------					
Red Deer Tongue	1.13	11.22	2.03	0.83	0.30	0.21	107	1051	193	81	28	19
Focea	1.06	10.77	1.84	0.64	0.32	0.18	115	1340	199	71	34	19
Australe	1.02	9.84	2.01	0.74	0.35	0.18	103	944	199	77	34	18
Forellenschluss	1.01	10.01	1.72	0.72	0.33	0.16	135	1278	225	97	42	21
Buttercrunch	1.01	9.22	2.14	0.73	0.32	0.16	130	1201	277	95	42	20
Simpson Elite	1.00	11.30	2.03	0.66	0.32	0.17	143	1590	289	96	45	24
Winter Density	1.00	9.52	1.98	0.71	0.34	0.16	134	1236	260	96	42	22
Black-Seeded Simpson	0.99	10.39	1.90	0.68	0.33	0.16	122	1259	232	88	40	20
Cosmo-Savoy	0.99	9.59	2.06	0.78	0.32	0.16	155	1473	331	128	49	25
Claremont	0.99	10.72	1.78	0.73	0.31	0.17	121	1291	222	91	37	20
Salad Bowl	0.98	10.92	1.93	0.67	0.35	0.16	138	1478	272	98	47	22
Two Star	0.98	11.27	2.01	0.71	0.33	0.16	132	1480	265	98	43	22
Tom Thumb	0.95	10.23	1.88	0.70	0.36	0.16	63	649	119	41	23	11
Tropicana	0.94	11.10	1.91	0.63	0.32	0.17	149	1724	302	103	49	27
Adriana	0.92	10.40	1.90	0.67	0.30	0.17	112	1246	235	86	36	21
Coastal Star	0.92	9.41	1.92	0.75	0.29	0.16	146	1448	297	121	43	26
Red Rosie	0.91	9.98	1.89	0.73	0.27	0.16	89	954	184	72	26	16
Bronze Mignonette	0.87	10.19	2.13	0.92	0.39	0.16	78	871	183	82	33	14
LSD (0.05)	0.07	0.82	0.19	0.05	0.02	0.01	22	205	32	14	5	3

Table 3.6. Concentration and accumulation of micronutrients in cultivars of lettuce

Cultivar	Micronutrient concentration					Total nutrient accumulation				
	Zn	B	Mn	Cu	Fe	Zn	B	Mn	Cu	Fe
	----------mg/kg dry wt--------					---------------µg/head---------------				
Focea	80	38	134	31	294	889	399	1553	355	2190
Black-Seeded Simpson	79	35	116	31	340	1046	414	1529	390	2980
Salad Bowl	77	34	141	30	412	1149	459	2066	426	3630
Buttercrunch	76	30	118	31	327	1021	383	1620	419	3176
Tom Thumb	75	35	140	35	709	504	224	960	233	3518
Adriana	74	38	121	32	337	959	460	1593	406	2967
Forellenschluss	74	33	119	33	268	1003	410	1638	451	2237
Bronze Mignonette	72	35	134	34	534	673	294	1201	313	3134
Simpson Elite	70	35	122	29	371	1077	490	1935	431	3503
Australe	69	40	145	34	417	718	377	1488	362	2707
Two Star	67	35	98	26	308	915	447	1370	358	3054
Claremont	66	38	112	31	259	833	460	1454	393	2611
Red Rosie	66	38	103	32	288	647	350	1006	318	2306
Red Deer Tongue	64	39	117	34	524	656	342	1195	348	3064
Winter Density	62	34	123	28	312	842	439	1702	378	2965
Cosmo-Savoy	61	34	114	29	232	980	506	1880	468	2673
Coastal Star	61	26	97	26	198	1025	384	1593	428	1919
Tropicana	60	34	116	25	344	1005	515	2023	412	3903
LSD (0.05)	10	3	18	3	126	204	69	297	74	1131

Table 3.7. Results of analysis of variance showing *F* values for fresh weight, dry weight, macronutrients, and mi
affected by genetics (heritage or modern), phenotypes (butterhead, romaine, loose leaf), fertility regimes (chemic
cultivars and their interactions

Effect	Head wt		Macronutrient concentration						Micronutrien		
	Fresh	Dry	P	K	Ca	Mg	S	Na	Zn	B	
G[†]	0.04	0.22	0.03	2.35	0.40	62**	9.38*	2.09	0.14	9*	0
F	91***	92***	4**	54***	4*	4*	18**	4*	7*	6*	2
T	37***	11**	11**	26***	0.68	1.13	15**	2.63	4*	0.35	14
C	10***	11***	6***	2*	3**	3***	12***	5***	3**	7***	4
G*T	0.78	0.13	0.14	0.52	0.17	0.15	0.19	0.60	2.44	0.39	0
F*T	1.89	0.67	1.22	1.41	1.57	0.20	1.44	0.23	2.04	0.19	5.
G*F	10**	24**	5*	0.01	2.35	3.29	2.36	4.80*	4.18	4.27	1
T*C	1.90*	1.57	0.65	1.14	1.43	0.96	1.11	1.02	0.67	0.48	1
G*F*T	1.76	1.49	0.54	1.45	1.14	2.67	2.23	0.56	2.14	0.34	

[†] G, Genetics of lettuce; F, Phenotypic form of lettuce; C, Cultivar of lettuce; T, Fertility regimes.

*, **, *** Significant at $P \leq 0.05, 0.01, 0.001$, respectively.

Table 3.8. Top five ranking cultivars for accumulation of high concentrations of nutrients

		Macronutrients		
Phosphorus	**Potassium**	**Calcium**	**Magnesium**	**Sulfur**
Red Deer Tongue	Simpson Elite	Buttercrunch	Bronze Mignonette	Bronze Mignonette
Focea	Two Star	Bronze Mignonette	Red Deer Tongue	Tom Thumb
Australe	Red Deer Tongue	Cosmo Savoy	Cosmo Savoy	Australe
Forellenschluss	Tropicana	Simpson Elite	Coastal Star	Black Seeded Simpson,
Buttercrunch	Salad Bowl	Two Star	Red Rosie,	Forellenschluss,
			Buttercrunch (tied)	Two Star (3-way tie)

		Micronutrients		
Zinc	**Manganese**	**Copper**	**Iron**	**Boron**
Focea	Australe	Tom Thumb	Tom Thumb	Australe
Black Seeded Simpson	Salad Bowl	Red Deer Tongue	Bronze Mignonette	Red Deer Tongu
Salad Bowl	Tom Thumb	Bronze Mignonette	Red Deer Tongue	Red Rosie
Buttercrunch	Focea	Australe	Salad Bowl	Adriana
Tom Thumb	Bronze Mignonette	Focea	Australe	Claremont

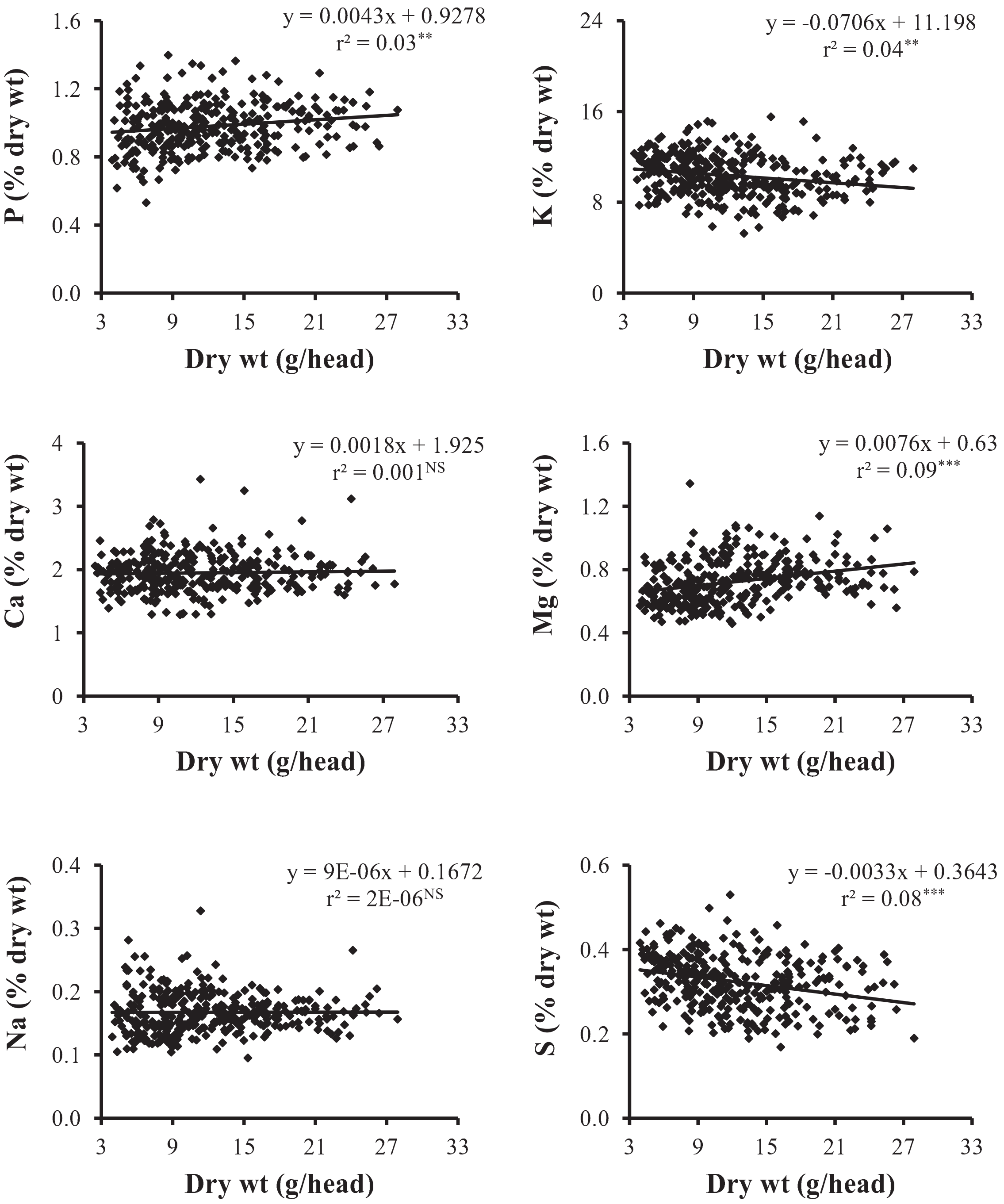

Fig. 3.1. Macronutrient concentrations as a function of head dry weight in lettuce. Elements include P, K, Ca, Mg, Na, and S. Lines are plots of the linear regression models. NS or ***, coefficient of determination is nonsignificant, $P>0.05$, or is significant, $P \leq 0.001$

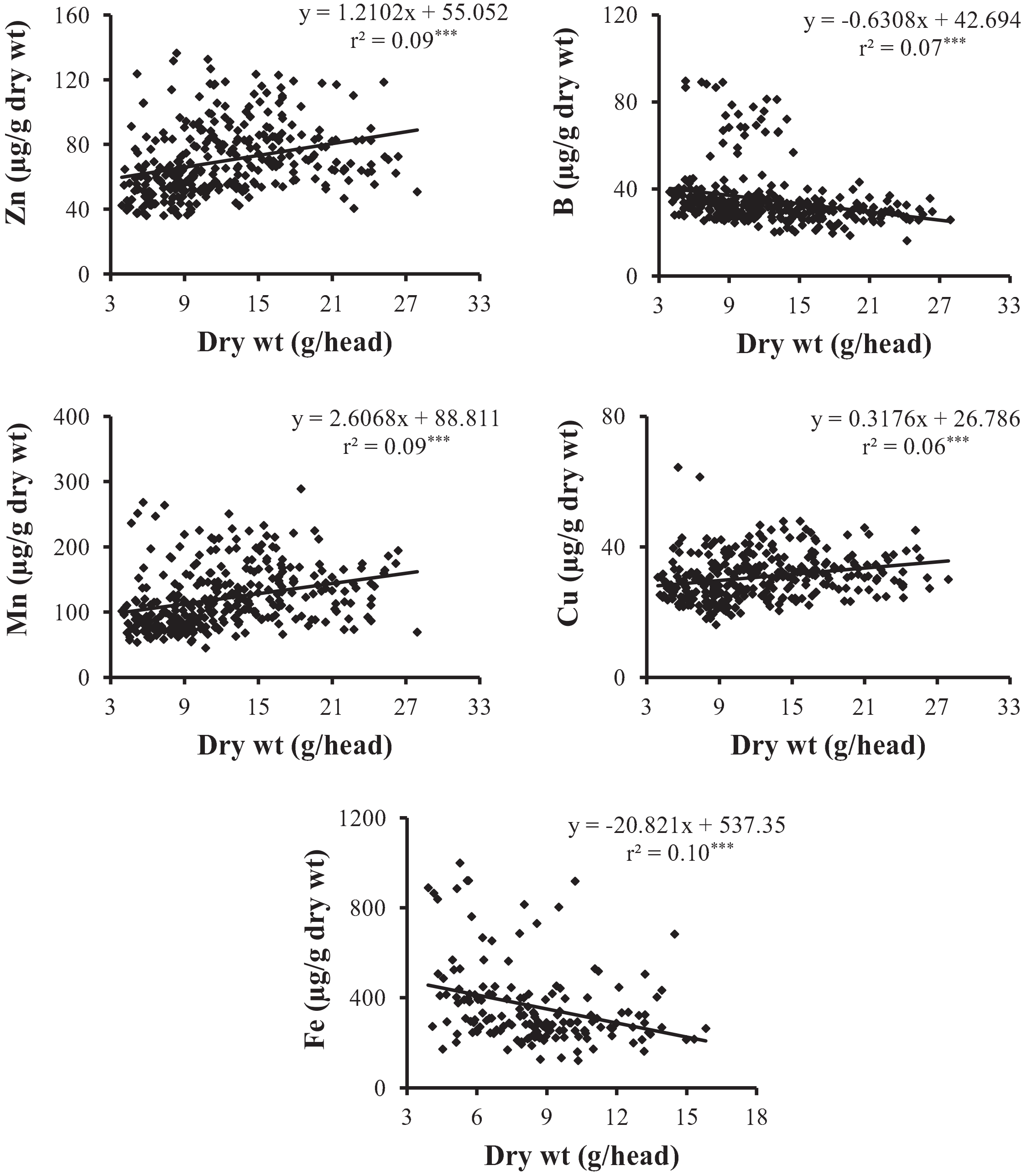

Fig. 3.2. Micronutrients concentration as a function of head dry wt in lettuce. Elements include Zn, B, Mn, Cu, and Fe. Lines are plots of the linear regression models. *** coefficient of determination significant, $P \leq 0.001$

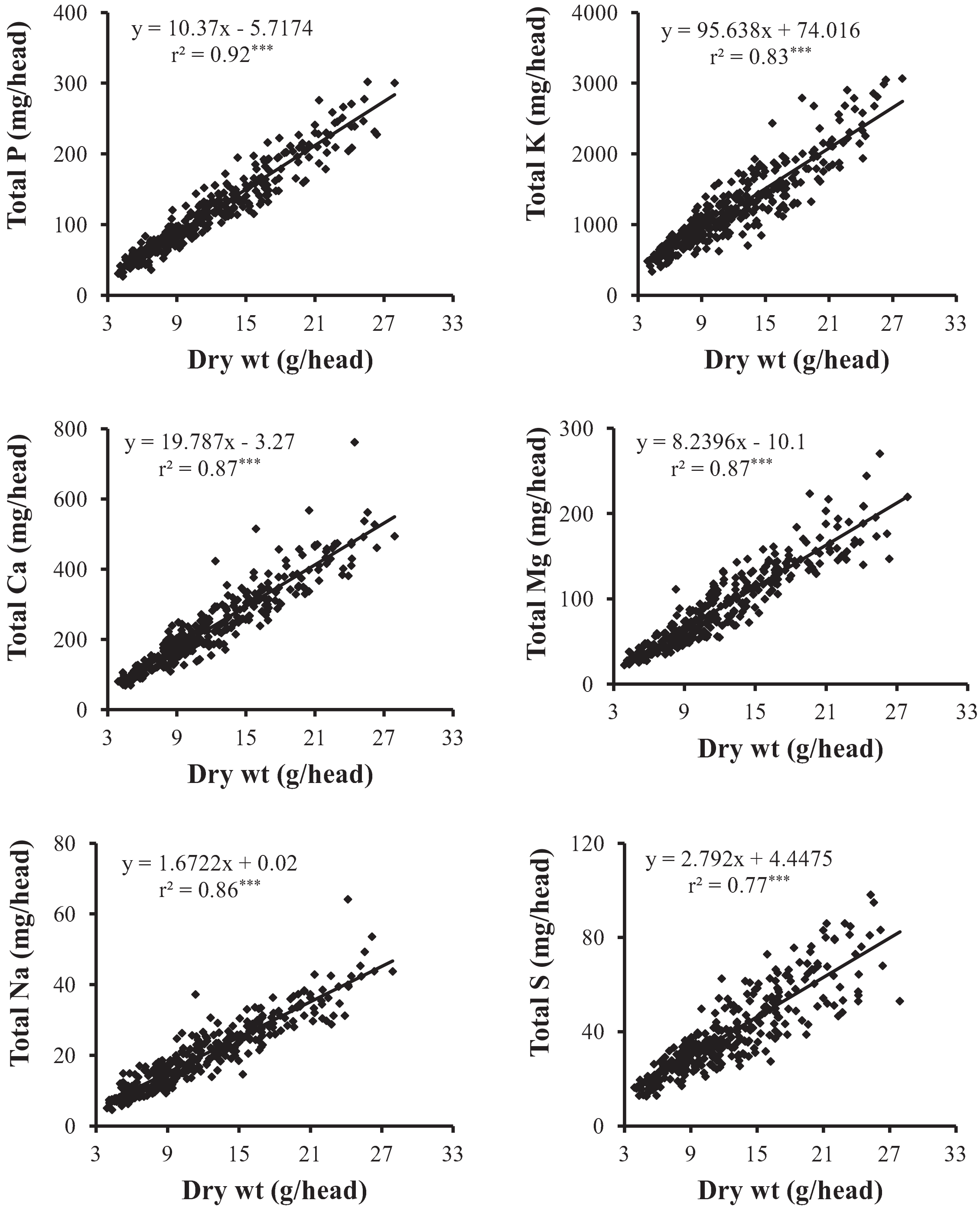

Fig. 3.3. Macronutrients accumulations as a function of head dry weight in lettuce. Elements include Total P, K, Ca, Mg, Na, and S. Lines are plots of the linear regression models. *** coefficient of determination significant, $P \leq 0.001$

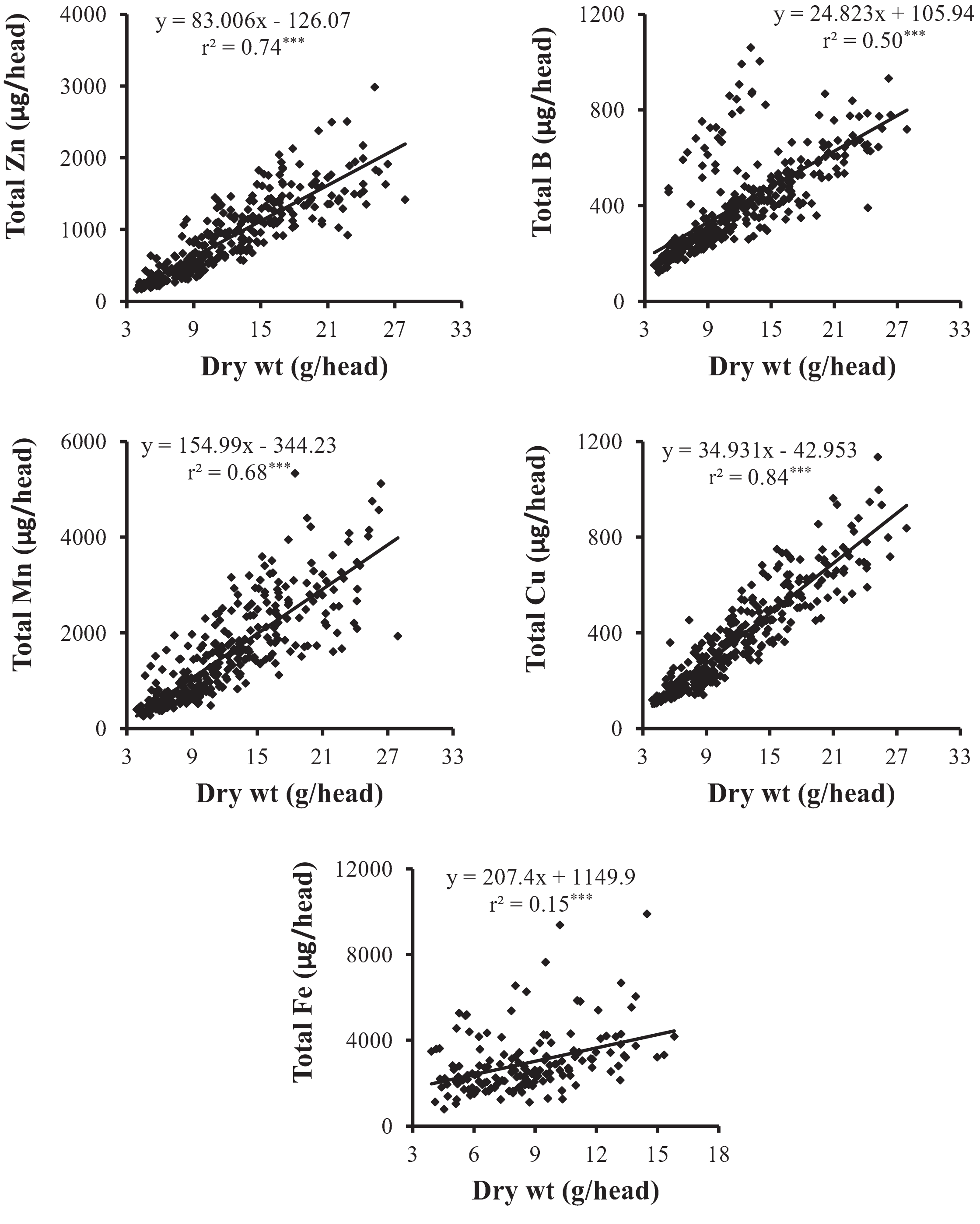

Fig. 3.4. Micronutrient accumulations as a function of head dry weight in lettuce. Element include Total Zn, B, Mn, Cu, and Fe. Lines are plots of the linear regression models. *** coefficient of determination significant, $P \leq 0.001$

CHAPTER 4

NUTRIENT DENSITY IN LETTUCE CULTIVARS GROWN WITH ORGANIC OR CONVENTIONAL FERTILIZATION WITH ELEVATED CALCIUM CONCENTRATIONS

Abstract

Calcium-rich vegetables in the diet could ameliorate the potential for calcium (Ca) deficiency in human nutrition. This study investigated the prospect of increasing Ca density of lettuce (*Lactuca sativa* L.) through cultivar selection and nutrient management in a greenhouse. Eighteen lettuce cultivars including butterhead, romaine, and loose-leaf phenotypes of heritage and modern genetics were tested. Organic fertilizer (3-0.7-3.3 NPK) and commercial conventional fertilizer (20-4.4-16.6 NPK) factored with three Ca levels (50, 100, 200 $mg^{\cdot}L^{-1}$ as $CaCl_2$) were the fertilizer regimes. Calcium in whole shoots was analyzed by atomic absorption spectrometry of oven-ashed samples. Heritage cultivars had a significantly higher Ca concentration (1.93% dry wt) than modern cultivars (1.54%). Loose-leaf phenotypes had the highest Ca concentration (2.06%) followed by butterhead (1.66%) and romaine (1.49%). Accumulation of Ca was higher with the conventional fertilizer (1.90%) than with the organic fertilizer (1.58%). Elevated Ca level in the fertility regimes raised the Ca concentration in lettuce from 1.56% at 50 mg/L to a mean of 1.82% at 100 $mg^{\cdot}L^{-1}$ and 200 $mg^{\cdot}L^{-1}$. Large differences in Ca concentration occurred among individual cultivars with ranges from 1.27% to 3.05%. ‘Salad Bowl’, ‘Red Deer Tongue’, ‘Buttercrunch’, and ‘Bronze Mignonette’ were the top in cultivar ranking with mean Ca concentration of 2.50%, whereas ‘Adriana’, ‘Australe’

‘Coastal Star’, and ‘Forellenschluss’ were low accumulators with a mean of 1.33%. Head size of cultivars had no correlation with Ca concentration. This experiment indicates that selection of nutrient regimes and cultivars can be utilized to increase Ca accumulation in lettuce.

Additional index words: *Lactuca sativa*; macronutrients; calcium accumulation; fertilizer regimes; lettuce genetics; lettuce phenotypes; cultivar selection

Introduction

Critical functions in the human body require mineral nutrients that are obtained through consumption of food. Calcium is required for constructing and maintaining bones, clotting of blood, and for function of hormones and enzymes (Department of Health & Human Services, 2000; Ervin et al., 2004; Karll, 2000; Krause and Mahan, 1984; National Research Council, 1989). Diets rich in Ca minimize Ca deficiency and maximize good health and well-being (Greenwald et al., 2001). The recommended daily intake of Ca for most men and women is 1,000 mg per day (Meacham et al., 2008); however, for elderly individuals, the required Ca intake should total 1300 to1700 mg per day (Heaney, 2001). A study suggested that it is possible to improve dietary Ca intake by increasing consumption of fruits, vegetables, and Ca-rich foods (Bernstein et al., 2002). However, fresh fruits are high in vitamins but are often low in minerals (Ashmead, 1982); on the other hand, fresh leafy vegetables are major sources of minerals (Kamchan et al., 2004). Vegetables such as kale, celery, collard, Chinese cabbage, and soybean sprouts contain high levels of Ca (Kamchan et al., 2004).

Lettuce (*Lactuca sativa* L.) is an important vegetable crop with high market value, and its nutritional characteristics have been studied throughout the world (Ashkar and Ries, 1971; Keat et al., 1999). The Ca content of lettuce is affected by relative humidity, which affects Ca distribution in the shoots, thereby resulting in the deficiency disorder of tipburn (Collier and Tibbitts, 1984; Schlagnhaufer et al., 1987).

Degree Brix, a measure of sucrose, is used commonly for expressing the quality of juices, fruits, and vegetables (Hale et al., 2005; Ikeda et al., 2013; Widodo et al., 1996). This usage has been expanded to include the concept that foods with high Brix readings also are high in mineral nutrients and that Brix readings are a general indicator of nutritional qualities of foods (Anderson, 2009; Frank, ; Sullivan, 2012).

This study was conducted to determine whether the nutrient concentration of lettuce varied among cultivars and if nutritional regimes could be managed to increase the Ca contents in the produce. Brix was measured in this investigation to assess whether a correlation occurred between this reading and calcium concentration in lettuce.

Materials and Methods

Plant materials

Eighteen lettuce cultivars of heritage and modern genetics with butterhead, romaine, and loose-leaf phenotypes were studied. Seeds were obtained from Seeds of Change (Rancho Dominguez, CA.) and Johnny's Seeds (Winslow, ME). All seeds were planted in peat moss-based medium (Fafard Growing Mix 1-PV, Conrad Fafard Inc, Agawam, MA). Seedlings at the three-leaf stage were transplanted to 15-cm round standard pots filled with the same peat moss medium. Temperatures generally ranged from 23 to 30^{o}C during day and 18 to 24^{o}C at night. Light conditions in the greenhouse, at the University of Massachusetts, Amherst (42.37°N, 72.53°W), were from sunlight during the season of production in June and July 2010 with about 13 hours of daylight and 11 hours of night.

Treatments

Two fertilizers factored with three elevated Ca concentrations were fertility regimes for this study. One regime was conventional, peat-lite professional fertilizer (20-4.4-16.6 NPK with micronutrients reported; Peters Fertilizer Products, J.R. Peters, Inc., Allentown, PA). The conventional fertilizer solution was prepared at 1 gm.L^{-1}, and 4 ml

0.5M $MgSO_4$ were added per liter. The second fertilizer regime was a commercial organic fertilizer (3-0.7-3.3 NPK with micronutrients not reported; Pure Blend Pro Grow, Pure Blend Pro Fertilizer Products, Chandler, AZ). The organic fertilizer solution was prepared at 6.7 $ml{\cdot}L^{-1}$ to approximate the supply form the conventional fertilizer. The factorial design consisted of three concentrations of Ca (50, 100, 200 $mg{\cdot}L^{-1}$ as $CaCl_2$) with three replicates in randomized complete blocks. The two fertilizers solutions were applied at 100 mL/plant for the first week then 200 mL/plant until harvest at three weeks later. The nutrient solution drained through the medium during application to avoid salt accumulation in the medium and to ensure a constant supply of nutrients.

Measurements

At harvest, fresh weights of plants were recorded, and the samples were washed once in tap water and twice in deionized water and oven-dried to a constant weight at 70°C with dry weights being recorded. For tissue analysis of Ca, 0.5 g of ground samples was ashed at 500°C for 8 h in a furnace, and the ash was dissolved in 10% (v/v) HCl prepared with distilled H_2O and concentrated HCl. The solutions were analyzed for Ca by atomic absorption spectrometry (Kalra, 1998).

Brix

Two leaves were selected from each head, one leaf from the center of the head and one from the outside. Juice was squeezed by hand into a plastic cup, mixed, and measured by a refractometer in sunlight (Pocket Refractometer PAL-1, ATAGO, Tokyo, Japan). One degree Bx is equivalent to 1 g of sucrose in 100 mL water. No correlation of Bx with calcium or other nutrient contents in plants is available.

Tipburn

Tipburn was assessed just before harvest by examination of each head of lettuce and ranking the severity of tipburn with an index of 0, no tipburn, 1, slight tipburn, 2, moderate tipburn, and 3, severe tipburn, based on judgment of the investigators.

Statistical Analyses

The statistical analyses of data were performed by analysis of variance or regression analysis (Steel and Torrie, 1980) with data processing by SAS software (SAS v. 9.2, Cary, NC). Means were separated by Duncan's New Multiple Range Test for main effects of treatments and by least significant difference for interactions of treatments (Steel and Torrie, 1980).

Results and Discussion

Results

Head weights

Fresh and dry weights of heads varied with cultivars (Table 4.1). The five largest headed cultivars had a mean fresh weight that was 40% larger and a mean dry weight that was 60% larger than the five smallest headed cultivars. 'Tropicana', 'Cosmo-Savoy', 'Buttercrunch', 'Coastal Star', 'Adriana', and 'Two Star' were grouped with high dry weights, and 'Tom Thumb', 'Winter Density', 'Black-Seeded Simpson', 'Bronze Mignonette', and 'Focea' had the lowest dry weights among the cultivars.

Heads of heritage (143 g/head) and modern (158 g) cultivars differed in fresh weights, and modern cultivars (10.2 g/head) also had higher dry weights than heritage cultivars (8.4 g) (Table 4.2). Loose-leaf phenotype had the highest fresh weight (154 g/head) followed by romaine (153 g) and butterhead (145 g) (Table 4.2). The dry weights of the phenotypic groups differed significantly in the same order as fresh weights. The organic and conventional regimes of fertilization had no significant effect on head weights (Table 4.2). Fresh and dry weights were slightly higher at 100 mg Ca L^{-1} than at 50 or 200 mg Ca L^{-1} (Table 4.2); however, regression analysis showed no significant trend between head weights and Ca in the medium.

A significant interaction of genetic group and phenotype occurred for fresh weights and dry weights with modern cultivars of butterhead and loose leaf phenotypes being larger than heritage cultivars whereas romaine phenotypes of heritage cultivars were larger than modern cultivars (Table 4.3). The type of fertilizer and the interaction of fertilizer x phenotype had no effect on head weights (Table 4.3). However, the levels of 100 or 200 mg Ca L^{-1} in the chemical fertilizer resulted in higher fresh or dry weights than the 50 mg treatment (Tables 4.4 and 4.5). With the organic regime, 200 mg Ca L^{-1} suppressed growth relative to the lower supplies of Ca. The interaction of Ca level with phenotype or with genetic grouping (heritage or modern) was nonsignificant and is not reported.

Calcium concentration

Calcium concentrations varied widely among the cultivars ranging from 1.20% to 2.54% with organic fertilization and from 1.35% to 3.56% with conventional fertilization (Table 4.6). The interaction of fertilizer by variety was significant, although the ranking of cultivars did not differ within the fertilizers (Table 4.6). The Ca concentration was always higher with conventional fertilization but the elevation with conventional over organic fertilization ranged from 4% to 49% among cultivars. Mean Ca concentration was about 20% higher with conventional fertilization (1.90%) than with organic fertilization (1.58%) (Tables 4.2 and 4.6). Heritage cultivars had about 25% higher Ca concentration (1.93%) than modern cultivars (1.54%) (Table 4.2). Loose-leaf phenotypes had the highest Ca concentrations (2.06%) followed by butterhead (1.66%) and romaine (1.49%) phenotypes (Table 4.2). The Ca concentrations with 100 or 200 mg Ca L^{-1} (mean 1.82%) were higher than that occurring at 50 mg Ca L^{-1} (Table 4.2). The interaction of genetics with Ca level was significant (Table 4.7). Heritage cultivars were always higher in Ca than modern cultivars, but the difference ranged from 14% to 32%. The interaction of cultivar with Ca level was significant with cultivars showing different trends in accumulation of Ca as Ca level increased (Table 4.8). Interactions other than fertilizer x cultivar, genetics x Ca level, and cultivar x Ca level had no significant effect on Ca concentration and are not reported.

Total Ca accumulation

Total Ca accumulation among the cultivars varied from 0.07 to 0.28 g/head (Table 4.9). Cultivars with notably high accumulation were ‘Salad Bowl’, ‘Buttercrunch’, and ‘Red Deer Tongue’. Cultivars with notably low accumulation were ‘Tom Thumb’, ‘Winter Density’, and ‘Black-Seeded Simpson’. Total Ca did not differ among heritage and modern cultivars (Table 4.2), but loose-leaf (0.18 g/head) had higher accumulation than butterhead or romaine (mean 0.14g/head) phenotype (Table 4.2). Total Ca was higher with conventional fertilization (0.18 g/head) than with organic fertilization (0.14 g/head) (Table 4.2). Total Ca was highest at 100 mg Ca L^{-1} (0.18 g/head) followed by the 200 (0.16 g) and 50 mg Ca L^{-1} treatments (0.14g) (Table 4.2) in a quadratic trend. An interaction of genetics x Ca level was significant with heritage cultivars showing an increase in total Ca with each increase in Ca level, and modern varieties showing highest

Ca at 100 mg L^{-1} (Table 4.8). An interaction of cultivar x Ca level was significant showing that cultivars ranged in responses to increasing Ca supply with an increase, no increase, or a peak in accumulation at 100 mg Ca L^{-1} as Ca supply increased from 50 to 200 mg L^{-1} (Table 4.9).

Calcium accumulation and head size

Overall, Ca concentration did not differ with head dry weights of cultivars, averaging 1.74 % (Fig. 4.1), but total Ca in the cultivars widely and significantly differed with increasing total dry weight of the cultivars and ranged from 0.07 to 0.28 g/head (Fig. 4.2).

Brix

Refractometer (°Bx) readings differed with cultivars (Table 4.10). ‘Forellenschluss’, ‘Two Star’, ‘Salad Bowl’, ‘Adriana’, and ‘Costal Star’ had °Bx of 4.5 or higher, whereas ‘Winter Density’, ‘Buttercrunch’, and ‘Tom Thumb’ had °Bx of about 3.0 or below. Heritage (3.84) and modern (4.01) cultivars differed in °Bx (Table 4.2), and differences also occurred among phenotypes with loose leaf (4.23) having the highest °Bx followed by romaine (3.85) and butterhead (3.43) cultivars. The two fertilizer groups differed significantly with the conventional (3.97) fertilizer giving higher °Bx than organic fertilizer (3.71) (Table 4.2). The levels of 50 or 100 mg Ca L^{-1} (4.02 and 4.10, respectively) resulted in higher °Bx than 200 mg Ca L^{-1} (3.40). Interactions were nonsignificant or presented no responses to suggest that any of these interactions were important factors in affecting °Bx in lettuce. Polynomial regression analysis showed no significant relationship of % Ca and °Bx.

Tipburn

Cultivars differed with expression of tipburn ranging from no tipburn to severe tipburn (Table 4.11). Heritage and modern cultivars did not differ in expression of tipburn (Table 2), but loose leaf cultivars (rating 0.93) had a lower ranking of tipburn than butterhead or romaine phenotypes (each rating 2.06) (Table 4.2). Tipburn did not differ with treatments of conventional or organic fertilization (Table 4.2). No interactive effects were significant. The relationship between % Ca in heads and expression of tipburn was nonsignificant by regression analysis.

Discussion

Modern cultivars had higher fresh weights than heritage cultivars because of genetic improvement in head sizes. Because of their smaller head sizes, heritage cultivars often are perceived to have higher mineral nutrient contents than modern cultivars (Mou, 2005; 2009). However, results of this investigation showed that Ca concentration did not differ overall with head size although total accumulation (g/head) varied, increasing as dry weight increased. Loose-leaf and romaine phenotypes had greater fresh weights than butterhead. Head weights did not differ in response to organic or chemical nutritional regimes. An increase in Ca nutrition from 50 to 200 mg L^{-1} increased head size in the chemical regime but had little effect in the organic regime. Loose-leaf cultivars ‘Tropicana’, ‘Cosmo Savoy’, ‘Buttercrunch’, ‘Coastal Star’, and ‘Two Star’ had greater fresh weights than other cultivars. This difference in growth has been attributed to differences in transpiration and the resulting nutrient accumulation (Mou, 2009). Perhaps, loose-leaf and romaine phenotypes because of their morphology transpired more water than butterhead and therefore had increased nutrient uptake transport and higher growth.

The nutritional value of the lettuce cultivars differed with phenotypes. Loose-leaf lettuce cultivars contained higher Ca concentration and total Ca than butterhead or romaine types. Transpiration affects the delivery and distribution of Ca to lettuce leaves as young leaves of the developing heads can develop tipburn because the outer leaves are transpiring more than the young leaves, thereby depriving the young leaves of Ca (Collier and Tibbitts, 1982; 1984). Loose leaf cultivars had less tipburn than romaine or butterhead phenotypes, again suggesting differences in delivery of Ca to the young leaves by transpiration (Hylmö, 1953).

A great variation occurred for Ca accumulation among cultivars. Loose leaf cultivars ‘Salad Bowl’ and ‘Red Deer Tongue’ had the highest Ca concentrations. Butterhead cultivars ‘Buttercrunch’ and ‘Bronze Mignonette’ had high Ca concentrations. The semi-open head of Buttercrunch and Bronze Mignonette perhaps allows transpiration of water into head and contributed to their higher Ca concentrations. In contrast, ‘Winter Density’, ‘Red Rosie’, and ‘Cosmo-Savoy’ accumulated moderate levels of Ca concentration within romaine types. The thick and semi-open leaves of romaine heads possibly obstruct the transpiration of water, thereby leading to lower Ca concentration than with loose-leaf and butterhead cultivars (Collier and Tibbitts, 1984). Butterhead cultivars, ‘Adriana’ and ‘Australe’, and romaine cultivars, ‘Coastal Star’ and ‘Forellenschluss’ had much lower Ca concentration than other romaine and butterhead cultivars. ‘Adriana’, ‘Australe’, ‘Coastal Star’, and ‘Forellenschluss’ have partially closed heads, whereas others in romaine and butterhead phenotypes have open heads. The closed head perhaps obstructs the transpiration of water, resulting in lower Ca concentration in the leaves (Barta and Tibbitts, 1991).

Tipburn varied among the phenotypes and cultivars. Loose leaf phenotypes expressed a lesser index of tipburn than romaine or butterhead structures. ‘Forellenschluss’, a heritage, romaine phenotype, expressed the highest tipburn index, whereas ‘Salad Bowl’ and ‘Tropicana’, heritage and modern loose leaf cultivars, respectively, had the lowest symptoms. Calcium concentration in the whole heads was not related to severity of tipburn. This lack of relationship is due to the fact that total Ca in the entire head was determined rather that Ca being determined in the affected leaves only.

Concentration of Ca did not vary with head weight, but total Ca accumulation increased with head weight. These results are important since they demonstrate that a dilution of Ca does not occur with an increase in dry mass and that consumers will receive the same amount of Ca from servings of lettuce regardless of head weight.

Conclusions

Heritage cultivars accumulated higher Ca concentration but were not superior in accumulation of total Ca from modern cultivars due modern cultivars having larger head weights. Loose-leaf cultivars accumulated higher Ca than romaine or butterhead phenotypes. Loose-leaf cultivars also had larger head fresh weights. Accumulation of Ca concentration or total Ca was higher in the chemical regime than in the organic regime. Cultivars differed widely in Ca accumulation with ‘Salad Bowl’, ‘Red Deer Tongue’, ‘Buttercrunch’, and ‘Bronze Mignonette’ ranking in the top among all cultivars in Ca concentration. A wide range of variability in Ca concentration occurred among different cultivars of lettuce including differing phenotypes and introductions. Therefore, improvement of nutrient density with lettuce through breeding and selection is a potentiality. Enhancing the mineral nutrition levels of lettuce would improve the nutrient uptake without requiring an increase in produce consumption.

Table 4.1. Head weight of cultivars in descending order by fresh weight and listing of genetics and phenotypes of lettuce

Cultivar	Genetics	Phenotype	Head wt[z], g/head	
			Fresh	Dry
Tropicana	Modern	Loose Leaf	177 a	11.6 ab
Cosmo-Savoy	Heritage	Romaine	175 ab	10.8 bc
Buttercrunch	Heritage	Butterhead	171 ab	11.5 ab
Coastal Star	Modern	Romaine	169 abc	12.0 a
Adriana	Modern	Butterhead	166 abc	11.4 ab
Two Star	Modern	Loose Leaf	165 abcd	11.3 ab
Forellenschluss	Heritage	Romaine	162 abcde	9.6 de
Claremont	Modern	Romaine	158 abcde	9.0 ef
Simpson Elite	Modern	Loose leaf	156 bcde	9.1 ef
Red Deer Tongue	Heritage	Loose leaf	150 cde	7.1 gh
Salad Bowl	Heritage	Loose leaf	146 def	9.4 de
Australe	Modern	Butterhead	145 ef	9.1 ef
Red Rosie	Modern	Romaine	145 ef	10.1 cd
Focea	Modern	Butterhead	145 ef	8.5 f
Bronze Mignonette	Heritage	Butterhead	143 ef	8.5 f
Black-Seeded Simpson	Heritage	Loose Leaf	129 f	7.3 g
Winter Density	Heritage	Romaine	112 g	6.3 h
Tom Thumb	Heritage	Butterhead	97 g	4.9 i

[z] Means of cultivar fresh or dry weights with different letters in columns are significantly different by Duncan New Multiple Range Test, $P = 0.05$.

Table 4.2. Fresh weight, dry weights, Ca concentration, total Ca, and tipburn of lettuce heads as a function of heritage genetics, butterhead, romaine, or loose leaf phenotype, organic or conventional fertilization, and Ca s

Measurement	Genetics		Phenotype			Fertilizer		Ca
	Heritage	Modern	Butterhead	Romaine	Loose Leaf	Organic	Conventional	50
Fresh wt, g/head	143	158*	145b	153a	154a	153	149^{NS}	143
Dry wt, g/head	8.4	10.2*	8.9b	9.6a	9.3a	9.2	9.4^{NS}	9.0b
% Ca, dry wt	1.93	1.54*	1.66b	1.49c	2.06a	1.58	1.90*	1.56
Total Ca, g/head	0.16	0.16^{NS}	0.15b	0.14b	0.18a	0.14	0.18*	0.14
Brix, °B	3.68	4.01*	3.43c	3.86b	4.23a	3.71	3.97*	4.02
Tipburn[z]	1.68	1.69^{NS}	2.06a	2.06a	0.93b	1.68	1.69^{NS}	1.78

[z] Visual index scale of 0, no tipburn, 1, slight tipburn, 2, moderate tipburn, and 3, severe tipburn.

For genetics and fertilizers, ^{NS,} *Means of measurements are not significantly different, *P*>0.05, or are signific F-test, *P*≤0.05, respectively. For phenotypes and calcium levels, means followed by different letters are signif Duncan's New Multiple Range Test, *P* = 0.05.

Table 4.3. Interaction of genetics and phenotypes and fertilizers on fresh weight of heads of lettuce

Cultivar	Genetics^z						
	Heritage				Modern		
	Phenotype			Mean	Phenotype		
	Butterhead	Romaine	Loose leaf		Butterhead	Romaine	Loose leaf
	-- Fresh wt, g/head ---------------------------						
Organic	134	152	152	146	156	154	166
Conventional	140	147	131	139^{NS}	148	160	165

^{z}LSD(0.05) for interaction of fertilizers x genetics x phenotypes = 12. ^{NS}Means of fertilizers are n significantly different by F-test, *P*>0.05.

Means of phenotypes are reported in Table 4.2.

Table 4.4. Interaction of type of fertilizer and Ca level on fresh weight of lettuce listed in descending orde
weights of cultivars

Cultivar	Type of Fertilizer[z]							
	Organic				Conventional			
	Ca level			Mean	Ca level			Mean
	50	100	200		50	100	200	
	--Fresh wt, g/head-------------------							
Tropicana	192	198	154	182	138	174	201	171
Cosmo-Savoy	181	200	159	180	147	190	170	169
Buttercrunch	183	200	139	174	139	169	199	169
Coastal Star	162	172	166	167	135	179	199	171
Adriana	181	195	147	174	128	151	195	158
Two Star	179	160	159	166	146	155	191	164
Forellenschluss	172	167	158	166	151	148	175	158
Claremont	175	153	136	154	151	170	163	161
Simpson Elite	162	163	130	152	126	160	195	160
Red Deer Tongue	162	162	146	157	146	132	149	142
Salad Bowl	168	188	123	160	123	121	154	133
Australe	151	172	129	150	131	149	141	140
Red Rosie	144	158	121	141	123	158	166	149
Focea	160	138	133	144	127	145	165	146
Bronze Mignonette	121	166	116	135	132	153	171	152
Black-Seeded Simpson	134	135	151	140	131	130	96	119
Winter Density	127	110	97	111	92	117	128	112
Tom Thumb	79	92	114	95	77	108	111	99

[z] LSD(0.05) for interaction of fertilizers x Ca level x cultivars = 12

[y] Cultivar means followed with different letters are significantly different by Duncan's New Multiple Ran
0.05. These means are presented also in Table 1. Means of fertilizers calcium levels are reported in Table

Table 4.5. Fertilizers and Ca level interaction with cultivars listed in descending order of mean dry wei
cultivars

Cultivar	Type of Fertilizer[z]							
	Organic				Conventional			
	Ca level			Mean	Ca level			Mean
	50	100	200		50	100	200	
	--- Dry wt, g/head ----------------							
Coastal Star	12.6	11.8	9.9	11.4	9.8	13.1	14.8	12.6
Tropicana	13.1	13.1	9.1	11.8	8.6	12.7	13.1	11.5
Buttercrunch	12.8	13.7	8.4	11.6	9.2	11.3	13.6	11.4
Adriana	12.5	13.4	8.8	11.5	8.7	11.9	13.0	11.2
Two Star	12.3	10.9	10.4	11.2	9.8	11.6	13.1	11.5
Cosmo-Savoy	11.7	12.2	8.9	11.0	8.4	12.5	10.9	10.6
Red Rosie	10.4	11.1	8.0	9.8	8.2	11.6	11.5	10.4
Forellenschluss	9.8	9.4	9.4	9.5	10.1	9.1	10.1	9.7
Salad Bowl	10.9	10.7	7.1	9.6	7.8	9.5	10.3	9.2
Australe	9.7	10.9	7.8	9.5	7.6	9.8	8.9	8.7
Simpson Elite	9.3	9.3	7.0	8.5	8.0	10.3	10.7	9.7
Claremont	10.4	9.1	7.2	9.9	8.3	9.7	9.1	9.0
Bronze Mignonette	7.1	9.3	6.3	7.5	8.1	9.9	10.6	9.5
Focea	9.4	8.2	7.5	8.4	7.5	9.1	9.4	8.7
Black-Seeded Simpson	7.3	7.6	8.1	7.6	7.8	7.4	5.4	6.9
Red Deer Tongue	7.4	7.2	7.0	7.2	7.1	6.6	6.8	6.8
Winter Density	7.4	6.6	5.2	6.4	5.0	7.1	6.8	6.3
Tom Thumb	4.5	4.7	3.9	4.4	4.1	5.6	6.4	5.4

[z] LSD(0.05) for interaction of fertilizers x Ca level x cultivar = 0.7.

[y] Cultivar means followed with different letters are significantly different by Duncan's New Multiple R
= 0.05. These data are presented also in Table 1. Means for fertilizers and calcium levels are reported T

Table 4.6. Interaction of cultivar and fertilizer on the calcium concentration in lettuce arranged in descending order of mean calcium concentration in the cultivars

Cultivar	Fertilizer^z		Cultivar mean^y
	Organic	Conventional	
	------Calcium concentration, % dry wt------		
Salad Bowl	2.54	3.56	3.05 a
Red Deer Tongue	2.53	3.51	3.02 a
Buttercrunch	1.83	2.17	2.00 b
Bronze Mignonette	1.95	2.03	1.99 b
Focea	1.71	1.79	1.75 c
Simpson Elite	1.52	1.97	1.73 cd
Two Star	1.39	1.84	1.61 cde
Winter Density	1.44	1.76	1.60 cde
Red Rosie	1.42	1.77	1.59 cdef
Claremont	1.28	1.91	1.59 cdef
Cosmo-Savoy	1.37	1.46	1.58 cdef
Tropicana	1.38	1.66	1.52 def
Tom Thumb	1.38	1.54	1.46 efg
Black-Seeded Simpson	1.40	1.49	1.44 efg
Adriana	1.22	1.54	1.38 fg
Australe	1.31	1.45	1.38 fg
Coastal Star	1.21	1.54	1.28 g
Forellenschluss	1.20	1.35	1.27 g

^{z}LSD(0.05) for interaction is 0.31.

^{y}Cultivar means followed by different letters are significantly different Duncan's New Multiple Range Test, *P* = 0.05. Means of fertilizers are Table 4.2.

Table 4.7. Interaction of genetics with calcium levels on calcium concentration and total calcium accumulation in lettuce

Ca level, mg/L	Genetics^z	
	Heritage	Modern
	-----Ca concentration, % dry wt-------	
50	1.70	1.43
100	2.05	1.66
200	2.06	1.52
	--------------Total Ca, g/head-----------	
50	0.14	0.14
100	0.18	0.18
200	0.17	0.15

[z] LSD for Ca level × genetics interaction on %Ca = 0.13 and on total Ca = 0.01. Means for main effects of Ca levels and modern and heritage cultivars are reported in Table 4.2

Table 4.8. Interaction of cultivar and calcium level on calcium concentration of lettuce

Cultivar	Ca level[z]		
	50	100	200
	----Ca concentration, % dry wt----		
Salad Bowl	2.76	3.71	2.69
Red Deer Tongue	2.47	3.22	3.36
Buttercrunch	1.71	1.99	2.30
Bronze Mignonette	1.57	1.90	2.50
Focea	1.58	2.00	1.68
Simpson Elite	1.69	1.91	1.58
Two Star	1.76	1.74	1.35
Winter Density	1.40	1.77	1.64
Red Rosie	1.47	1.73	1.60
Claremont	1.39	1.87	1.52
Cosmo-Savoy	1.53	1.66	1.56
Tropicana	1.38	1.48	1.71
Tom Thumb	1.37	1.46	1.56
Black-Seeded Simpson	1.32	1.48	1.53
Adriana	1.24	1.64	1.27
Australe	1.24	1.38	1.52
Coastal Star	1.14	1.24	1.47
Forellenschluss	1.19	1.25	1.39

[z] LSD(0.05) for interaction of cultivars × Ca level = 0.39
Means of main effects of cultivars and levels of calcium are reported in Table 4.2.

Table 4.9. Interaction of cultivar and calcium level on total calcium accumulation by lettuce listed in order of descending accumulation

Cultivar	Ca level			Cultivar mean
	50	100	200	
	------------------g Ca/head------------------			
Salad Bowl	0.24	0.37	0.23	0.28 a
Buttercrunch	0.19	0.25	0.26	0.23 b
Red Deer Tongue	0.18	0.22	0.23	0.21 b
Two Star	0.19	0.19	0.16	0.18 c
Tropicana	0.15	0.19	0.20	0.18 c
Cosmo-Savoy	0.16	0.21	0.16	0.17 cd
Bronze Mignonette	0.12	0.19	0.21	0.17 cd
Red Rosie	0.13	0.20	0.16	0.16 cd
Simpson Elite	0.15	0.19	0.14	0.16 cd
Adriana	0.13	0.21	0.14	0.16 cd
Coastal Star	0.13	0.15	0.18	0.15 cd
Focea	0.14	0.18	0.14	0.15 de
Claremont	0.14	0.18	0.13	0.15 def
Australe	0.11	0.15	0.13	0.13 efg
Forellenschluss	0.12	0.12	0.14	0.12 fg
Black-Seeded Simpson	0.10	0.11	0.10	0.10 g
Winter Density	0.09	0.13	0.10	0.10 g
Tom Thumb	0.06	0.08	0.09	0.07 h

[z] LSD(0.05) for interaction of cultivar × Ca level = 0.05

[y] Means followed by different letters for cultivars in column and fertilizers in row are significantly different by Duncan's New Multiple Range Test, *P* = 0.05. Means for Ca level are reported in Table 4.2.

Table 4.10. Refractometer readings among cultivars with ranking from highest to lowest brix (°Bx)

Refractometer reading[z], °Bx					
Forellenschluss	4.76 a	Tropicana	4.16 bcd	Cosmo-Savoy	3.58 f
Two Star	4.68 a	Simpson Elite	4.03 cde	Australe	3.57 f
Salad Bowl	4.51 ab	Black-Seeded Simpson	3.77 def	Focea	3.34 fg
Adriana	4.50 ab	Bronze Mignonette	3.67 ef	Winter Density	3.03 g
Coastal Star	4.50 ab	Red Rosie	3.65ef	Buttercrunch	2.98 g
Red Deer Tongue	4.23 bc	Claremont	3.64 ef	Tom Thumb	2.54 h

[z] °Bx followed by different letters are significantly different by Duncan's New Multiple Range Test (*P*=0.05).

Table 4.11. Expression of tipburn among cultivars with ranking from highest to lowest symptoms

Cultivar and tipburn rating, 0 to 3 scale^z					
Forellenschluss	3.00a	Adriana	2.17bcd	Simpson Elite	1.25fg
Red Deer Tongue	2.75a	Claremont	2.17bcd	Buttercrunch	1.08g
Focea	2.67ab	Red Rosie	1.92cde	Coastal Star	0.83g
Winter Density	2.58ab	Cosmo-Savoy	1.83cdef	Two Star	0.17h
Tom Thumb	2.42abc	Bronze Mignonette	1.75def	Salad Bowl	0.08h
Australe	2.25bcd	Black-Seeded Simpson	1.33efg	Tropicana	0.00h

^{z}Visual index of 0, no tipburn; 1, slight tipburn; 2, moderate tipburn; 3, severe tipburn. Tipburn ratings followed by different letters are significantly different by Duncan's New M Range Test (*P*=0.05).

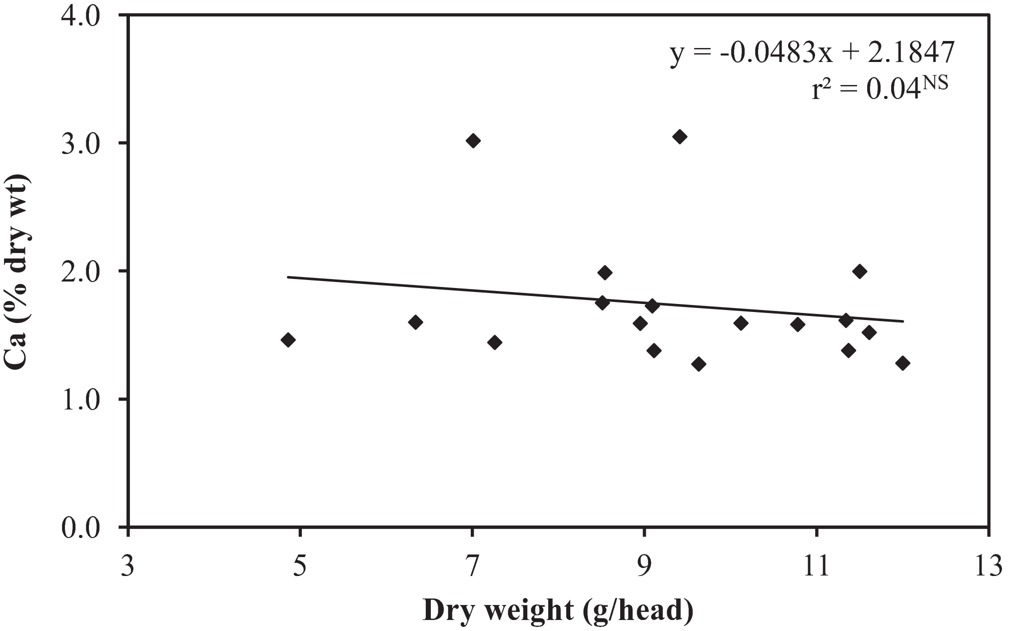

Fig. 4.1. Calcium concentration as a function of head dry weights of lettuce. Equations are linear regression models. Calcium concentration did not vary with dry weights as dry weight increased.

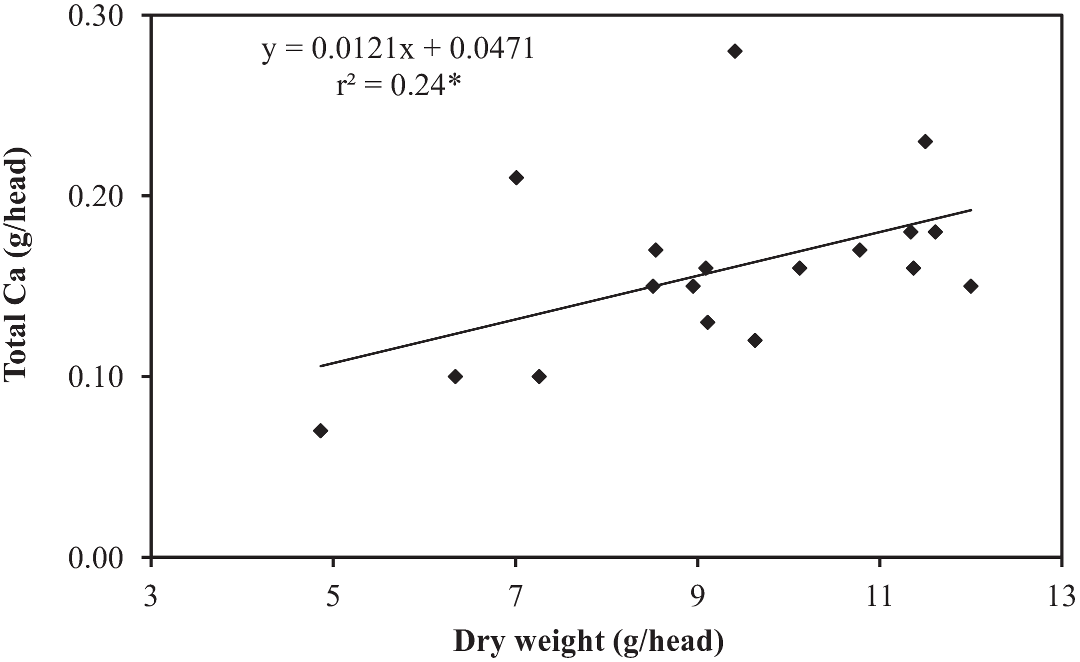

Fig. 4.2. Accumulation of total Ca as a function of head dry weights of lettuce. Equations are linear regression models. Total Ca accumulation varied with dry weight as dry weight increased.

CHAPTER 5

ZINC ACCUMULATION IN LETTUCE CULTIVARS GROWN WITH ORGANIC OR CHEMICAL BASED NUTRITIONAL REGIMES

Abstract

This study determined the potential to increase Zn density of lettuce (*Lactuca sativa* L.) through cultivar selection and nutrient management. Organic fertilizer and Hoagland and Arnon no.1 solution factored with three Zn levels provided as $ZnSO_4$ were the fertilizer regimes in a greenhouse experiment. Modern cultivars had a 32% higher fresh head weight than heritage cultivars, but each accumulated the same Zn concentration (65 mg/kg dry wt). Butterhead phenotypes had a 38% lower yield than loose-leaf and had the highest Zn concentration (78 mg/kg dry wt) followed by romaine (66 mg/kg dry wt) and loose-leaf (53 mg/kg dry wt). Concentration of Zn did not differ between fertility regimes, being about 66 mg/kg dry wt with each regime. Differences in Zn concentrations were significant among individual cultivars with ranges from 42 mg/kg dry wt to 91 mg/kg dry wt. 'Tom Thumb', 'Adriana', 'Claremont', and 'Focea' were the top in cultivar ranking with mean Zn concentration of 63 mg/kg dry wt. The results signify that selection of cultivars may be utilized to increase Zn accumulation in lettuce but that nutritional regimes had little effect on accumulation.

Key words: Micronutrients; zinc accumulation; nutritional regimes; *Lactuca sativa*; nutrient density.

Introduction

Microelements are required for growth and good health of the human body. Fruits and vegetables are perceived as principal sources for these microelements; however, fruits and vegetables are high in vitamins but are often low in essential minerals (Ashmead, 1982). Zinc is vital for many biological functions and critically plays role in more than 300 enzymes in the human body (Coleman, 1992) . Zinc has a role in growth and cell division of babies during pregnancy (Sorensen et al., 1999). The role of Zn in DNA replication and gene expression recently has gotten attention in the scientific community (Coleman, 1992). Superoxide dismutase (SOD) activity, which protects cells from auto-oxidation, decreases in absence of Zn (Cakmak and Marschner, 1988). Zinc deficiency has been associated with complications of diabetes (Golik et al., 1993; Thompson and Godin, 1995), with development of skin problems (Richard et al., 1993), with risk of cancer (Abdulla and Gruber, 2000; Chan et al., 1998), and chronic diseases (Prasad, 2003).

The recommended daily intakes of Zn are 5 mg for infants, 12 mg for women, 15 mg for pregnant women, and 15 mg for men (Institute of Medicine, 2001). Although the main dietary source of Zn is red meat, nutrient-dense green, leafy vegetables can be excellent dietary sources. Lettuce is an important vegetable crop with high market value, and its nutritional characteristics have been studied throughout the world (Ashkar and Ries, 1971; Keat et al., 1999). Tissue mineral concentrations in lettuce vary from 14 to 200 mg Zn/kg dry mass (Tambasco et al., 2000).

Deficiency of Zn in plants gives stunted growth as a result shortening of internodes (rosetting) and causes die back disease (Arce et al., 1992; Moraghan, 1978).

Shoot growth is more inhibited than root growth with Zn deficiency (Zhang et al., 1991). On the other hand, Zn toxicity inhibited root elongation (Ruano et al., 1988) and photosynthesis (Van Assche and Clijsters, 1986).

This study was performed to determine whether the Zn densities of lettuce varied among selected cultivars and nutritional regimes and if densities can be increased through increasing the Zn-nutrient contents in the medium.

Materials and Methods

Plant Materials

Twelve lettuce cultivars of heritage or modern genetics with four each from butterhead, romaine, and loose-leaf phenotypes were used for the study. Heritage seeds were from Seeds of Change (Spicer, MN), and modern seeds were from Johnny Selected Seeds (Winslow, ME). Seedlings of lettuce were grown in a greenhouse at the University of Massachusetts, Amherst (N42º 23 ´ , W72º 31 ´). All seedlings were started in a peat-lite medium (Fafard Growing Mix 1-PV, Conrad Fafard Inc, Agawam, MA) with temperature maintained at 20° C (68° F). At four weeks from seeding, same-sized seedlings were transplanted to 15-cm round standard pots filled with the peat-lite medium. The lettuce was grown in the greenhouse for about 3 weeks from transplanting to marketable size.

Treatments

Two fertilizers factored with three Zn concentrations were fertility regimes for this study. One fertilizer regime was Hoagland no. 1 solution (Hoagland and Arnon, 1950), and other fertilizer regime was Lady-bug Organic (3-1.5-2 NPK) providing 200 mg N/L (John’s Recipe Fertilizer Products, Dallas, TX). Zinc was added at 0.05, 0.10, or

0.15 mg Zn/L as $ZnSO_4$ in each regime. Micronutrients and iron at Hoagland and Arnon (1950) concentrations were added in each regime. The treatments were applied daily at 200 mL/plant from first week of transplanting until harvestable head size.

Measurements

At harvest, fresh weights of plant were recorded. Whole heads were sampled, dried, weighed, and ground to pass a 30-mesh screen for analysis of Zn. Dried ground samples were ashed in a muffle furnace at 500^{o}C. The ash was dissolved in 10% HCl (v/v) and analyzed by atomic absorption spectrophotometry (Kalra, 1998).

Statistical Analyses

The statistical analysis was performed by analysis of variance with statistical software (SAS, Cary, NC) with mean separation by least significant difference (Steel and Torrie, 1980).

Results and Discussion

Results

Genetics and phenotypics

Modern cultivars had significantly higher fresh weight (80 g) than heritage cultivars (61 g), but the Zn concentration did not differ with genetics (Table 1) ranging from 55 to 67 mg/kg Zn. Modern cultivars (341 µg Zn/head) did not differ in total Zn accumulation from heritage cultivars (285 µg/head). Loose-leaf phenotype had higher fresh weight (82 g/head) than romaine (70 g) or butterhead (59 g) (Table 2). However, the dry weight of the three phenotypic groups did not vary, ranging 4.14 to 5.72 g per head. The butterhead phenotypes accumulated the highest concentration of Zn (78 mg/kg) followed by romaine (66 mg/kg) and loose-leaf (53 mg/kg), but the total accumulation of Zn did not differ ranging 296 to 333 µg Zn per head.

Fertilizer evaluation

The Hoagland fertility regime and the organic regime did not differ in fresh or dry weight production (Table 3). Also, the Zn concentration and total Zn accumulation did not differ between fertility regimes. The Zn levels did not affect fresh or dry weights or Zn contents in the heads (Table 4).

Cultivar evaluation

Two Star (143 g/head), Bronze Mignonette (87 g), Coastal Star (85 g), and Simpson Elite (82 g) had the highest fresh weights (Table 5). Black Seeded Simpson (35 g/head), Forellenschluss, Tom Thumb (45 g), and Focea (48 g) had very low fresh weights.

Tom Thumb (91 mg Zn/kg), Adriana (82 mg/kg), Claremont (79 mg/kg), Focea (75 mg/kg), and Coastal Star (70 mg/kg) had higher Zn concentrations than Two Star (42 mg/kg), Black Seeded Simpson (52 mg/kg), Simpson Elite (55 mg/kg), and Winter Density (56 mg/kg). Other cultivars Red Deer Tongue, Bronze Mignonette, and Forellenschluss were moderate Zn accumulators with concentrations ranging from 60 to 64 mg/kg. Total Zn accumulation in the cultivars significantly differed among cultivars ranging from 134 to 414 µg per head. Coastal Star had high total Zn accumulation (414 µg/head) in spite of low Zn concentration (70 mg/kg). Tom Thumb had moderate total Zn accumulation (280 µg/head) in spite of high Zn concentration (91 mg/kg). Zinc concentration in the cultivars did not differ as a function of dry weight, averaging 66 mg/kg (Figure 1), but accumulation of total Zn in the cultivars rose with increasing total dry weight of the cultivars averaging 313 μg Zn/head (Figure 2).

Discussion

Modern cultivars had higher plant fresh weights than heritage cultivars. Modern and heritage cultivars did not differ significantly in Zn concentrations or total accumulation although modern cultivars had an apparently higher total Zn accumulation because of larger head size. Loose-leaf phenotypes had larger head sizes than romaine or butterhead. A report suggests that loose-leaf varieties due to their open-headed morphology absorb more nutrients than butterhead varieties as a result of higher transpiration rates (Marschner, 1986). Growth may be improved by this accumulation of nutrients (Van Assche and Clijsters, 1986). Loose leaf lettuce also has good, direct contact with sunlight, and photosynthesis might promote vigorous plant growth. The butterhead group had higher Zn concentrations than loose-leaf or romaine phenotypes, but phenotypes did not differ in total accumulation. These differences appear to be related to head size and to the effects of dry matter accumulation on Zn concentration and total accumulation.

Regimes of Hoagland-based or organic nutrition did not differ in effects on plant growth or Zn contents in heads of lettuce suggesting that either regime supplied adequate nutrition for the crop. These results suggest that as long as adequate nutrition is provided growers can be secure in growing lettuce with chemical or organic supplies of nutrients. Addition Zn of 0.10 or 0.15 mg/L did not enhance growth or affect Zn accumulation in lettuce relative to the 0.05 mg/L addition. Perhaps, higher levels of Zn nutrition would have enhanced Zn accumulation, but higher concentrations must be investigated for toxic effects (Hamlin and Barker, 2006).

A wide variation occurred for plant fresh and dry weights, and Zn accumulation among cultivars. Variation in these parameters was 100% or more from the smallest to the largest growth or Zn accumulation. No clear association of these characteristics could be associated with genetics of phenotype. These results show that selection can be made among lettuce cultivars to obtain the best performers with regards to growth and nutrient accumulation.

Conclusions

Lettuce grown chemically or organically did not differ in plant fresh or dry weight production and did not differ in Zn concentration or total accumulation. Supplemental Zn additions of 0.10 mg/L or 0.15 mg/L level did not elevate Zn content over a supply of 0.05 mg/L. Modern and heritage genetics did not differ in Zn contents. Butterhead phenotype accumulated a higher Zn concentration than romaine or loose leaf, but total Zn accumulation did not vary with phenotype. Individual cultivars differed widely in Zn contents. Cultivars Tom Thumb and Adriana exhibited high Zn accumulation, but their total Zn did not have this ranking because Tom Thumb and Adriana are small headed. Cultivars Two Star and Black Seeded Simpson exhibited the lowest Zn accumulation, but total Zn of Two Star had the top ranking because total dry weight. Therefore, plant dry weight was an important factor affecting Zn concentration and total accumulation among cultivars. Selection of cultivars is a significant factor for improving nutritional status of Zn. Zinc impairment in diet could be corrected by using nutrient-dense lettuce cultivars without requiring additional consumption or enrichment of produce.

Table 5.1. Head weights and content of zinc in heritage and modern cultivars of lettuce.

Genetics	Head weight, g/plant		Zinc Content	
	Fresh	Dry	(mg/kg)	(µg/head)
Heritage	61	4.25	67	285
Modern	80	5.61	65	341
LSD (*P*=0.05)	16	1.11	8	90

Table 5.2. Head weight and content of Zn in phenotypes of lettuce.

Phenotype	Head weight, g/plant		Zinc content	
	Fresh	Dry	(mg/kg)	(µg/head)
Butterhead	59	4.14	78	311
Romaine	70	4.93	66	333
Loose leaf	82	5.72	53	296
LSD (*P* = 0.05)	19	1.30	3	109

Table 5.3. Head weights and content of Zn in lettuce grown on Hoagland or organic fertilizers.

Fertilizer	Head weight, g/plant		Zinc content	
	Fresh	Dry	(mg/kg)	(µg/head)
Hoagland	75	5.26	65	329
Organic	66	4.60	66	297
LSD (*P* = 0.05)	13	0.89	13	84

Table 5.4. Head weights and content of Zn in lettuce grown on Hoagland or organic fertilizers with elevated

Zinc, mg/L	Head weights, g/plant				Zinc conte		
	Fresh		Dry		(mg/kg)		
	Hoagland	Organic	Hoagland	Organic	Hoagland	Organic	Ho
0.05	75	66	5.21	4.61	66	64	
0.10	81	65	5.65	4.55	63	68	
0.15	70	66	4.90	4.65	66	67	
Mean	75	65^{NS}	5.26	4.60^{NS}	65	66^{NS}	
LSD (*P* = 0.05) Interaction	14		0.97		6		

^{NS} Nonsignificant (*P > 0.05*), test of means of Hoagland and organic by F-test.

Table 5.5. Head weight and zinc content of twelve cultivars of lettuce arranged in order of descending zinc concentrations (mg/kg) in the heads.

Cultivars	Genetics	Phenotype	Head weight, (g/plant)		Zinc content	
			Fresh	Dry	(mg/kg)	(µg/head)
Tom Thumb	Heritage	Butterhead	45	3.1	91	280
Adriana	Modern	Butterhead	57	4.0	82	322
Claremont	Modern	Romaine	67	4.7	79	381
Focea	Modern	Butterhead	48	3.4	75	258
Coastal Star	Modern	Romaine	85	6.0	70	414
Red Deer Tongue	Heritage	Loose leaf	67	4.7	64	308
Bronze Mignonette	Heritage	Butterhead	87	6.1	62	382
Forellenschluss	Heritage	Romaine	45	3.2	60	193
Winter Density	Heritage	Romaine	85	6.0	56	343
Simpson Elite	Modern	Loose leaf	82	5.7	55	328
Black Seeded Simpson	Heritage	Loose leaf	35	2.5	52	134
Two Star	Modern	Loose leaf	143	10.0	42	412
LSD (*P = 0.05*)			18	1.3	9	119

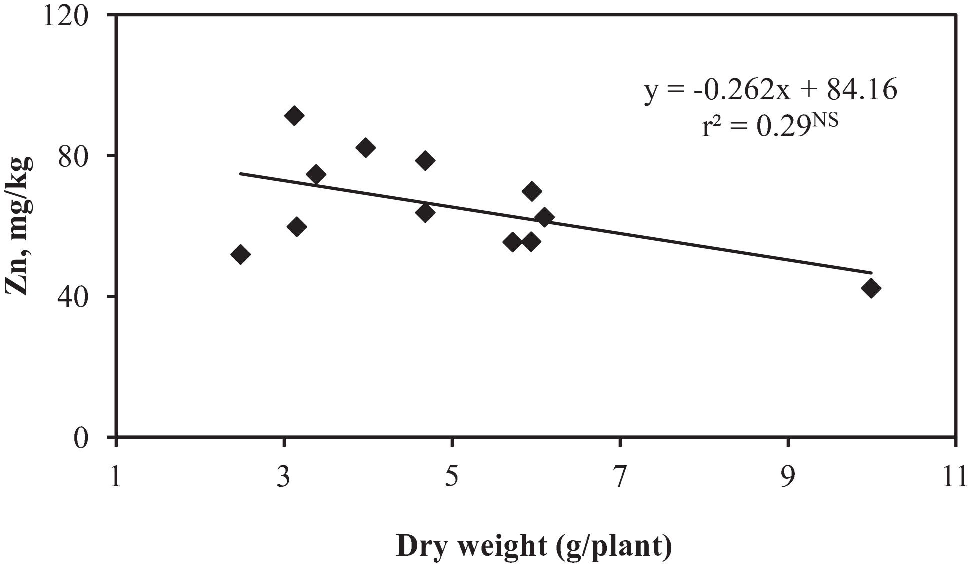

Fig. 5.1. Zinc concentration as a function of dry weight of heads of lettuce. Equations are linear regression models. Zinc concentration did not vary with dry weights as dry weight increased

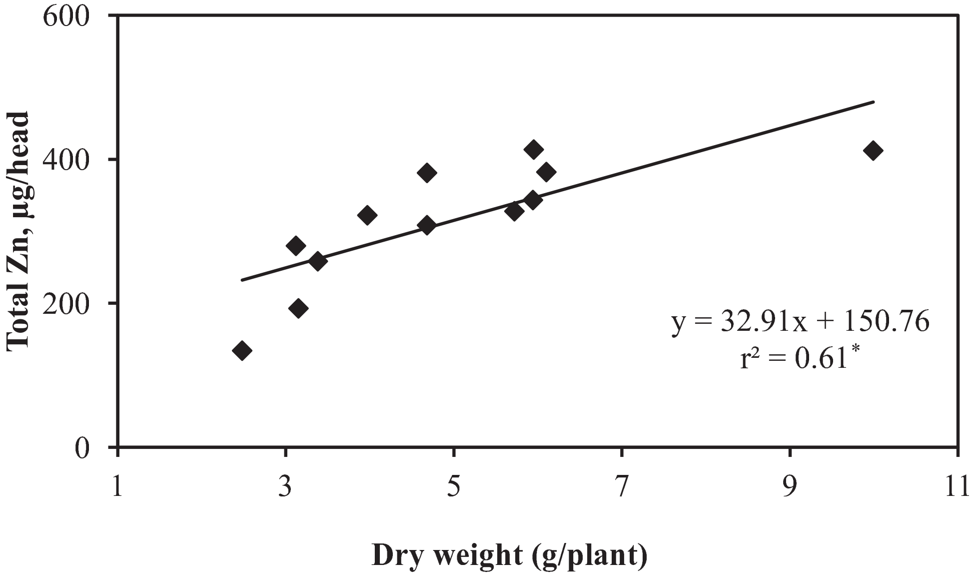

Fig. 5.2. Total accumulation of zinc as a function of dry weight of heads of lettuce. Equations are linear regression models. Total Zinc accumulation varied with dry weights as dry weight increased

CHAPTER 6

ASSESSMENT OF THE GENETIC DIVERSITY IN SELECTED LETTUCE CULTIVARS WITH SIMPLE SEQUENCE REPEAT MARKERS

Abstract

Lettuce (*Lactuca sativa* L.) is a widely used leafy vegetable and highly economic crops in U.S. Previously, the cultivars were classified on the basis of phenotypic traits. However, the identification of the cultivars was required with molecular analysis as well. Understanding the genetic diversity of lettuce (*Lactuca sativa* L.) is attractive to scientists with an interest in cultivar development. This study assessed the genetic diversity among 18 lettuce cultivars including butterhead, romaine, and loose-leaf phenotypes of heritage and modern genetic groups with mineral status. The cultivars were screened with fifteen previously described Expressed Sequence Tag-Simple Sequence Repeat (EST-SSR) primers. In total, 32 polymorphic alleles were detected at 15 EST-SSR loci. The polymorphic information content (PIC) or marker heterozygosity (*UHe)* averaged 0.37, and the number of different alleles per locus (*Na*) averaged 2.13. The average marker heterozygosity was highest in loose-leaf (0.344) followed by butterhead (0.303) and romaine (0.258) phenotypes. Along with high marker heterozygosity, loose-leaf or butterhead had high Ca, K, Mg, Zn, and Fe contents than romaine cultivars. These data suggest that the genetics of cultivar groups were distinguishable, a property that may be of utility in plant breeding efforts for nutrient-rich lettuce.

Keywords: *Lactuca sativa*; EST-SSR; genetic diversity; heritage cultivars; heirloom cultivars; modern cultivars; lettuce phenotypes.

Introduction

Lettuce is a genus of Asteraceae (Compositae, sunflower family) (Funk et al., 2005; Judd et al., 1999) and one of the oldest domesticated (8,000 to 4,000 BC) plants (Hancock, 2004). The genus *Lactuca* L. comprises approximately 100 wild species; however, detailed information about the biogeography and ecobiology of most of these species is not available (Lebeda et al., 2004). The genus *Lactuca* has four named species, the cultivated *L. sativa* L. and three wild species, which are *L. serriola* L., *L. saligna* L., and *L. virosa* L. *Lactuca serriola* is believed to be the primary progenitor of cultivated lettuce and is the species most related to *L. sativa* (de Vries, 1997). Lettuce comprises seven main groups including oil seed type that differ phenotypically (described as morphotypes) (Kristkova et al., 2008). Cultivated lettuce is grouped commonly into two genetic types, heritage and modern, that include five horticultural phenotypes, butterhead, romaine, loose-leaf, crisphead, and stem lettuce. Lettuce is a major economic horticultural crop worldwide, with production of over 21 million tons in 2004 and an estimated $2.06 billion farm value in the USA (Truco et al., 2007). Principal producers of lettuce are the United States, England, Australia, Israel, Spain, Italy, France, Germany, and Greece (Katz and Weaver, 2003). Lettuce is considered to be a poor nutrient source,(Triplett, 2005) but is rich in Ca, K, and Fe and somewhat rich in vitamins A and C (Herbst, 2001).

Lettuce has been investigated as one of the subjects of classical and molecular studies in the Compositae Genome Project that includes genetic analysis for qualitative and quantitative traits and identification of potential genes from extensive expressed sequence tag (EST) database. The ability to control gene expression and redirect metabolic output will make possible the production of novel materials (i.e., flavonoids) in plants at economically sound levels (Caetano-Anolles and Gresshoff, 1997). Also, the molecular basis for specific characters (i.e., accumulate high nutrient content) is becoming better known, so it is increasingly possible to associate target genes, discovered in ideal species, with corresponding loci in crop plants. The primary gene pool of *Lactuca* is represented by *L. sativa*, with its enormous morphological and genetic variation, numerous cultivars, and many landraces (Phillips and Rix, 1993; Rodenburg, 1960; Stickland, 1998). All of the various *L. sativa* (cultivated lettuce) morphotypes are interfertile and form a primary gene pool together with the wild species *L. serriola* (de Vries and van Raamsdonk, 1994).

Molecular markers became widely used in plant genetic fingerprinting and breeding programs during the 1980s; thus, genotype-based genetic orientation has been adopted rather than phenotype-based genetic approaches. Several molecular markers are used regularly for cultivar genotyping, linkage mapping, and population structure, such as EST-SSRs (expressed sequence tag-simple sequence repeat) markers (Scheef et al., 2003; Simko, 2009; Yu and Pauls, 2004), RFLP (restriction fragment length polymorphism) (Ooijen et al., 1994; Sandbrink et al., 1995), RAPD (random amplification of polymorphic DNA) (Curley and Jung, 2004; Qian et al., 2001; Stevens et al., 1995), ISSR (inter simple sequence repeat) (Joshi et al., 2000; Zietkiewicz et al., 1994), AFLP (amplified fragment length polymorphism) (Vos et al., 1995), and microsatellite polymorphism (Panaud et al., 1996). SSR markers are becoming the preferred molecular markers in crop breeding because of their properties of genetic co-dominance, high reproducibility, and multi-allelic variation. They are the most practical markers for genomic mapping, variety identification, and marker-assisted selection. For lettuce, a database containing of over 135,000 EST-SSR generated from genotypes *L. sativa* ‘Salinas’ and *L. serriola* ‘UC96US23’ (http://cgpdb.ucdavis.edu) is publically available. Thus, an EST-SSR that represents candidate genes for phenotypic traits of interest (e.g., disease resistance, stress response, plant development) can be identified easily. Recently, the vast majority of studies of lettuce genes have focused on disease resistance in genetic map studies (Kesseli et al., 1994; Michelmore et al., 1994; Paran and Michelmore, 1993). In genetic diversity studies, amplicons are scored as presence or absence and converted into measurements of similarity or dissimilarity, based on the test used. This kind of tests of genetic distance provide a crucial method of exploring divergence (or similarity) between individuals or taxa (Breyne et al., 1999). In the current research, relationships between the entities were screened and then used to construct a matrix of pair-wise distances and analyzed using one of the several different clustering algorithms including N-J (neighbor joining) clustering methods. The data are presented as a dendrogram, providing graphical representations of the similarity between groups of entities, or using taxonomic units (Rohlf, 1997; Saal and Wricke, 1999; Tamura et al., 2007). This study assessed the genetic diversity of eighteen selected *Lactuca sativa* cultivars to characterize the differences and nutritional status of lettuce using fifteen previously described EST-SSR primers.

Materials and Methods

Plant material and DNA extraction

Eighteen cultivars (Table 6.1) including butterhead, romaine, and loose-leaf phenotypes of heritage and modern genetics were studied in this experiment. Seeds of heritage and modern cultivars were obtained from Seeds of Change, Spicer, MN, and from Johnny's Selected Seeds, Winslow, ME. Heritage cultivars commonly are understood as being introduced in or before 1950. The vendors identified the heritage cultivars, which were verified against a data base (http://compositdb.ucdavis.edu/), when possible. All cultivars were not in the data base or dates of introduction were not given (Table 6.1). All of the cultivars are readily available from seed vendors. All available lettuce cultivars were chosen because of growers concern. The lettuce cultivars were grown with a day temperature of 24 ± 3°C and a night temperature of 18 ± 3°C in a greenhouse at the University of Massachusetts Amherst. Approximately 500 mg of young leaves of month-old plants were collected for total genomic DNA extraction following the method described by Scheef et al. (2003) and Yu and Pauls (2004).

In brief, four fresh disks obtained by cutting leaves from each cultivar with a sharp knife were put into 2.0-ml, screw-top microcentrifuge tubes. Then 600 μL potassium ethylxanthogenate (PEX) buffer were added, and 2 copper beads were placed in each tube. For breaking the leaf tissue, the tubes were placed in a tissue grinder (MiniBeadbeater, Biospec Products, Bartlesville, OK) for two min. The sample tubes were put in a water bath for 30 min at 65°C and then were centrifuged for 10 min at 17,530 x g. The supernatant was poured into new snap-top microcentrifuge tubes, which were then filled with 6:1 mixture(v:v) of ethanol (ETOH) [98%] and 7.5M ammonium acetate and mixed by inverting the tubes. After 30 min, the tubes were then shaken by inverting, and the nucleic acids were pelleted by spinning for 10 minutes at 1,431 x g. The supernatant was decanted, and 400 μL of TE + RNAase (TRIS-EDTA RNAase) were added to suspend the pellet, which was then incubated at 37°C for one hour. The samples were centrifuged for 1 min at 17,530 x g in the next day. The supernatant was transferred into clean microcentrifuge tubes, and then the tubes were filled with 10:1 mixture (v:v) of ETOH (98%) and 3M sodium acetate and mixed by inverting. After 30 min, the tubes were shaken well to break up the precipitate before eluting and spinning the samples for 5 min at 2,236 x g. The supernatant was then discarded, and the pellet was washed by filling the tube with 70% ETOH and vortexing until the pellet came off the tube. The pellet was then spun for 15 seconds at 17,530 x g. The ETOH was decanted, and 100 μL of 1xTE were added in the samples, which were stored in the refrigerator at 7°C until quantification. The DNA quantification was conducted with a fluorometer (TKO 100 Fluorometer, Hoefer Scientific Instruments, San Francisco, CA) (Mes, 1993). Concentration of DNA yield ranged between 50-100 ng/μL.

Primer selection

Thirty-three EST-SSR primers pairs were selected based on published data (Simko, 2009) where the repeat is located at least 50 base pairs (bp) from the 5′ and the 3′ ends of the sequence. Simple Sequence Repeats (SSRs) were identified from ESTs generated from *L. sativa* cv. S*alinas* and *L. serriola* accession "*UC96US23*" and are publicly available at the Compositae Genome Project database (CGPDB) (Michelmore et al., 2003). Cultivars Salinas and UC96US23 are the two genotypes that had been used as parents for an interspecific cross to develop the molecular linkage map of lettuce (Johnson et al., 2000; Truco et al., 2007) that is also available at the CGPDB website (McHale et al., 2009). The SSRs are at least 20 bp long with repeat motif length of 2 to 8 bp. All selected EST-SSRs oligonucleotides were synthesized commercially by Integrated DNA Technology, Inc. (IDT; Coralville, Iowa).

PCR amplification

The PCR reactions were performed in 96-well plates using a thermal cycler (PTC-200, Peltier Thermal Cycler, MJ Research, Waltham, MA) according to the protocol of Williams et al. (1990) and Zietkiewicz et al. (1994). Each 10-µL reaction mixture (with 10% pipetting loss) contained about 2.3 µL ultrapure water (Milli-Q, Millipore Corporation, Bellerica, MA), 5 µL of genomic DNA, 0.4 µL of forward and reverse primers, 0.4 µL of dNTP (deoxynucleotide triphosphate), 2.2 µL of 5x PCR buffer (containing 50 mM of TRIS-HCl at pH 8.5, 10 mM of KCl, and 2 mM $MgCl_2$) and 0.13 µL of *Taq* polymerase. The PCR (Williams et al., 1990; Zietkiewicz et al., 1994) amplification conditions were programmed according to Simko (2009) as follows: one cycle of denaturation at 94°C for 5 min, followed by 40 cycles amplification with a 30 s denaturing at 94°C, a 30 s annealing at 57°C, a 30 s extension at 72°C, and the final extension at 72°C for 10 min. After PCR amplification, the products were electrophoresed on a 3% metaphor agarose at a constant power of 168 V for 2 h. A 100 bp DNA ladder (New England BioLabs, Inc., Beverly, MA) also was loaded along with the samples for each run to estimate the sizes of the separated DNA fragments. The gel was stained in 1% ethidium bromide solution for 20 min and then was detained in distilled H_2O for 40 min. The gel was photographed with a high-resolution electrophoresis gel imaging device for visualization and isolations of the DNA fragments.

Data analysis

All 18 cultivars from different geographic origins were used to screen the SSR primers for PCR amplification and product-length polymorphism. For primers that produced the expected fragments after PCR reactions, the number of alleles was recorded, and the polymorphism information content (PIC) of an SSR locus was calculated as described by Saal and Wricke (1999).

$$PIC = 1 - \sum_{i=1}^{k} pi^2$$

where, *pi* is the frequency of the *i*th allele out of the total number of alleles at an SSR locus, and *k* is the total number of different alleles for that locus. For phylogenetic analysis, only the data for the polymorphic SSR loci were entered for all DNA samples, and a "1" or "0" was used if an allele was present or absent for a cultivar, respectively. The data matrix based on the shared proportion of ancestry between individuals was used subsequently for construction of a dendrogram by the N-J method (Nei and Li, 1979; Saitou and Nei, 1987) implemented in the program MEGA 4 (Tamura et al., 2007). The genetic-distance estimation was based on the method described by Nei and Li (1979).

The EST-SSRs data for statistical analysis were conducted using the software GenAIEx 6.1 (Peakall and Smouse, 2006) for co-dominant markers. Null and missing alleles were excluded from the analysis. The unbiased estimate of genetic heterozygosity "*UHe*" (Nei, 1978) and observed number of different alleles (*Na*) were used to measure marker informative value. The principal component analysis plot was conducted using the XLSTAT 2011.2.04 - Principal Component Analysis (PCA) program (Fahmy, 2011). Fifteen functional EST-SSRs (detecting a total of 32 alleles) were used for rank correlation analysis using principal component correlation in XLSTAT. PCA was attempted to identify underlying variables that explain the pattern of correlations within set of observed variables. PCA is often used in data reduction to identify a small number of factors that describes variance observed in a set of variables.

Results and Discussion

DNA polymorphism

Each EST-SSR primer pair was amplified in DNA of all 18 lettuce cultivars. There was 45.45% polymorphism among the amplified fragments. In a total of 33 EST-SSR markers tested, 15 produced polymorphic amplicons (Table 6.2) within the expected size from all 18 cultivars of lettuce. A fairly low level of polymorphism was observed compared with other self-pollinated species, e.g., wheat (*Triticum aestivum*) (Gupta et al., 2003) and Jute (*Corchorus capsularis*) (Mir et al., 2008). Among phenotypes (butterhead, romaine, and loose-leaf), a small number of alleles (*Na*), ranging from 2 or 3 alleles with an average of 2.13 was observed. The highest marker polymorphism (*UHe*) was in loose-leaf (0.344) and butterhead (0.303) whereas the lowest *UHe* was in romaine (0.258) (Table 6.2). Overall the average marker polymorphism was 0.37, with the lowest and highest values at 0.11 (SML-028) and 0.61 (SML-001), respectively (Table 2). Almost one-third of the markers had polymorphism less than 0.30, whereas one-third had polymorphism near or above 0.50. Generally low level of polymorphism (<0.30) was observed only when most (or all) of the *L. sativa* cultivars were monomorphic for the marker allele, and different alleles originated from wild species only.

Fairly similar allelic forms were observed when comparing our phenotypic results with those published (Simko, 2009). The case of null alleles may be attributed either to sequence divergence in the primer binding sites or to an absence of specific SSR loci in the respective genotypes (Gupta et al., 2003). Among the functional EST-SSRs, 50% also was discriminated between *L. sativa* horticultural species. In the total functional primer pairs, 75% amplified unique alleles from *L. sativa* (heritage) and *L. sativa* (modern), and

25% of primers pairs amplified common alleles shared with *L. sativa* biotypes. This result concluded that these markers appear useful in discriminating *L. sativa* biotypes. The estimate of polymorphism within each horticultural type showed that the highest heterozygosity was observed in loose-leaf types of green and red lettuce (Salad Bowl and Red Salad Bowl). The level of marker polymorphism assessed with EST-SSRs supports morphological observations on different horticultural types. Romaine cultivars have limited phenotypic variability and a low level of marker polymorphism, whereas loose-leaf types of lettuce show a wide range of leaf shape, size, and texture together with high marker polymorphism.

Diversity analysis using EST-SSR

The genetic association among *L. sativa* cultivars was calculated based on the combined of 15 functional EST-SSR markers. The genetic dissimilarity (GD) coefficient values (Table 6.3) for all possible 153 pairs of genotypes ranged from 2 (between 'Red Deer Tongue' and 'Winter Density') to 22 (between 'Cosmo Savoy' and 'Salad Bowl') with a mean of 11.67. All 18 cultivars were grouped into 2 main clusters (Fig. 6.1). Each cluster was further subdivided into 3 subclusters. Heritage and modern cultivars did not group separately from each other. Interestingly, the clustering pattern observed in the dendrogram corresponded poorly with horticultural distribution of butterhead, romaine, and loose-leaf. Additionally, 3 of 5 cultivars in cluster 1 ('Australe', 'Tom Thumb', and 'Bronze Mignonette'), and 6 of 13 cultivars in cluster 2 ('Red Deer Tongue', 'Focea', 'Forellenschluss', 'Winter Density', 'Cosmo Savoy', and 'Simpson Elite') had high nutrient content, whereas 4 of 18 cultivars in clusters 1 and 2 ('Red Rosie', 'Tropicana', 'Two Star', and 'Coastal Star') had low nutrient content. The clustering pattern suggested that most of the *L. sativa* cultivars grouped in the same clusters based on the high or low nutrient content, whereas none of the horticultural groups [butterhead, romaine, and loose-leaf] explained the observed clustering pattern.

The genetic distances among 6 lettuce phenotypic groups also were estimated on the basis of genetic dissimilarity. Co-efficient values for all possible 15 pairs of genotypes ranged from 14 (between butterhead-modern and romaine-modern) to 24 (between butterhead-modern and romaine-heritage) with a mean of 19. All 6 phenotypes were grouped into 3 clusters (Fig. 6.2). Each cluster was again subdivided into 2 subclusters. As expected, cluster 1 romaine-heritage and romaine-modern grouped distinctly from clusters 2 and 3. Interestingly, butterhead-modern grouped separately in cluster 2 from butterhead-heritage in cluster 3. On the other hand, loose-leaf-heritage grouped separately in cluster 2 from loose-leaf-modern in cluster 3. The clustering pattern in the dendrogram noted differences in genetic groups. The data agrees that in cluster 1, romaine-heritage and modern phenotypes showed individuality from clusters 2 and 3 and showed variability with genetic groups. However, butterhead-modern and loose-leaf-heritage grouped in same cluster that concluded each cultivar may have same ancestor but variable genetic resemblance. Similarly, butterhead-heritage and loose-leaf-modern grouped in same cluster that also showed variable genetic compatibility.

Grouping analysis

Five cultivars groups were formed by the neighbor-joining methods. Group V, the largest group contained six cultivars (Fig. 6.1). Group I, IV, and III contained five, four, and two cultivars respectively, whereas group II contained only one cultivar. The greatest genetic similarity was observed among cultivars ‘Red Rosie’, ‘Tom Thumb’, and ‘Salad

Bowl', which were in group I together with 'Bronze Mignonette' and 'Australe'. Cultivar 'Focea' was one of the most divergent and formed group II alone. Cultivars 'Red Deer Tongue' and 'Winter Density' jointly made up group III. The consideration is supported by the fact that the great majority of the cultivar pairs from same locality were not dissociated in distinct groups. For example: cultivars 'Red Rosie' and 'Salad Bowl', derived, respectively from romaine and loose-leaf types, were present in group I.

Four groups were set up by the neighbor joining method (Fig. 6.1) taking as base the discrepant changes of level in the dendrogram, where group V united the cultivars belonging to groups IV and V by the neighbor joining method. Cultivars 'Red Deer Tongue' and 'Winter Density' formed the second dendrogram group, whereas in neighbor joining grouping, those cultivars was represent on group III. Cultivars 'Black Seeded Simpson', 'Simpson Elite', 'Two Star', 'Adriana', 'Claremont', and 'Buttercrunch' formed in the fifth group in both dendrogram and neighbor joining methods. The group IV was composed by the cultivars 'Coastal Star', 'Cosmo Savoy', 'Forellenschluss', and 'Tropicana'. The group IV was not equivalent to group I; thus, the results obtained by the neighbor joining methods were very different from those having different genetic similarity.

The perfect genetic similarity between cultivars 'Red Rosie', 'Tom Thumb', and 'Salad Bowl' was not expected as these materials are phenotypically divergent in literature, and it can be denoted for example, that 'Red Rosie' is classified as a classic example of a romaine variety, 'Tom Thumb' is classified as a heritage example of a butterhead type, whereas 'Salad Bowl' is a heritage example of a loose-leaf variety. However, during the course of this study, the 'Tom Thumb' plants morphoagronomically showed a small round formation in the field. In germplasm collection, cultivars 'Tom Thumb' and 'Salad Bowl' may constitute identical genomes for lettuce genotyping.

Genetic distance between lettuce cultivars with SSR-based PCA groupings

The molecular marker derived genetic distances between 18 lettuce cultivars is graphically presented with a Principal Component Analysis (PCA) plot (Fig. 6.3). The plot approximately 42 % of the total variance in the genetic distance data was explained by two PCA coordinates. Further, the *x* coordinate of the PCA explained 24.76 % of the total variance, while the *y* coordinate explained 16.86 % of the total variance. The cultivars appears fairly heterogeneous as a group, and do not appear to group uniformity. There are three big areas of tight grouping of cultivars suggesting that some cultivars are more genetically related than other, which may be expected if several cultivars were derived from common gene pools.

The PCA plots shows the genetic distance among cultivars in the 3 phenotypic groups (Fig. 6.3). Three areas of tight grouping in the PCA plot did not appear to represent cultivars belonging to the same phenotypic groups. The phenotypic groups in the cultivar formed three distinct groups in the PCA plot, while some cultivars mixed with others phenotypic groups which may be expected if cultivars were derived from same ancestor. No distinct patterns appeared, indicating that cultivars with genetic relatedness and morphological groupings exist only for butterhead, loose-leaf, and possibly common types. Butterhead cultivars Tom Thumb (TT), Bronze Mignonette (BM), and Australe (AS) were grouped together and showed higher genetic variability (Fig. 3). Further, loose-leaf cultivars Salad Bowl (SB), Simpson Elite (SE), and Black-Seeded Simpson (SM) had also higher genetic variability. Cultivars TT, BM,, AS, SB, SE, and SM were found higher Ca, K, Mg, Zn, and Fe mineral accumulation compared to other cultivars. The high mineral accumulation was satisfied in the nutrient density experiments using with same cultivars (Meagy et al., 2013a; Meagy et al., 2013b). This result suggests that the cultivars with high genetic variability might have high nutrient accumulation ability. Further, the higher polymorphism found in loose-leaf and butterhead phenotype. The correspondence results agree with most of the loose-leaf and butterhead cultivars for this PCA grouping and mineral accumulation.

Within the group, Romaine cultivars Coastal Star (CS), Forellenschluss (FR), Cosmo Savoy (CV), and Claremont (CL), loose-leaf cultivars Red Deer Tongue (RD) and Tropicana (TP), and butterhead cultivars Buttercrunch (BC) and Focea (FC) were found in the same area of distribution in a PCA plot (Fig. 6.3). The result suggests that the cultivars in this group were found lower genetic variability and found in a tight grouping. Genetically, these cultivars might be closed with each other and derived from the same originator. In this grouping, cultivars did not show lower nutrient accumulation except RD found high nutrient accumulator. This is because RD is belonged to the loose-leaf phenotypic group and loose-leaf cultivars had high mineral accumulation than romaine and butterhead. More studies may be required for this group of cultivars to enrich the ability of the nutrient accumulation. However, Red Rosie (RR) was sit separately in PCA plot (Fig. 3) and accumulated low amount of minerals compared to other cultivars. Cultivar RR also showed low genetic variability and might need more attention for developing mineral status.

In this study, cultivars Adriana (AR), Two Star (TS), and Winter Density (WD) were grouped together in the PCA plot (Fig. 6.3). This correspondence suggests that these cultivars might have more genetic relatedness compared to other cultivars. The result further suggests that upon with genetic similarity these were derived from the same ancestor. For developing nutrient status, it might need more studies to develop these cultivars' nutrient status.

Conclusions

The current results report genetic diversity of selected *L. sativa* cultivars using EST-SSR markers from *Lactuca* species. From previously reported 33 EST-SSRs markers for lettuce, of which 15 have showed polymorphism in this study. Testing the set of 18 cultivars showed presence of subpopulations that were relatively distinct with the classification based on horticultural types. The phenotypic classification into horticultural types differs to some extent from genotypic relatedness. As a result, molecular markers indicate that the cultivar groups are highly similar to romaine and loose-leaf types. The phylogenetic data showed genetic distinction of 18 cultivars, which differ from 6 horticultural types. However, in the PCA plot, cultivars TT, BM, AS, SB, SE, and SM showed genetically divergent with high minerals accumulation. This result will help for studying in mineral nutrients accumulation because cultivars with high genetic variability may potential for high minerals accumulation. Along with high genetic variability, loose-leaf and butterhead had high Ca, K, Mg, Zn, and Fe mineral contents than romaine cultivars that supported with coordinated mineral nutrient studies. The EST-SSRs markers used in this study can be implemented in population structure studies in more lettuce species. Future research may concentrate on validating of these EST-SSR markers on genetic diversity for developing nutrient-rich lettuce cultivars followed by genetic mapping.

Table 6.1. List of lettuce cultivars including genetics and phenotypes used in this molecular study

Cultivar	Type		Year of[†] Release	Seed source
	Phenotype	Genetic		
Buttercrunch (BC)	Butterhead	Heritage	1963	Seeds of Change
Bronze Mignonette (BM)	Butterhead	Heritage	1898	Seeds of Change
Tom Thumb (TT)	Butterhead	Heritage	1850	Seeds of Change
Winter Density (WD)	Romaine	Heritage	1930	Johnny's Seeds
Cosmo Savoy (CV)	Romaine	Heritage	-	Seeds of Change
Forellenschluss (FR)	Romaine	Heritage	-	Seeds of Change
Salad Bowl (SB)	Loose-leaf	Heritage	1950	Johnny's Seeds
Black-Seeded Simpson (SM)	Loose-leaf	Heritage	1850	Seeds of Change
Red Deer Tongue (RD)	Loose-leaf	Heritage	-	Seeds of Change
Adriana (AR)	Butterhead	Modern	-	Johnny's Seeds
Focea (FC)	Butterhead	Modern	-	Johnny's Seeds
Australe (AS)	Butterhead	Modern	-	Johnny's Seeds
Red Rosie (RR)	Romaine	Modern	-	Johnny's Seeds
Claremont (CL)	Romaine	Modern	-	Johnny's Seeds
Coastal Star (CS)	Romaine	Modern	1999	Johnny's Seeds
Tropicana (TP)	Loose-leaf	Modern	2008	Johnny's Seeds
Simpson Elite (SE)	Loose-leaf	Modern	1999	Johnny's Seeds
Two Star (TS)	Loose-leaf	Modern	1995	Johnny's Seeds

[†] Years indicating the release or introduction time period of cultivars for production. Release in or before 1950 are considered heritage cultivars and release after 1950 are considered modern cultivars. However, some cultivars have no record of release time but vendors were conformed the cultivars for either heritage or modern genetics.

Table 6.2. Details of EST-SSR primers (Simko, 2009) identified from *L. sativa* and *L. serriola* ESTs used for est
diversity of 18 lettuce cultivars.

Primer	Primers sequences		Allele size (bp)	PIC or M Polymorp (*UHe*
SML-001	F: CCATGGATCCTGTGTGAAGA	R:CACCATGTTCCACTTCCACTT	196 - 220	0.61
SML-003	F: CGGGCTGGTTTTGATTTTTA	R: TGTCAAATCGTCACGTGGTT	110 - 120	0.44
SML-007	F: ACACTTGCCGATTCCTTCAC	R: ACCCGTGTTGAAAATGGAGA	200 - 236	0.20
SML-013	F: TCCCATGATGGAGAGACTCA	R: CCCAAAAGGGAATAGCAACC	246 - 276	0.41
SML-015	F: TTGAGGAGGGCATTTACGTC	R: GAGGCGTATCTCCAAGGTGT	168 - 263	0.50
SML-019	F: AAGGAGGAAAGTATGGTGAGGA	R: TGAAATGAAGCAACACACGA	162 - 172	0.44
SML-026	F: GGGTTCTCATTGGCTGACAT	R: TGTCTTCCAACCAAAACATACA	179 - 224	0.20
SML-028	F: TGATCCAGGCTCTCCAGAAT	R: CACGACCATGAATGATAAGTGC	189 - 201	0.11
SML-039	F: ATTACCCCTGGCCTTATGCT	R: TCGTATCTTGGCTGCTCCAT	237 - 255	0.57
SML-042	F: CATGAAGTGTTTTGGGGTGA	R: GGCCTTTCATTTCTTCCTCA	182 - 207	0.28
SML-043	F: TTCTTTCGCCCATCTGAAAC	R: AAACAGGGGGCTAACGATCT	200 - 212	0.49
SML-045	F: ACAAAACCGTTTCACCCAAA	R: AGCCCTGTCCTCTTCAGGAT	232 - 256	0.34
SML-055	F: CTGCGTGTTTTAAGCCGTTT	R: TCCATAATAATATAATCGCACCAA	215 - 236	0.48
SML-056	F: GCCTGATCCAATTGCTTTGC	R: CAGCTTAACATACTTTTGTTCATTCA	181 - 202	0.34
SML-061	F: GAGTGCTGAGAAAGCCCAAG	R: TAAGCTGCTCTTCCCTCCTG	203 - 207	0.11

Table 6.3. Genetic dissimilarity coefficient values of 18 lettuce cultivars (see Table 6.1 for genotype

	BC	BM	TT	CS	CV	FR	SB	SM	RD	AR	FC	AS	RR	CL	WD	TP	SE
Operational Taxonomic Unit (OTU)	0																
	14	0															
	16	8	0														
	10	18	18	0													
	6	16	18	4	0												
	6	16	14	8	4	0											
	22	12	16	20	22	20	0										
	10	16	18	10	10	12	12	0									
	6	12	18	12	8	8	20	16	0								
	6	14	14	10	10	12	18	8	12	0							
	10	10	10	8	8	8	18	12	8	8	0						
	14	4	12	18	16	16	12	16	12	14	10	0					
	12	12	12	10	14	10	12	10	14	10	10	12	0				
	6	14	14	10	10	12	18	12	8	4	8	14	14	0			
	8	10	16	10	6	6	18	14	2	14	6	10	12	10	0		
	6	12	10	12	8	4	16	12	8	12	8	12	10	8	6	0	
	10	20	18	18	14	12	16	12	12	12	20	20	14	12	14	12	0
	4	14	16	8	4	6	18	6	10	6	10	14	12	6	8	6	10

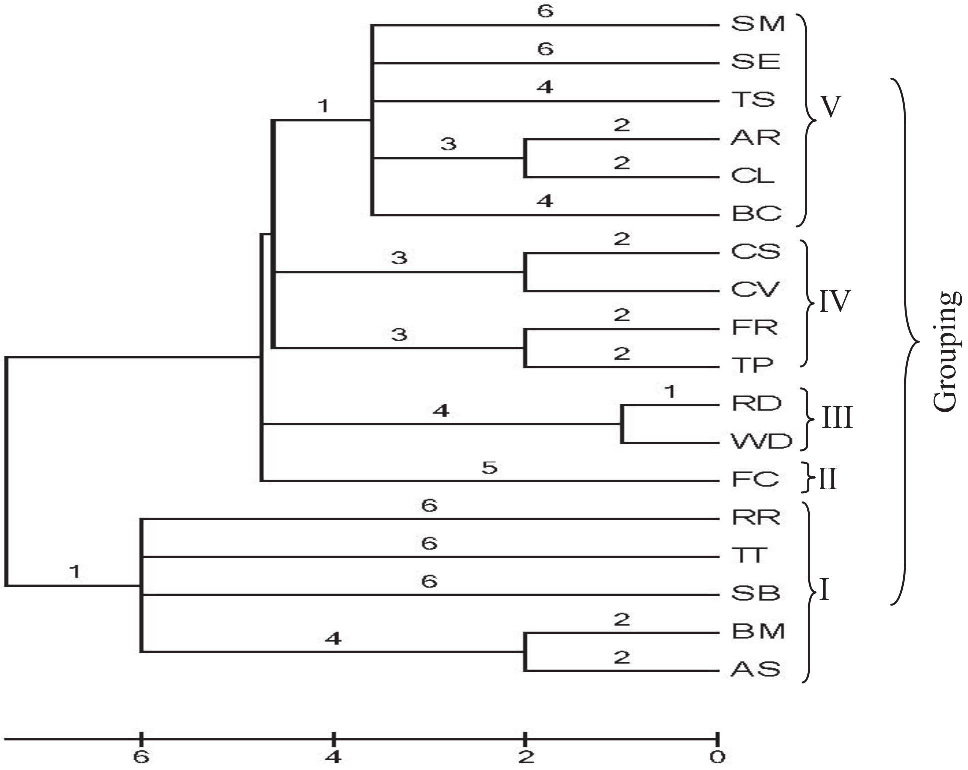

Fig. 6.1. A dendrogram showing clustering pattern of 18 lettuce cultivars (for genotype details, see Table 6.1) based on genetic distance estimated from EST-SSR polymorphism generated with the neighbor-joining clustering method

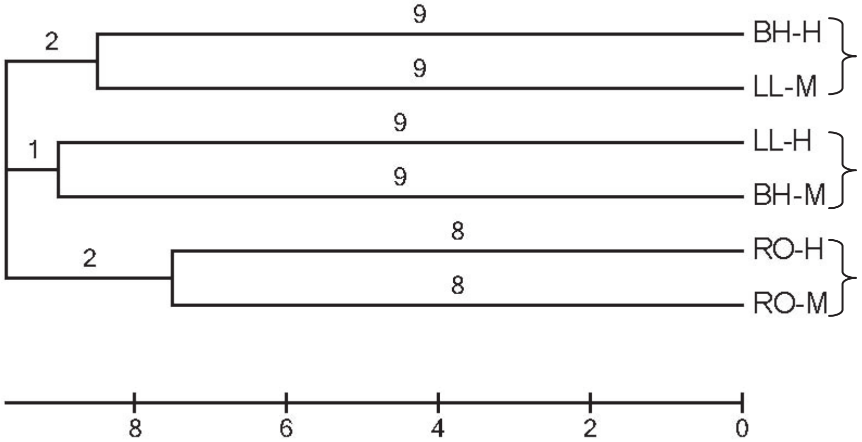

Fig. 6.2. A dendrogram showing clustering pattern of 18 lettuce cultivar into 6 groups (for phenotypic and genetic grouping details, see Table 6.1) based on genetic distances estimated from EST-SSR polymorphism generated with the neighbor-joining clustering method. Where BH-H, Butterhead-Heritage; BH-M, Butterhead-Modern; RO-H, Romaine-Heritage; RO-M, Romaine-Modern; LL-H, Loose-leaf-Heritage; LL-M, Loose-leaf-Modern.

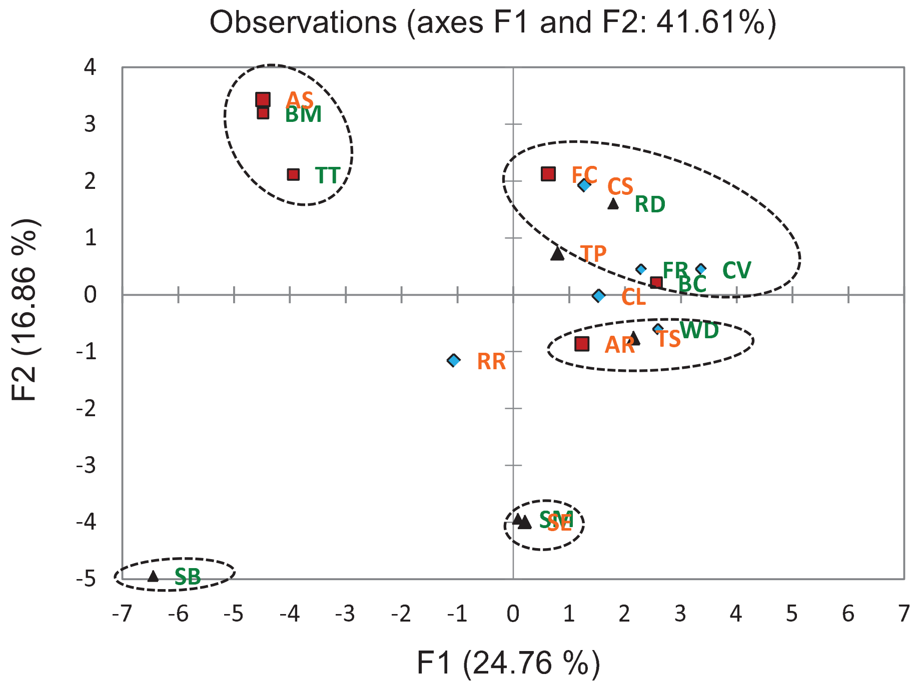

Fig. 6.3. Principal component analysis plot of the genetic distance matrix computed from SSR data generated from 18 Lettuce cultivars (for cultivar name, see Table 6.1), classified into 3 phenotypic groups, each group presented by different symbol and color within 2 genetic groups, indication orange front (Heritage) and green front (Modern).

CHAPTER 7

OVERALL SUMMARY AND CONCLUSIONS

Accumulation of nutrients did not differ substantially with heritage and modern cultivars. However, concentrations (% dry wt) of P, K, Ca, Mg, and S were higher in the heritage cultivars than in the modern cultivars. Phenotype had significant effect with nutrient concentration and total accumulation, but the effect appeared to be due to head size and morphology with loose-leaf and butterhead phenotypes being higher accumulators of nutrients than romaine phenotypes. Romaine and butterhead cultivars had same head weights, but loose-leaf generally had higher head weights and total nutrient accumulation. Loose-leaf cultivars accumulated higher Ca concentrations and total accumulation than romaine or butterhead phenotypes. On the other hand, butterhead phenotype accumulated a higher Zn concentration than romaine or loose leaf, but total Zn accumulation did not vary with phenotype.

Fertilizer regime appeared not to be a major factor in nutrient accumulation, but each regime provided accumulation of nutrients at amounts needed for optimum of yields. Overall, chemical or Hoagland no. 1 regime had higher nutrients concentration or accumulation than organic or compost. Supplemental Zn additions of 0.10 mg/L or 0.15 mg/L level did not elevate Zn content over a supply of 0.05 mg/L. Levels of 100 or 200 mg Ca L^{-1} in the chemical fertilizer gave the highest fresh weights and enhanced total nutrient accumulation and concentration.

Individual cultivars differed widely in nutrient density with a wide range of variability in mineral nutrients concentrations occurring among different. High concentrations of P, K, Ca, Mg, S, Na were in ‘Tom Thumb’, ‘Focea’, and ‘Bronze Mignonette’ of the butterhead group, in ‘Forellenschluss’ and ‘Winter Density’ of the romaine group, and ‘Red Deer Tongue’, ‘Salad Bowl’, and ‘Black Seeded Simpson’ of the loose-leaf phenotypes. A wide difference occurred with micronutrients Zn, B, Mn, Cu, and Fe concentration among cultivars. ‘Tom Thumb’, ‘Focea’, ‘Simpson Black Seeded’, ‘Winter Density’, ‘Bronze Mignonette’, and ‘Salad Bowl’ were nutrient rich perhaps because of being loose leaf or semi-loose leaf phenotypes. Cultivars differed widely in Ca accumulation with ‘Salad Bowl’, ‘Red Deer Tongue’, ‘Buttercrunch’, and ‘Bronze Mignonette’ ranking in the top among all cultivars in Ca concentration. ‘Adriana’, ‘Australe’, ‘Coastal Star’, and ‘Forellenschluss’ accumulated much lower concentrations of Ca. A wide range of variability in Ca concentration occurred among different cultivars of lettuce including differing phenotypes.

Cultivars ‘Tom Thumb’ and ‘Adriana’ exhibited high Zn accumulation, but their total Zn did not have this ranking because ‘Tom Thumb’ and ‘Adriana’ are small headed. Cultivars ‘Two Star’ and ‘Black Seeded Simpson’ exhibited the lowest Zn accumulation, but total Zn of ‘Two Star’ had the top ranking because of high total dry weight. Selection of cultivars is a significant factor for improving nutritional of status of Zn. So zinc impairment in diet could be corrected by using nutrient-dense lettuce cultivars without requiring additional consumption or enrichment of produce. Therefore, the potential for mineral nutritional improvement of different types of cultivated lettuce through breeding and selection is apparent. Improving the mineral nutrition levels of lettuce will enhance nutrient uptake in diet without requiring an increase in consumption, as nutrient density was not a factor of large heads having a lower nutrient concentration than small heads.

Simultaneously, head wt had an effect on higher accumulation but small heads lettuce was containing higher concentration of mineral.

Overall no interaction occurred between fertilizers and genetics, fertilizer and phenotypes, and fertilizers and cultivars with fresh weight, dry weight, P, K, Ca, Mg, S, Na, Zn, B, Cu, and Fe accumulation. Only weak correlations occurred between Ca and other nutrients (P, K, Mg, S, Zn, B, Mn, and Fe) suggesting that measurement of Ca accumulation is not a good indicator of the accumulation of other nutrients. The accumulations of mineral nutrients also were correlated weakly with Zn accumulation, indicating that Zn accumulation also was not a good index of accumulation of other nutrients. Hence, it is necessary to evaluate accumulation of each nutrient separately. Accumulations of total elements per head were highly significant as a function of dry weight of heads

Genetic diversity observed in the selected *Lactuca sativa* cultivars with using EST-SSR markers. From reported 33 EST-SSRs markers for lettuce, of which 15 have showed polymorphism in this study. The phenotypic classification into horticultural types differs to some extent from genotypic relatedness. As a result, molecular markers indicate that the cultivar groups are highly similar to romaine or loose-leaf types. The phylogenetic data showed genetic distinction of 18 cultivars which differ from 6 horticultural types. The EST-SSRs markers used in this study can be implemented in population structure studies in more lettuce species. Eventually, these markers may prove to be associated with important agricultural traits of the lettuce crops, which will encourage a wider application of these types of markers for DNA marker-assisted selection in commercial breeding program.

APPENDIX A. INDUCTIVELY COUPLED PLASMA (ICP)

Introduction

Inductively Coupled Plasma (ICP) is an analytical technique used for the detection of trace metals in plant and environmental samples. The primary goal of ICP is to get elements to emit characteristic wavelength specific light which can then be measured. The technology for the ICP method was first employed in the early 1960's with the intention of improving upon crystal growing techniques. Recently, ICP has been refined and used in conjunction with other procedures for quantitative analysis.

Workings of an ICP

ICP hardware is designed to generate plasma, which is a gas in which atoms are present in an ionized state. The basic set up of an ICP consists of three concentric tubes, most often made of silica. These tubes, termed outer loop, intermediate loop, and inner loop, collectively make up the torch of the ICP. The torch is situated within a water-cooled coil of a radio frequency (RF) generator. As flowing gases are introduced into the torch, the RF field is activated and the gas in the coil region is made electrically conductive. This sequence of events forms the plasma. The formation of the plasma is dependent upon an adequate magnetic field strength and the pattern of the gas streams follows a particular rotationally symmetrically pattern. The plasma is maintained by inductive heating of the flowing gases.

ICP typically includes:

- Sample introduction system (nebulizer)
- ICP torch

- High frequency generator
- Transfer optics and spectrometer
- Computer interface

APPENDIX B. HIGH TEMPERATURE OXIDATION (DRY-ASHING)

Procedure

Half of gram of dried and ground (30 mesh) plant tissue was weighed into a 30 ml high form porcelain crucibles. The crucibles were then placed in a cool muffle furnace. The temperature control of the furnace was set at 500^{o}C. After approximately 8 hours of muffling at 500^{o}C, the crucibles were removed from the furnace and allowed to cool. 10% HCl acid solution were used to extract the ashed plant tissue. The ashed tissue sample then was extracted using 25 ml 10 % HCl. The extract afterwards was filtered using Whitman no. 1 filter paper. The filtered sample was stored for elemental analysis in ICP.

Atomic Absorption Procedure for Calcium and Zinc Analysis

Reagents:

a. Lanthanum solution: 50000 PPM
b. Calcium sample solution: 25 ml 10% HCl
c. Zinc sample solution: 25 ml 10% HCl
d. Calcium standard stock solution: 1000 PPM
e. Zinc standard stock solution: 100 PPM

Preparation of Standards:

The standards were prepared in distilled water as directed bellow and used to establish a calibration curve in atomic absorption spectrometer.

Calcium Standards:

Ca Standard (PPM)	Volume of Ca from 1000 PPM	Volume of La_2O_3 from 50000 PPM	Volume of 10 % HCl	Final volume of Standard (ml)
0	0.0 ml	4 ml	96.0 ml	100
2	0.2 ml	4 ml	95.8 ml	100
4	0.4 ml	4 ml	95.6 ml	100
6	0.6 ml	4 ml	95.4 ml	100

Zinc Standards:

Zn Standard (PPM)	Volume of Zn from 100 PPM	Volume of 10 % HCl	Final volume of Standard (ml)
0.0	0.0 ml	100 ml	100
0.2	0.2 ml	99.8 ml	100
0.4	0.4 ml	99.6 ml	100
0.6	0.6 ml	99.4 ml	100
0.8	0.8 ml	99.2 ml	100
1.0	1.0 ml	99.0 ml	100

Procedure for Preparation of Ca Sample for AA Spectrophotometer

At first 0.25 ml from 25 ml calcium sample solution were transferred into 25 ml volumetric flask. Then one ml of 50000 PPM lanthanum solution were added and the solution were diluted to volume with distilled water (10 % HCl). Thereafter calcium concentration was determined with the atomic absorption spectrophotometer as of following manufacture's guidelines. Finally, there was done a calculation for estimation of calcium in the plant sample.

Calculation for Ca Estimation:

[Ca] in Plant Tissue sample (%) = $\frac{\text{Meter Reading of Ca } (\mu g/ml) \times \text{Ca sample } (25 \text{ ml}) \times (100)}{\text{Plant tissue sample } (0.5 \text{ g})}$

$$= \frac{\text{Meter Reading of Ca } \times 5000}{10000} \ \mu g/g$$

$$= \text{Meter reading of Ca} \times 0.5$$

$$= \% \text{ Ca}$$

Procedure for Preparation of Zn Sample for AA Spectrophotometer

25 ml Zn sample (extracted in10% HCl) solution were prepared for estimation of zinc concentration in plant tissue sample. Thereafter zinc concentration was determined with the atomic absorption spectrophotometer as of following manufacture's guidelines. Finally, a calculation was done for estimation of zinc in the plant sample.

Calculation for Zn Estimation:

[Zn] in Plant Tissue sample (mg/kg),

$$= \frac{\text{Meter Reading of Zn } (\mu g/ml) \times \text{Zn sample}}{\text{Plant tissue sample}}$$

$$= \frac{\text{Meter Reading of Zn } \times 25 \text{ ml}}{0.5 \text{ g}} \ \mu g/g$$

$$= \text{Meter reading of Zn} \times 50$$

$$= \mu g/g \text{ Zn or mg/kg Zn}$$

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