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Genotypic variability and inheritance of iron and zinc in sweetpotato

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GENOTYPIC VARIABILITY AND INHERITANCE OF IRON AND ZINC IN SWEETPOTATO

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by
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DEDICATION

To my wonderful family and friends, and especially to my grandmother, Celestine Polit, who thought I'd be a horticulturist one day.

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ABSTRACT

Sweetpotato [*Ipomœa batatas* (L.) Lam.] is a major subsistence crop in Sub-Saharan Africa, where iron and zinc deficiency in humans is an important health problem. A sweetpotato cultivar suited for subsistence farming, with high iron and zinc concentration, would be important in alleviating these deficiencies.

The main objective of this research was to identify the extent of genetic variability of iron and zinc concentration in sweetpotato germplasm. An important subcomponent of this research was to determine the heritability of iron and zinc in sweetpotato. Protocol development studies were also conducted to aid in determining proper sampling technique.

The results of the protocol development study indicated there was a significant replication effect between plot replicates but that no significant variation existed among roots from a plant, among plants from a given replicate plot, or among different root zones. In general, most of the genetic variability present was attributable to the difference in genotype. Therefore, one root from each replicate is sufficient for determining iron and zinc concentration in sweetpotato.

A three-fold difference between high- and low-yielding cultivars for iron and zinc for ~80 cultivars was observed. The cultivars with the highest iron concentration were 'Kyukei No. 63' and 'Pata de Oso', both with ~7 ppm iron, fwb, from Japan and Peru, respectively. This compares with cultivars 'Pung-mi' and 'Chuquimanco' from Korea and Peru, respectively, both with ~3 ppm iron, fwb. These results suggest that sweetpotato with the highest levels of iron and zinc could provide about 30% and 15% of the daily dietary intact of these micronutrients, respectively. This is based on daily consumption of one 300-gram root. Iron and zinc in sweetpotato is highly available to humans given low phytic acid and high ascorbic acid concentration in orange flesh varieties.

The heritability study showed high broad-sense heritability for iron ($H^2 = 0.74$), zinc ($H^2 = 0.82$), and dry matter concentration ($H^2 = 0.93$) among half-sib families. These results and those which showed a positive correlation between iron and zinc concentration suggest that traditional breeding strategies like mass selection could improve the nutritional value of sweetpotato.

CHAPTER 1: INTRODUCTION

1.1 Iron and Zinc in Human Nutrition

Malnutrition is a large and growing problem in the developing world. Over three billion people currently suffer micronutrient malnutrition (Welch and Graham, 2004). Iron deficiency may affect three billion people worldwide (Long *et al.,* 2004). It is estimated that 49% of the world's population is at risk for low zinc intake (Cichy *et al.,* 2005), while vitamin A deficiency is estimated to affect over 140 million children under five (*Biofortified Sweetpotato,* 2006). These micronutrient deficiencies are concentrated in the semi-arid tropics, particularly in South and Southeast Asia and sub-Saharan Africa (Reddy *et al.,* 2005). Attempts have been made to alleviate these deficiencies through the use of supplements and food fortification, but these strategies do not reach most of those suffering from deficiency and have not proven to be sustainable (Römheld, 1998). Therefore, biofortification of a crop that is a staple in these areas could be an important means of reducing iron and zinc malnutrition worldwide.

1.1.1 Iron in Human Physiology and the Symptoms of Iron Deficiency

The importance of iron as the central atom of hemoglobin, and the anemia caused by the lack of it, are well known (Tuman and Doisy, 1978). Iron is also a component of myoglobin (Zhang, *et al.,* 2004), which has a function in the storage of oxygen in muscle tissue, and of the cytochrome system (Tuman and Doisy, 1978), which is important in the derivation of energy from cellular respiration. Iron, with zinc and selenium, has an immunomodulating function (Lyons, *et al.,* 2004). In addition to causing anemia, lack of sufficient iron can cause impaired cognitive development and physical coördination in children under two years of age, limitation of the ability to perform endurance physical activity, impairment of the immune system, and a number of other symptoms (Lynch, 2003). Iron deficiency has also been shown to reduce the effectiveness of iodine supplementation (Lyons, *et al.,* 2004).

1.1.2 Zinc in Human Physiology and the Symptoms of Zinc Deficiency

Zinc is required for virtually all aspects of cellular metabolism (Ruz, 2003); among other functions, zinc forms the prosthetic group of numerous enzymes, as well as the receptor proteins for steroid and thyroid hormones and vitamins A and D (Bender, 1999). Because zinc in excess of short-term metabolic needs is either excluded from absorption or excreted, the human organism lives with a perpetually marginal zinc nutriture (Solomons, 2003); therefore, it is obvious that insufficient zinc in the diet will quickly have adverse consequences. Zinc malnutrition has been linked to a number of symptoms, including behavioral alterations such as anorexia, depression, and psychosis;

impaired growth and development; altered reproductive biology; gastrointestinal problems such as diarrhea and impairment of nutrient absorption; and impaired immunity (Solomons, 2003). In juveniles, zinc deficiency can lead to slow growth or even periods of arrested growth, and to the delay of sexual maturity. Zinc deficiency can also contribute to Vitamin A deficiency, since lack of zinc can impair the synthesis of Retinol Binding Protein (Bender, 1999).

1.1.3 Complicating Factors

There are a number of substances common in foodstuffs that either interfere with or assist in the uptake of micronutrients in the human diet. These are known as inhibitors and promoters, respectively; some of those common in plant food sources are listed in Table 1.1. Promoters and inhibitors can play an crucial dietary role in areas where micronutrient nutrition is already marginal. (Raboy, 2002).

Table 1.1. Iron and zinc uptake inhibitors and promoters in the human diet common in plant food sources.

Substance	Micronutrient Affected	Common Sources
Inhibitors		
Phytic acid	Iron and Zinc	Legumes and cereal grains
Dietary fiber	Iron and Zinc	Whole-grain cereal products
Oxalic acid	Iron and Zinc	Spinach, chocolate, rhubarb, certain nuts
Goitrogens	Iron and Zinc	Brassicas, alliums, cassava, soybeans
Heavy metals	Iron and Zinc	Contaminated leafy roots and vegetables
Promoters		
Certain organic acids (*e.g.,* ascorbic acid, fumerate, malate, citrate)	Iron and/or Zinc	Fresh fruits and vegetables
Hemoglobin and certain amino acids (*e.g.,* methionine, cysteine, histidine, lysine)	Iron	Animal meats
Long-chain fatty acids	Iron and Zinc	Human breast milk
Beta-carotene	Iron	Green and orange vegetables
Inulin and other non-digestible carbohydrates	Iron and Zinc	Chicory, garlic, onion, wheat, Jerusalem artichoke
Table Summarized from Welch, 2002, and Bender, 1999.		

Two important inhibitors of iron and zinc uptake are phytic acid and oxalic acid. Phytic acid is the principle means of phosphorus storage in a number of crop plants (Raboy, 2002), including cereal grains, particularly in the bran, and legumes (Bender, 1999). In fact, marginal zinc deficiency has been found to be widespread in people who maintain diets rich in legumes (Cichy, *et al.,* 2005). Another inhibitor, oxalic acid, is found in spinach, chocolate, and nuts; in large amounts, it accounts for the toxicity of rhubarb leaves (Bender, 1999). Two important promoters of iron and zinc are ascorbic acid and ß-carotene (Table 1.1). Ascorbic acid, or vitamin C, is an antioxidant found in many fresh fruits and vegetables. ß-carotene is a precursor of vitamin A and is found in green and orange vegetables (Welch, 2002 and Bender, 1999).

1.2 Iron and Zinc in Plants

1.2.1 Physiology

As with humans and other animals, iron and zinc are essential for plant health and proper growth and development. Thus, plant foods are significant sources of iron and zinc for humans. Iron is a catalyst in chlorophyll formation, is a component of ferredoxin, and is present in several peroxidase, catalase, and cytochrome oxidase enzymes (Brady, 2002). Iron deficiency in plants is manifested as interveinal chlorosis on new leaves (Aquaah, 2002). Zinc promotes growth hormone biosynthesis, the formation of starch, and seed production and maturation (Brady, 2002). Plants that are deficient in zinc have reduced leaf size and shortened internodes. Interveinal chlorosis may appear in young leaves, as is the case with iron deficiency (Aquaah, 2002).

1.2.2 Iron and Zinc Uptake

Since iron and zinc are usually present in soil in adequate to excess amounts, deficiency is caused by their presence in an unavailable form rather than by their lack, and a plant can improve its iron and zinc uptake by using strategies solubilize the iron and zinc present in the soil (Rengel, 2001). For the most part, plants acquire micronutrients by absorbing them from the soil solution; therefore, the availability of micronutrients to plants is closely related to the solubility of the forms in which they appear (Aquaah, 2002). Several environmental factors can affect the solubility of micronutrients. Leached, acid, sandy soils, organic soils, soils that have supported intensive cropping, soils with high pH, and eroded soils all tend to be low in available iron and zinc (Brady, 2002).

Uptake efficiency of soil-grown plants may consist of increased capacity to solubilize non-available nutrient forms into forms that are available to the plant, and/or increased capacity to transport nutrients across the plasma membrane. However, it appears that increased conversion capacity is of greater importance for efficient uptake, especially for nutrients that are transported to roots by diffusion (Rengel, 2001).

Table 1.2. Typical iron and zinc levels in staple crops.

Crop	Iron (mg/100g)	Zinc (mg/100g)	Ascorbic Acid (mg/100g)	ß-carotene (µg/100g)
Wheat, durum *(Triticum durum)*	3.52	4.16	0.0	n/a
Rice, white, glutinous, cooked *(Oriza sativa)*	0.14	0.41	0.0	0.0
Corn, sweet, yellow, raw *(Zea mays)*	0.52	0.45	6.8	52
Potatoes, russet, flesh and skin, raw *(Solanum tuberosum)*	0.86	0.29	19.7	0
Barley, hulled *(Hordeum vulgare)*	3.60	2.77	0.0	13
Beans, white, mature, cooked, boiled, without salt *(Phaseolus vulgaris)*	3.70	1.38	0.0	0
Beans, pinto, mature, cooked, boiled, without salt *(Phaseolus vulgaris)*	2.09	0.98	0.8	0
Table summarized from http://www.nal.usda.gov/fnic/foodcomp/search/				

(table continued)

Crop	Iron (mg/100g)	Zinc (mg/100g)	Ascorbic Acid (mg/100g)	ß-carotene (µg/100g)
Cassava, raw *(Manihot esculenta)*	0.27	0.34	20.6	8
Sweetpotato, raw, unprepared *(Ipomœa batatas)*	0.61	0.30	2.4	8509
Sweetpotato leaves, raw *(Ipomœa batatas)*	1.01	0.29	11.0	n/a
Taro, raw *(Colocasia esculentum)*	0.55	0.23	4.5	35
Taro leaves, raw *(Colocasia esculentum)*	2.25	0.41	52.0	2895
Yam, raw *(Dioscorea* ssp.)	0.54	0.24	17.1	83
Yambean (jicama), raw *(Dioscorea* ssp.)	0.60	0.16	20.2	13
Table summarized from http://www.nal.usda.gov/fnic/foodcomp/search/				

1.2.3 Iron Deficiency in Plants

Iron deficiency in plants is a major problem worldwide because of low iron availability in the aerobic environment and at biological pH, especially in the calcareous soils that cover about one-third of the surface of the earth (Yang and Römheld, 1999; Rengel, 2005).

There were two major strategies by which plants can overcome iron deficiency. For Strategy I plants, dicotyledons and non-graminaceous monocotyledons, iron efficiency is a function of a number of induced responses by plant roots; primarily, an increased rate of reduction reactions (Fe^{3+} to Fe^{2+}) at the root surface, an increased rate of rhizosphere acidification, increased release of phenolic compounds, e.g., caffeic and chlorogenic acid, and the accumulation of citric acid in plant roots (Yang and Römheld, 1999; Hell and Stephan, 2003). Three types of root membrane-bound Fe(III) reductases have been suggested for strategy I plants. There is a standard reductase, which occurs in the plasma membranes of all higher plant species but does not reduce chelated iron compounds, and inducible and constitutive reductases, which can reduce Fe(III) in chelates from various origins. Apparently, inducible reductase takes effect upon the increased activity of constitutive reductase under iron stress conditions (Rengel, 2002). Strategy II plants, which consist of the *graminaceæ*, respond to iron deficiency by the increased release of phytosiderophores (Rengel, 2002). Strategy II plants also possess membrane-bound standard reductases that are capable of reducing electron donor molecules such as ferricyanide, but they do not possess the inducible and constitutive reducatases of Strategy I plants (Yang and Römheld, 1999).

1.2.4 Zinc Deficiency in Plants

It has been estimated that zinc deficiency is the most widespread micronutrient deficiency affecting production and quality of cereals, such as wheat, rice, and other crops. Genotypes of plants vary widely in their tolerance of zinc-deficient soils. Tolerance to zinc deficiency is termed "zinc efficiency," and defined as the ability of a genotype to grow and yield well in soils too deficient in available zinc for a standard cultivar (Yang and Römheld, 1999).

Zinc enters the plant mainly via root absorption of Zn^{2+} from the soil solution. Because of the low zinc concentration in the soil solution, supply of zinc by mass flow is limited and diffusion is the major process by which zinc reaches the roots. Therefore, root morphology and vitality characteristics are crucial in how efficiently the plant explores for zinc in the soil. Less work has been done on understanding the mechanisms of zinc uptake than iron uptake in higher plants; however, zinc uptake appears to be a function of transport across the plasma membrane, which is largely metabolism-dependent and genetically controlled (Yang and Römheld, 1999). For example, zinc-efficient wheat genotypes release more phytosiderophores than do inefficient genotypes (Rengel, 2001). The speculated mechanisms of zinc uptake in the plant include thermodynamic transport of zinc, driven by an electrochemical potential gradient across the membrane; transport through an H^+-ATP-ase ion pump; the involvement of zinc-chelate transport system; and ion channels (Yang and Römheld, 1999). A number of attributes are characteristic of zinc-efficient genotypes, such as more and finer small roots (≤0.2 mm), the release of zinc-chelating phytosiderophores, and the efficient use and compartmentalization of zinc within cells (Rengel, 2001).

1.3 Improvement of Iron and Zinc Concentration in Plants

Staple crops that are micronutrient-enriched, either through traditional breeding or molecular biological techniques, are powerful tools that can help the people who are most vulnerable to micronutrient malnutrition (Welch, 2002). Increasing the amounts of micronutrient metals stored in seeds and grains of staple food crops increases the yield potential of these crops when they are sown in the micronutrient-poor soils so prevalent in the developing world (Welch, 2002). Available research has indicated that micronutrient enrichment traits are available within the genomes of major crops; as a result, improvements in micronutrient concentration can be made without adversely affecting yield. Furthermore, enrichment traits appear to be stable across soil types and climatic environments (Welch and Graham, 2002). Further research is needed to determine if increasing levels of micronutrients in staple foods can significantly improve the nutritional status of people suffering deficiency (Welch and Graham, 2002).

1.3.1 Current Biofortification Efforts

There were a number of programs ongoing focussed on improving micronutrient density in staple crops. Researchers at CIAT have been studying the genetic variability in iron and zinc concentration in common bean *(Phaseolus vulgaris)*. Their data suggest there is sufficient genetic variability to increase iron concentrations by

approximately 80% and zinc concentrations by approximately 50%, and they have found a highly significant positive correlation between iron and zinc concentrations across genotypes (Gregorio, 2002).

A wide range of wheat germplasm is being studied at CIMMYT to determine the range of iron and zinc concentrations in whole grains as well as the effect of environmental conditions on these concentrations. Their data suggest there is enough genetic variability to substantially increase iron and zinc concentrations in wheat grain, and though there was a significant genotype by environment interaction, there was also a high correlation between iron and zinc uptake in the lines studied. This indicates that it should be possible to improve iron and zinc concentration simultaneously in wheat grain. Additional research has shown no negative linkage between grain yield and iron and zinc concentration (Gregorio, 2002).

Researchers at CIMMYT have also evaluated grain concentration of iron and zinc for nearly two thousand maize core germplasm and breeding populations. Iron concentrations varied more than six-fold and zinc concentrations more than four-fold; these differences were attributed to both genetic and environmental factors (Gregorio, 2002).

Researchers at IRRI have been evaluating the genetic variability of iron and zinc concentration in rice grain. Roughly four-fold differences were found in concentrations of both micronutrients, which suggests there is genetic potential to increase the concentration of these micronutrients in rice grain. However, the effects of rice grain processing on iron and zinc levels in the edible product and the bioavailability of the iron and zinc in the rice grains are still being studied (Gregorio, 2002).

1.3.2 Heritability Estimates

In order to determine whether iron and zinc concentration in a particular crop can be improved by traditional breeding methods, it must be known to what extent these traits are heritable. Heritability is a measure of the extent to which observed phenotypic differences for a trait are due to genetic differences (Klug and Cummings, 2005). There are two commonly used measures of heritability, broad-sense (H^2) and narrow-sense (h^2) heritability. Broad-sense heritability measures the proportion of phenotypic difference (V_p) that is due to variation in genetic factors for a single population under the limits of the environment during the experiment. An estimate of broad-sense heritability near 1.0 indicates that environmental conditions have little impact on the phenotypic differences observed in the population; an estimate near 0.0 indicates that the environment is almost solely responsible for the differences (Klug

and Cummings, 2005). Broad-sense heritability is considered to be the sum of additive variance (V_A), dominance variance (V_D) and interactive variance (V_I); thus, $H^2=V_A+V_D+V_I$. Broad-sense heritability estimates are less accurate than narrow-sense ones in estimating the selection potential of quantitative traits since H^2 calculations take into account all forms of genetic variation, not just additive genetic effects. Conversely, narrow-sense heritability excludes dominance and interactive variance, leaving only additive variance; thus, $h^2=V_A=V_A/V_P$. Narrow-sense heritability estimates are useful for predicting the phenotypes of offspring during selection procedures; the closer h^2 is to 1.0, the greater one's ability to make an accurate prediction of the phenotype of the offspring based on the knowledge of parental phenotypes (Klug and Cummings, 2005).

There are two major methods for estimating heritability; one uses correlation and regression among related individuals to estimate heritability and can be calculated by parent/offspring regression, full-sibling and half-sibling comparisons, twin studies, and the use of large, complex pedigrees; the other major class uses analysis of variance (Klug, 2005). The technique used to estimate heritability in this project is analysis of regression among half-sibling families. The data resulting from the assay were analyzed using a technique adapted from one used to estimate heritability in sorghum (Cisse & Ejeta, 2003). Analyses of variance were used to examine differences in iron and zinc concentration by fresh matter, and in dry matter. Broad-sense heritability based on family means was calculated using the formula: H^2=[MS(among families) – MS(year*family)]/MS(among families).

1.3.3 About Sweetpotatoes

Sweetpotato (*Ipomœa batatas* L.[Lam.]) is a dicotyledonous root crop and a member of the family *Convulvulaceæ* (Woolfe, 1992). Its exact origin is unknown but the available evidence suggests southern Mexico as a likely place of origin (Gichuki, *et al.,* 2003). By weight, sweetpotato is the seventh most important food crop worldwide, after wheat, rice, maize, potato, barley and cassava (Woolfe, 1992). It is the only member of *Ipomœa* of major economic importance (Woolfe, 1992). China accounts for 84% of the world's sweetpotato production. The United States is one the few developed countries that produce sweet potatoes (Rubatzky and Yamaguchi, 1997 and La Bonte and Cannon, 1998). The primary importance of sweetpotato is in poor regions of the world. It is the fourth most important food crop in developing tropical countries and is grown in most of the tropical and subtropical regions of the earth, where the vine, as well as the roots, is consumed by humans and livestock (Woolfe, 1992). While yields in the United States were about 12-13 MT/hectare, in tropical countries yields can be about 35-40 MT/hectare (Woolfe, 1992).

Sweetpotato is a highly heterozygous natural hexaploid; the germplasm of the crop includes more diversity than cassava *(Manihot esculenta)*, yam *(Dioscorea* spp.), cocoyam *(Colocasia* and *Xanthosoma* spp.), or even potato *(Solanum tuberosum)* (Woolfe, 1992). The sweetpotato genome is made up of ninety chromosomes and sweetpotato is the only know hexaploid morning glory. Most wild species are diploid; occasionally triploid or tetraploid examples are found in collections (Jones, *et al.,* 1986). Interspecific crosses are difficult to make in sweetpotato; however, since it is a hexaploid organism and is so genetically diverse, there is extensive variability within the species available for exploitation by plant breeders. Genotypes differ in root flesh color, root skin color, in the size and shape of roots and leaves, the depth of rooting, the time to maturity, disease resistance, and in the texture of the flesh (Woolfe, 1992). It is not known, however, to what extent iron and zinc levels vary among genotypes.

The complicated nature of sweetpotato genetics makes them difficult for breeders to manipulate. Almost all traits are quantitatively inherited, and mass selection is used to rapidly aggregate desirable alleles (Jones, *et al.,* 1986). Sweetpotato is propagated asexually so any advances made in breeding can be passed on to the producer and consumer without the need for achieving homozygosity.

There are a number of reasons sweetpotatoes biofortified with iron and zinc could be a powerful tool in the fight against iron and zinc malnutrition. It is an important staple crop in areas in which iron and zinc deficiencies are a particular problem. It is low in inhibitors (e.g., phytates) and high in promoters (e.g., ascorbic acid), so even a small increase in iron and zinc concentration will pay dividends in the health of the consumers. Sweetpotato makes a large yield per area per unit of time, and is capable of yielding even in marginal conditions. This makes it an ideal sustainable crop for production in developing countries, where population growth has decreased the amount of arable land per person and increased the use of marginal land for food production (Woolfe, 1992). While yields of sweetpotato were still low in many countries, it has been shown that there is tremendous potential for increasing yield by the introduction of improved clones and more efficient cultivating practices. Finally, sweetpotato produces two useful foods from the same plant; both the roots and tips are used as a nutritious food for human and animal consumption (Woolfe, 1992).

Sweetpotato is also a dependable subsistence crop (Woolfe, 1992). It does not require high levels of input, and can grow and produce under relatively dry conditions, making irrigation less necessary. Also, sweetpotato does not "mature" as such and will continue growing as long as the environment allows, so a farmer is able to use all of an

unusually long growing season, or produce a partial crop even in a season too short for other crops to mature in. This characteristic also makes it possible to produce two crops per year in some areas (Woolfe, 1992).

1.3.4 Objectives

There are three main objectives to this research. In the first part, protocols for the sampling of iron and zinc concentration in sweetpotato will be developed, and estimates for variability from root to root of the same plant, from plant to plant of the same plot, and from proximal to distal end and from cortex to cambium of the same root, will be made. In the second part, estimates of the ranges of iron and zinc concentrations of a number of genotypes of sweetpotato, representing the world's germplasm collection, will be made. In the third part, broad-sense heritability will be estimated.

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CHAPTER 2: PROTOCOL DEVELOPMENT FOR IRON AND ZINC IN SWEETPOTATO

2.1 Introduction

Malnutrition is a large and growing problem in the developing world. Over three billion people currently suffer micronutrient malnutrition (Welch and Graham, 2004). Iron deficiency may affect three billion people worldwide (Long *et al.,* 2004). It is estimated that 49% of the world's population is at risk for low zinc intake (Cichy *et al.,* 2005) while vitamin A deficiency affects over 140 million children under five (*Biofortified Sweetpotato,* 2006). These micronutrient deficiencies were concentrated in the semi-arid tropics, particularly in South and Southeast Asia and sub-Saharan Africa (Reddy *et al.,* 2005). Attempts have been made to alleviate these deficiencies by the use of supplements and food fortification, but these strategies do not reach all those suffering from deficiency and have not proven to be sustainable (Römheld, 1998).

There were a number of reasons sweetpotatoes biofortified with iron and zinc could be a powerful tool in the fight against iron and zinc malnutrition. It is an important staple crop in areas in which iron and zinc deficiencies were a particular problem. It is low in inhibitors (e.g., phytates) and high in promoters (e.g., ascorbic acid), so even a small increase in iron and zinc concentration will pay dividends in the health of the consumers. Sweetpotato makes a large yield per area per unit of time, and is capable of yielding even in marginal conditions. This makes it an ideal sustainable crop for production in developing countries, where population growth has decreased the amount of arable land per person and increased the use of marginal land for food production (Woolfe, 1992). While yields of sweetpotato were still low in many countries, it has been shown that there is tremendous potential for increasing yield by the introduction of improved clones and more efficient cultivating practices. Finally, sweetpotato produces two useful foods from the same plant; both the roots and tips were used as a nutritious food for human and animal consumption (Woolfe, 1992).

Preliminary steps to biofortification of sweetpotatoes include estimating the heritability and genotypic variability of iron and zinc concentration. However, there were no published protocols for the analysis of these variables in this crop. It is neither known how to properly process the materials to avoid contamination, nor how much variation exists within roots or from root to root within a genotype. This study investigates proper sampling conditions for roots and tips, and attempts to discover proper protocols for such sampling. Variation in iron and zinc concentration from root to root of the same plant, from plant to plant in the same plot, from proximal to distal end

of the same root, and cambium to cortex of the same root were investigated in an effort to discover proper sampling procedures for roots. Variation in iron and zinc concentration in roots and tips that were processed by different methods were investigated in order to discover proper sampling conditions.

2.2 Materials and Methods

Field research was conducted at the Sweetpotato Research Center at Macon Ridge, Louisiana in 2004. The soil was a Gilbert silt loam (fine-silty, mixed, thermic, Typic Glossaqualf) with a pH of approximately 5.4. Typical soil analysis characteristics were presented in Appendix B. Plants of orange-fleshed 'Beauregard' and white-fleshed 'L01-29' were replicated four times in a randomized block design. There were seven plants per plot, spaced 0.3 m between plants and 1.5 m between plots; the five middle plants of each plot were considered for analysis. Breeding line 'L99-35' was added in 2005. Mostly, U.S. #1 grade (5.1-8.9 cm diameter and 7.6-22.9 cm long) were used and were harvested for analysis from the five middle plants. Roots were harvested for the varied studies September 21, 2004 and September 30, 2005, 120 and 115 days after planting, respectively.

Processing of tissue samples for zinc and iron analysis was based on the methods of Norbotten *et al.,* (2000). Harvested roots were washed in tap water and allowed to air-dry before weighing. They were then rinsed in double-distilled water, peeled with a stainless steel knife, and rinsed in double distilled water again. The roots were then sectioned, weighed, and dried at 80°C for 48 hours, after which they were weighed again. Dry matter was based on the differential between the results of the two weighings, then the dry samples were pulverized using an IKA A10 Basic Analytical Mill (IKA Works, Inc, Wilmington, NC), bottled in Corning Snap-Seal tubes (product no. 1730, Corning, New York); and stored at 60°C until assayed for iron and zinc concentration.

Analysis for various minerals was based on the methods of Huang and Schulte (1980); and Havlin and Soltanpour (1980). In short, 1 g samples were digested in 5 ml of nitric acid added. The samples were placed on a Magnum 120 Plant/Soil Digester (Ivesdale, Il). After 45 minutes, a 3 ml aliquot of H_2O_2 was added to each sample, prior to the block reaching 90°C. The samples were heated until the volume was reduced to 0.5 ml, then diluted to 12.5 ml and filtered using Whatman #2 paper. The samples were then quantified for the various minerals via inductively coupled plasma mass spectrometry using a Spectro Ciros CCD (Kleve, Germany). For every twenty samples a National Institute for Standards and Technology (Gaithersburg, MD) 1547 peach sample was used for repeatability measurements.

In all cases, samples that showed aluminum levels above 3 ppm dwb, were considered to be contaminated and were discarded. A generic threshold of >5-6 ppm dwb (dry weight basis) was suggested by Pfeiffer and McClafferty (in press), but recent perspectives suggest >3 ppm to be more appropriate (Wolfgang Pfeiffer, 2006 personal communication.) Additional research is needed in the area of contamination thresholds.

2.2.1 Variation from Proximal to Distal End of the Same Root

Four roots were randomly selected from each of the varieties, harvested, processed as described above, and divided into five sections longitudinally from distal to proximal ends. Each section was processed and assayed separately. The dry weight calculations and the results of the assay were analyzed using a nested model in a generalized linear method (PROC GLM) (SAS 9.1, SAS Institute Inc, Cary, N.C.) to test variation in iron and zinc concentration based on fresh matter, and in dry matter concentration, from genotype to genotype, from root to root within each genotype, and from zone to zone within each root. Sample code for this experiment and the three following were presented in Appendix A.

2.2.2 Variation from Cambium to Cortex of the Same Root

Four roots were randomly harvested from each genotype, processed as described above, and divided into cortex and cambium. Each section was processed and assayed separately. The dry weight calculations and the results of the assay were analyzed using a nested model in PROC GLM (SAS 9.1, SAS Institute Inc, Cary, N.C.) to test variation in iron and zinc concentration based on fresh matter, and in dry matter concentration, from genotype to genotype, from root to root within each genotype, and from zone to zone within each root.

2.2.3 Variation from Root to Root of the Same Plant

Three roots were harvested from one plant among the five middle plants, in each replication of each genotype. The roots were processed as described above. The dry weight calculations and the results of the assay were analyzed using a nested model in PROC GLM (SAS 9.1, SAS Institute Inc, Cary, N.C.) to test variation in iron and zinc concentration based on fresh matter, and in dry matter concentration, from genotype to genotype, from plant to plant within each genotype, and from root to root in each plant.

2.2.4 Variation from Plant to Plant in the Same Plot

One root was harvested from each of the five middle plants in each replication of each genotype. The roots were processed as described above. The dry weight calculations and the results of the assay were analyzed using a

nested model in PROC GLM (SAS 9.1, SAS Institute Inc, Cary, N.C.) to test variation in iron and zinc concentration based on fresh matter, and in dry matter concentration, from genotype to genotype, from plot to plot within each genotype, and from plant to plant in each plot.

2.2.5 Root Contamination

Nine roots of the cultivar 'Beauregard' were used for this study. Three were processed as described above; three were processed exactly the same but tap water was substituted for purified water at every step; three were processed with no washing at all and from each of these samples one sample without peel and one sample with peel was taken. Otherwise these samples were processed as described above, and the results of the assay used to test for variation in aluminum, iron, and zinc concentration. The test was performed using an ANOVA model in PROC GLM (SAS 9.1, SAS Institute Inc, Cary, N.C.) Sample code is presented in Appendix A.

2.2.6 Tip Contamination

Field research was conducted at the Burden Research Center at Baton Rouge, Louisiana in 2005, using the genotypic variability plot. One plant from each of the four repetitions of the varieties 'Beauregard', 'Kyukei No. 63', 'Tanzania', 'Wagabolige', and 'Xushu 18' was staked to allow one shoot to grow upward to keep it free of soil splatter during rains. Tips were harvested from each staked plant, both from the shoot growing up the stake and from a shoot trailing along the ground. The ends of the shoot tips including the first three to four fully opened leaves were harvested. The samples were immediately placed into labeled, sealed plastic bags to prevent dehydration, and returned to the laboratory for processing.

Immediately upon returning to the laboratory, the tips were immediately weighed, washed in tap water and again in double-distilled water. They were then dried at 80°C for 48 hours, after which they were weighed again. Dry matter was based on the differential. Finally, the dry samples were pulverized in an IKA A10 Basic Analytical Mill (IKA Works, Inc, Wilmington, NC), bottled in Corning Snap-Seal tubes (product no. 1730); and stored in a dryer at 60°C until assayed for aluminum, iron and zinc concentration (fresh weight basis) using the method described above. The results of the assay were used to test for variation based on whether the plant was grown upright or prostrate, using an ANOVA model in PROC GLM (SAS 9.1, SAS Institute Inc, Cary, N.C.) Sample code is given in Appendix A.

2.3 Results and Discussion

2.3.1 Variation from Cambium to Cortex of the Same Root

Results for 2004 and 2005 were presented separately because year effects were significant for iron and dry matter concentration. For iron (Table 2.1), genotype was a significant source of variation in 2005, accounting for 35% of the variation in the model for that year, but was not significant in 2004. No other model term was significant in either year and none accounted for over 25% of the variation. Means by zone (Table 2.2) for the two varieties for 2004 were: 'Beauregard: 4.75 ppm and 3.73 ppm for cambium and cortex, respectively; and 'L01-29': 4.58 ppm and 5.70 ppm for cambium and cortex, respectively. For 2005, the pairs of means were 3.47 ppm and 3.36 ppm for 'Beauregard', 3.45 and 4.80 ppm for 'L99-35', and 4.41ppm and 4.90 ppm for 'L01-29" for cambium and cortex, respectively. The only pair that differed significantly in either year was the pair of means for cambium and cortex for 'L99-35' in 2005.

For zinc, as with iron, genotype provided the bulk of the variation in the model. Genotype was significant in both years and provided 68% and 48% of the variation in 2004 and 2005, respectively. No other term was significant in either year except root to root within genotype, which was significant in 2004 but only accounted for 6% of the variation. Zinc means for cambium and cortex tissue for 2004 were, respectively, 1.58 and 1.64 ppm for 'Beauregard' and 2.88 and 2.83 ppm for 'L01-29'; for 2005, the pairs of means were: 2.64 and 2.19 ppm for 'Beauregard', 2.82 and 2.91 ppm for 'L99-35', and 3.67 and 3.12 ppm for 'L01-29' for cambium and cortex, respectively. None of these pairs of means differed significantly.

For dry matter, genotype was a significant source of variation in both years, accounting for 73% of the variation in 2004 and 57% in 2005. No other effects were significant in either year, and no other term in either year accounted for more than 9% of the variation in dry matter. For 2004, means by zone for 'Beauregard' were 22.33% and 21.33% for cambium and cortex, respectively, and for 'L01-29', 30.25% and 34.25%, for cambium and cortex, respectively. In 2005, the pairs of means were: 21.50% and 21.25% for 'Beauregard', 23.75% and 26.00% for 'L99-35', and 27.25% and 31.33% for 'L01-29' for cortex and cambium, respectively. The pairs of means for 'L01-29' differed significantly in both years but no other pair of means differed significantly for dry matter in either year.

Table 2.1. Relative contributions and significance of dry matter, iron, and zinc among zones (cambium to cortex), roots, and genotypes in sweetpotato roots..

Variable	Year		Sources of Variation			
			genotype	root(genotype)	zone(root)	remainder
Iron (ppm)[z]	2004	Proportion	8%	16%	24%	51%
		p-value	0.3583	0.4337	0.5795	
	2005	Proportion	35%	25%	9%	31%
		p-value	**0.0275**	0.2764	0.5057	
Zinc (ppm)[z]	2004	Proportion	68%	6%	1%	26%
		p-value	**0.0006**	**0.0482**	0.5501	
	2005	Proportion	48%	22%	6%	25%
		p-value	**0.0081**	0.2503	0.6024	
Dry Matter	2004	Proportion	73%	1%	6%	20%
		p-value	**0.0061**	0.7000	0.5405	
	2005	Proportion	57%	9%	5%	29%
		p-value	**0.0019**	0.4446	0.5408	

[z]: Iron and zinc concentrations were corrected for dry matter.

Table 2.2. Cortex and cambium means, standard deviations, and statistical groupings by genotype for iron, zinc, and dry matter in sweetpotato roots.

Year	Genotype	Zone	Iron (ppm)[z]			Zinc (ppm)[z]			Dry Matter		
			Mean	SD	Group	Mean	SD	Group	Mean	SD	Group
2004	Beauregard	Cambium	4.75	1.57	A	1.58	0.25	A	22.33%	2.08%	A
		Cortex	3.73	0.26	A	1.64	0.31	A	21.33%	1.52%	A
	L01-29	Cambium	4.58	0.22	A	2.88	0.18	A	30.25%	0.50%	**A**
		Cortex	5.70	0.90	A	2.83	0.33	A	34.25%	1.50%	**B**
2005	Beauregard	Cambium	3.47	0.48	A	2.64	0.46	A	21.50%	1.29%	A
		Cortex	3.36	0.36	A	2.19	0.12	A	21.25%	1.26%	A
	L99-35	Cambium	3.45	0.40	**A**	2.82	0.28	A	23.75%	1.50%	A
		Cortex	4.80	0.97	**B**	2.91	0.52	A	26.00%	4.00%	A
	L01-29	Cambium	4.41	0.96	A	3.67	0.83	A	27.25%	1.89%	**A**
		Cortex	4.90	1.05	A	3.12	0.54	A	31.33%	1.15%	**B**

[z]: Iron and zinc concentrations were corrected for dry matter.

2.3.2 Variation from Proximal to Distal End of the Same Root

Results for 2004 and 2005 were presented separately due to significant year effects in the model for iron, zinc, and dry matter. For iron (Table 2.3), genotype was a significant source of variation in 2004 and 2005 and accounted for 43% and 48% of the variation, respectively, for the two years. Root within genotype was significant in 2005 but accounted for only 11% of the variation in the model, and was not significant in 2004. Zone within root was not significant in either year, though it did account for 31% of the variation in 2004 and 18% in 2005. For iron in 2004, 'Beauregard' (Table 2.4) showed a significantly higher iron concentration in the distal end (8.01 ppm) than in the rest of the root (4.34 ppm, at the proximal end). In 'L 01-29', iron was significantly higher at the proximal end (6.16 ppm) than at the distal end (4.39 ppm), though neither end was significantly different from any of the three middle zones.

For zinc, both genotype and root within genotype were significant in both years; genotype accounted for most of the variation: 49% (2004) and 37% (2005) while root to root variation within a genotype represented 12 % (2004) and 23 % (2005) of the variation in the model. Zone within root was not significant in either year; variation in zinc concentration from the proximal to the distal ends was minimal (Table 4).

For dry matter, all terms of the model were significant sources of variation in both years. As expected, genotype provided the bulk of the variation: 82% in 2004 and 75% in 2005. Zone within root accounted for 7% of the variation at most. As might be expected, zonal mean dry matter was usually highest for the fibrous end zones of the roots and lower in the middle sections; distal ends tended to be highest. In 2004, 'Beauregard' ranged from 27.67% dry matter in the medial zone to 32.67% in the distal zone; 'L01-29' ranged from 40.33% dry matter in the medio-proximal zone to 46.00% in the distal zone. In 2005, dry matter in 'Beauregard' ranged from 21.50% in the medio-proximal zone to 23.75% in the distal zone, in 'L99-35' it ranged from 24.00% in the medio-proximal zone to 25.33% in the distal zone, and in 'L01-29' it ranged from 29.25% in the medial zone to 32.25% in the distal zone.

In this experiment, the statistical analysis indicated no significant difference from zone to zone within a root for either iron or zinc; however, the fact that a few zonal means differ from others within certain genotypes suggests that if the entire root were available, it would be expedient to use a radial sample that proportionately represents the entire root from end to end. The incongruence between the ANOVA-based partitioning of variation and means comparison is due to two different statistical analyses. Dry matter was the one variable among the three that predictably differed significantly between the zones and likely impacted our estimates of micronutrients. Chapter 3 presents evidence that the higher the dry matter, the higher the iron and zinc concentration. In order to properly estimate dry matter concentration and micronutrients, it is necessary to obtain a proportionately representative sample of every root using a radial section. Still the overriding source of variation was the genotype and to a lesser extent the root and zone within a root.

Table 2.3. Relative contributions and significance of dry matter, iron, and zinc among zones (end-to-end), roots, and genotypes in sweetpotato roots.

Variable	Year		Source of Variation			
			genotype	root(genotype)	zone(root)	remainder
Iron (ppm)[z]	2004	Proportion	43%	1%	31%	24%
		p-value	**0.0004**	0.7917	0.2712	
	2005	Proportion	48%	11%	18%	23%
		p-value	**<0.0001**	**0.0080**	0.0630	

[z]: Iron and zinc concentrations were corrected for dry matter.

(table continued)

Variable	Year		Source of Variation			
			genotype	root(genotype)	zone(root)	remainder
Zinc (ppm)[z]	2004	Proportion	49%	12%	19%	20%
		p-value	**<0.0001**	**0.0288**	0.3513	
	2005	Proportion	37%	23%	8%	32%
		p-value	**<0.0001**	**<0.0001**	0.2284	
Dry Matter	2004	Proportion	82%	2%	7%	9%
		p-value	**<0.0001**	**0.0271**	**0.0438**	
	2005	Proportion	75%	15%	6%	4%
		p-value	**<0.0001**	**<0.0001**	**0.0010**	

[z]: Iron and zinc concentrations were corrected for dry matter.

Table 2.4. Means, standard deviations, and statistical groupings by genotype for five longitudinal zones for iron, zinc, and dry matter in sweetpotato roots.

Year	Genotype	Zone	Iron (ppm)[z]			Zinc (ppm)[z]			Dry Matter		
			Mean	SD	Group	Mean	SD	Group	Mean	SD	Group
2004	Beauregard	Proximal	4.34	0.35	**B**	2.21	0.30	A	31.67%	2.12%	A
		Medio-proximal	3.84	0.35	**B**	2.20	0.30	A	28.33%	2.12%	A
		Medial	4.02	0.35	**B**	2.05	0.30	A	27.67%	2.12%	A
		Medio-distal	5.38	0.35	**B**	2.64	0.30	A	29.67%	2.12%	A
		Distal	8.01	0.35	**A**	3.44	0.30	A	32.67%	2.12%	A
	LA01-29	Proximal	8.44	0.91	A	3.76	0.38	A	44.33%	1.32%	A
		Medio-proximal	7.05	0.91	A	3.67	0.38	A	40.33%	1.32%	A
		Medial	7.48	0.91	A	3.50	0.38	A	42.67%	1.32%	A
		Medio-distal	7.23	0.91	A	3.70	0.38	A	43.00%	1.32%	A
		Distal	8.96	0.91	A	3.90	0.38	A	46.00%	1.32%	A
2005	Beauregard	Proximal	4.51	0.35	A	2.64	0.26	A	21.75%	1.24%	A
		Medio-proximal	3.86	0.35	A	2.32	0.26	A	21.50%	1.24%	A
		Medial	3.73	0.35	A	2.47	0.26	A	22.00%	1.24%	A
		Medio-distal	3.51	0.35	A	2.41	0.26	A	22.50%	1.24%	A
		Distal	3.71	0.35	A	2.71	0.26	A	23.75%	1.24%	A
	LA99-35	Proximal	6.57	0.68	A	3.73	0.41	A	24.33%	1.16%	A
		Medio-proximal	6.09	0.68	A	3.22	0.41	A	24.00%	1.16%	A
		Medial	5.27	0.68	A	3.03	0.41	A	24.33%	1.16%	A
		Medio-distal	5.60	0.68	A	3.23	0.41	A	24.67%	1.16%	A
		Distal	6.11	0.68	A	3.25	0.41	A	25.33%	1.16%	A
	LA01-29	Proximal	6.16	0.38	**A**	3.74	0.31	A	29.75%	0.90%	A
		Medio-proximal	5.25	0.38	**AB**	3.28	0.31	A	30.00%	0.90%	A
		Medial	5.25	0.38	**AB**	3.23	0.31	A	29.25%	0.90%	A
		Medio-distal	4.50	0.38	**AB**	3.09	0.31	A	29.75%	0.90%	A
		Distal	4.39	0.38	**B**	3.05	0.31	A	31.25%	0.90%	A

[z]: Iron and zinc concentrations were corrected for dry matter.

2.3.3 Variation among Roots from the Same Plant

Results for 2004 and 2005 were presented separately because of significant year effects in the model for dry matter and iron. For iron (Table 2.5) genotype was significant in both years and, as in the previous experiments, provided

for most of the variation, 50% in 2005 and 68% in 2004. Plant to plant within genotype was significant in 2005 and accounted for 24% of the variation, but was not significant in 2004. Root to root variation from a given plant was not significant in 2004 or 2005.

For zinc, as with iron, genotype was significant in both years; it accounted for 66% of the variation in 2004 but a relatively low 39% in 2005. Plant to plant within genotype variation was not significant in either year and at most it accounted for 15% of the variation. Root to root within plant variation followed a similar pattern; it was not a significant source of variation in either year and at most it accounted for 14% of the variation.

For dry matter, genotype and plant to plant within genotype were both significant in both years; however, genotype accounted for the vast bulk of the variation with 87% in both 2004 and 2005. While plant to plant within genotype was significant in both years, it accounted for 7% of the variation at most. Root to root within plant was significant in 2005, with 5% of the variation, but was not significant in 2004. The ease and accuracy of determining dry matter concentration permits discrimination between samples even when only minor differences exist.

Taken together, these results indicate that the genotypes studied can reliably be differentiated by dry matter, iron, and zinc; that is, different genotypes differ significantly for all of these traits. This has little bearing on the present study of variation among roots from the same plant, but is encouraging in the larger sense that if genotypes reliably differ in iron and zinc levels, genetic variation does exist for these traits, thus allowing for breeding for higher levels of these nutrients. Differentiation among plants from the same genotype was less reliable. For all three variables, there is enough difference from plant to plant in at least one year of the study to require that more than one plant per genotype be sampled. This was expected. For variation among roots from the same plant, differentiation based on these variables was even less reliable; dry matter was significant in one year but neither iron nor zinc was significant in either. This indicates that for purposes of estimating iron and zinc, only one root per plant need be sampled in order to get a reliable estimate, though the relatively low p-values for both variables in one year of the study were bothersome. When sampling for dry matter, the significant result in 2005 indicates that more than one root per plant would need to be sampled in order to get a proper estimate.

Table 2.5. Relative contributions and significance of dry matter, iron, and zinc among roots, plants, and genotypes in sweetpotato roots.

Variable	Year		Source of Variation			
			genotype	plant(genotype)	root(plant)	remainder
Iron (ppm)[z]	2004	Proportion	68%	2%	14%	16%
		p-value	**0.0048**	0.7469	0.5983	
	2005	Proportion	50%	24%	11%	15%
		p-value	**<0.0001**	**0.0049**	0.1605	
Zinc (ppm)[z]	2004	Proportion	66%	5%	13%	16%
		p-value	**<0.0001**	0.0691	0.0594	
	2005	Proportion	39%	15%	14%	33%
		p-value	**0.0005**	0.2030	0.3899	
Dry Matter	2004	Proportion	87%	2%	1%	10%
		p-value	**<0.0001**	**0.0123**	0.5001	
	2005	Proportion	87%	7%	5%	2%
		p-value	**<0.0001**	**0.0025**	**0.0297**	

[z]: Iron and zinc concentrations were corrected for dry matter.

2.3.4 Variation among Plants within a Plot

Results for 2004 and 2005 were presented separately due to significant year effects in the models for iron, zinc, and dry matter. Iron in fresh matter varied significantly both by genotype and by plant to plant within genotype, though, again, genotype provided most of the variation: 66% in 2004 and 50% in 2005 (Table 2.6). Plot to plot within genotype, while still significant, accounted for modest amounts of variation, ranging from 6-14%. Plant to plant within plot was not a significant source of variation in iron concentration in either year.

Zinc in fresh matter varied significantly by genotype in both years. It accounted for 70% of the variation in 2004 but only 26% in 2005. Of note is the inclusion of an additional line in 2005 which may have affected partitioning. Neither plot to plot within genotype nor plant to plant within plot varied significantly in either year.

Genotype was the only significant source of variation in dry matter concentration in either year (Table 6) and accounted for the vast bulk of the variation in the model, 91% in 2004 and 81% in 2005. Neither plot to plot within genotype nor plant to plant within plot were significant sources of variation in either year.

These results, like those of the experiment to investigate variation among roots from the same plant, indicated that the genotypes studied can reliably be differentiated by dry matter, iron, and zinc. The results also showed that different plots (repetitions) of the same genotype can be differentiated by iron concentration but not by dry matter or zinc; that is, more than one plot per genotype would need to be studied in order to estimate iron, but not the other

two characteristics. The lack of any significant variation among plants from the same plot for any of these variables suggests that only one plant per plot need be sampled when investigating dry matter, iron, or zinc concentration.

Table 2.3. Relative contributions and significance of dry matter, iron, and zinc among plants, plots, and genotypes in sweetpotato roots.

Variable	Year		Source of Variation			
			genotype	plot(genotype)	plant(plot)	remainder
Iron (ppm)[z]	2004	Proportion	66%	6%	14%	14%
		p-value	**<0.0001**	**0.0490**	0.2075	
	2005	Proportion	50%	14%	10%	26%
		p-value	**<0.0001**	**0.0099**	0.5366	
Zinc (ppm)[z]	2004	Proportion	70%	3%	12%	15%
		p-value	**<0.0001**	0.3663	0.6730	
	2005	Proportion	26%	15%	15%	44%
		p-value	**0.0008**	0.1273	0.7786	
Dry Matter	2004	Proportion	91%	0%	2%	7%
		p-value	<.0001	.5566	.8287	
	2005	Proportion	81%	2%	6%	12%
		p-value	<.0001	.5374	.3295	

[z]: Iron and zinc concentrations were corrected for dry matter.

Collectively, these results showed that genotypic variation was the primary source of variation for iron, zinc, and dry matter. It is important to note that partitioning of the variation may vary greatly depending on the genotypes selected from which to make the comparisons.

In 2005, these three genotypes had iron concentrations of 3.5, 4.6, and 4.7 ppm for 'Beauregard', 'L99-35' and 'L01-29', respectively. Zinc levels for the three genotypes were 2.5, 3.0, and 3.3 ppm respectively, and dry matter levels were 22%, 23%, and 30% respectively. Genotypes with greater differences in micronutrient and dry matter concentration would presumably skew our results even further toward genotype as the primary source of variation.

Differentiation by variation among plants from the same genotype is less reliable. For all three variables, there is enough difference from plant to plant in at least one year of the study to require that more than one plant per genotype be sampled. This was expected. Variation in soil pH and fertility may account for such differences. Root to root variability from a given plant is minimal and suggests that one root from a given plant is sufficient for testing purposes for iron and zinc concentration. While dry matter concentration does vary significantly among roots from the same plant, the minimal contribution of this factor to the variation in the overall model suggests this is a minor concern.

2.3.5 Root Contamination

Our objective was to develop baseline information on the changes in aluminum, iron, and zinc concentration in sweetpotato roots that were processed differently. A control was processed using our standard protocol. One treatment varied only in that tap water was used. The two other treatments were designed to introduce significant amounts of soil contamination into the samples. In one treatment the roots were peeled but not washed, while in the other, the roots were neither washed nor peeled.

Our ability to compare data from the distilled water control was hampered because one of the three samples had significant levels of aluminum contamination (about 40 ppm dry weight basis, 7 ppm fresh weight basis). Data in Table 2.7 denotes the mean aluminum, iron, and zinc concentration for the three replicates. In parentheses, mean and standard deviation were presented for the two samples with minimum aluminum contamination. The data showed that regardless of whether or not we included the contaminated sample, there was little difference in mean iron concentration for the distilled water control treatment. Tap water does contain measurable iron which can bias samples, but in this study it was not a major contributing contaminant. Zinc levels were likewise similar. Roots that were peeled but not washed had mean aluminum concentration of more than 9 ppm fwb (about 45 ppm dwb) and iron levels approached a three-fold increase in comparison with the control. Aluminum levels were concomitantly high. Zinc levels were essentially unaffected.

This study presents results based on a minimal number of replicates and is considered preliminary in nature. It does present evidence that aluminum in excess of 9 ppm fwb (about 45 ppm dwb) is an indication of soil contamination which may affect iron levels.

Table 2.7. Variation in aluminum, iron, and zinc concentration among sweetpotato roots processed with differing levels of care to prevent contamination with soil.

Treatment	Variable and p-value					
	Aluminum (ppm)[z]: **p<0.0001**		Iron (ppm)[z]: **p<0.0001**		Zinc (ppm)[z]: p=0.7578	
	Mean	SD	Mean	SD	Mean	SD
purified water	2.84 *(0.95)*	3.29	5.52 *(5.26)*	0.51	2.70 *(2.41)*	0.64
tap water	0.49	0.13	5.34	1.28	2.28	0.44
peeled but not washed	9.34	6.08	14.21	6.65	2.48	0.39
not peeled or washed	34.12	4.49	37.96	2.94	2.54	0.34
[z]: Aluminum, iron, and zinc concentrations were corrected for dry matter.						

2.3.6 Tips Contamination

Results for the two harvest periods were presented in Table 2.8. There was a significant difference between samples taken during the two harvest periods, so the results were presented separately. Leaf iron concentration did

not vary significantly between treatments for either leaf harvest date. This lends support to the idea that aluminum concentrations ranging from 0.84 to 1.57 ppm (about 6.7 to 12.6 ppm, dwb) do not impact constituent iron concentration enough to alter significance levels. It would be interesting to estimate aluminum threshold levels that would significantly affect iron concentration and to document iron levels in samples with ~ 0 ppm of aluminum, but the exploratory nature of this study did not allow for answering this question. Leaf samples of varying degrees of contamination would aid in defining threshold levels.

Zinc concentration did vary significantly between treatments in the first harvest. Average zinc concentration was 3.69 ppm for staked plants, versus 3.24 ppm for unstaked plants, a ten per cent difference. It is worth noting that the staked plants had the higher levels of zinc in this study. Zinc concentration did not vary between treatments in the samples from the second harvest.

Staking did have a significant effect on dry matter concentration in samples from both harvest dates, and varied by about 2% in both cases. This was likely a result of staked leaves receiving direct sunlight, and unstaked leaves being nestled under the canopy and therefore being somewhat etiolated. Staking did not have an observable effect on aluminum concentration in leaves from the first harvest batch but did on those from the second.

Our intent was to show that leaves grown near the ground would have higher levels of aluminum and iron content because of their proximity to the ground and because they are subject to having soil splashed onto them during rains. Our study showed significant differences in aluminum content for staked (1.05ppm fwb and 7.50 ppm dwb) and unstaked (0.68 ppm (fwb) and 5.67 ppm, dwb) vines in 2005; however, contrary to expectation our study showed the higher aluminum content on the staked plants. In 2004, there was as wide a divergence between mean aluminum estimates, but because of a large standard deviation in the aluminum mean for the unstaked plants, there was no significant difference. We regard these results as inconclusive. Further research is warranted in determining iron concentration in leaves and contamination thresholds.

Table 2.8. Variation in dry matter and aluminum, iron, and zinc concentration between staked and unstaked sweet-potato tips for two harvest periods on a fresh weight basis.

Harvest 1				
	Dry Matter: **p=0.0031**		Aluminum (ppm)[z]: p=0.1906	
	Mean	SD	Mean	SD
Staked	13%	2%	0.84	0.44
Prostrate	11%	2%	1.57	1.88

(table continued)

	Iron (ppm)[z]: p=0.9241		Zinc (ppm)[z]: **p=0.0124**	
	Mean	SD	Mean	SD
Staked	5.07	0.02	3.69	0.66
Prostrate	4.88	2.20	3.24	0.69
Harvest 2				
	Dry Matter p=**0.0236**		Aluminum (ppm)[z]: **p=0.0370**	
	Mean	SD	Mean	SD
Staked	14%	4%	1.05	0.37
Prostrate	12%	3%	0.68	0.23
	Iron (ppm)[z]: p=0.5363		Zinc (ppm)[z]: p=0.8313	
	Mean	SD	Mean	SD
Staked	6.51	2.10	2.68	0.88
Prostrate	6.01	1.29	2.77	0.79
[z]: Aluminum, iron, and zinc concentrations were corrected for dry matter.				

The results of this study indicate that for purposes of estimating iron and zinc, there was no significant variation among roots from the same plant nor among plants from the same plot; therefore, one plant per plot and one root per plant could be used. However, this assumes no observations were lost to contamination. Using multiple roots per plant and/or multiple plants per plot ensures against the loss of an entire genotype for a repetition in the case of a single observation having to be discarded because of contamination.

Results showed that proportion of dry matter varied among roots from the same plant; however, most variability was due to the genotype and minimizes the practical impact of root to root variability on a plant. There was no evidence to indicate iron or zinc varies significantly between cortex and cambium or from end to end of the same root; however, while dry matter does not vary significantly from cortex to cambium, there was evidence that it varies from the proximal to distal end of a root. If only iron and zinc concentration were being estimated, it was not necessary to use any particular part of the root; however, if dry matter is being estimated as well, care should be taken to use a root sample that is as representative of the tissue from end to end as possible.

There was no preliminary evidence to suggest that tap water contamination of root tissue leads to elevated levels of iron contamination; however, iron contamination should be minimized at all opportunities. Our data simply suggests an errant tap water droplet will do minimal harm. There was, however, strong evidence to suggest that soil contamination strongly affects iron concentration, so great care should be taken that no soil contamination occurs when estimating iron concentration. There was no evidence that suggests soil contamination-at least with soil from these plots-affects zinc concentration, or, of course, dry matter. Further research is needed to determine threshold limits for iron via aluminum contamination.

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CHAPTER 3: GENOTYPIC VARIATION OF IRON AND ZINC CONCENTRATION IN SWEETPOTATO

3.1 Introduction

The HarvestPlus program is a global alliance of research institutions and implementing agencies that seek to develop staple food crops that were rich in iron, zinc, and vitamin A. The object of this effort is to reduce the effects of malnutrition in these nutrients, particularly in poorer areas of the world where deficiencies in these micronutrients were widespread. This process of genetically enhancing the nutritional properties of crops is called biofortification. The HarvestPlus program is coördinated by the International Center for Tropical Agriculture (CIAT) and the International Food Policy Research Institute (IFPRI). Phase I of the program focuses on biofortification of beans, cassava, maize, rice, sweetpotato, and wheat; Phase II of the program will focus on biofortification of crops such as millet, sorghum, potatoes, and a number of others, that generally make up a smaller proportion of the diet of the developing world (HarvestPlus.org).

There were two main strategies for the biofortification of crops: traditional breeding, and genetic transformation. Genetic transformation, such as the production of rice that is biofortified with ß-carotene (golden rice) can be extremely expensive, and can lead to consumer rejection due to concerns about the health of eating such foods. We were unaware of current consumer sentiment and adoption of golden rice. Traditional breeding does not present this problem, though it does require that genetic variation for the trait in question, in this case, iron and zinc density, exists in the germplasm for the crop. So, an important initial step in developing a biofortified crop is the screening of germplasm to see if this genetic variation exists. Such germplasm screenings have been undertaken for a number of crops. Studies with common bean (*Phaseolus vulgaris*), rice (*Oriza sativa*), wheat (*Triticum* spp.) have all found large (two- to three-fold) variation in iron and zinc concentration (Welch and Graham, 2004).

Presently little is known about the concentration of iron and zinc in sweetpotato. A range of 0.59 to 0.86 mg/100g (fresh weight) and a level of 0.24 mg/100g (fresh weight) for iron and zinc, respectively, were given in Woolfe (1992); the USDA gives 0.61 mg/100g and 0.30 mg/100g for iron and zinc concentration, respectively (http://www.nal.usda.gov/fnic/foodcomp/search/). Unfortunately, the sweetpotato cultivars for which these figures were intended to be descriptive were not given in the sources, and in the case of the USDA figures, were based on a very small number of data points. Furthermore, there is no published information on the genotypic range of iron and zinc concentration in sweetpotato. It is this deficiency that this study intends to remedy.

3.2 Materials and Methods

Field research was conducted at the Burden Research Center at Baton Rouge, Louisiana in 2004. The soil was a Loring silt loam (fine-silty, mixed thermic, Typic Fraguidalf) with a pH of approximately 5.1. Typical soil analysis characteristics were presented in Appendix B. Approximately 76 varieties (Table 3.1) were replicated four times in a randomized block design. There were seven plants per plot, spaced 0.3 m between plants and 1.5 m between plots; the five middle plants of each plot were considered for analysis. These experiments were repeated in the spring of 2005 in the same location, with some additional varieties (Table 3.2). Mostly, U.S. #1 grade roots (5.1-8.9 cm diameter and 7.6-22.9 cm long) were harvested for analysis from the five middle plants. Roots were harvested for the varied studies on November 4, 2004 and November 9, 2005, 121 and 120 days after planting.

Processing of tissue samples for zinc and iron analysis was based on the methods of Norbotten *et al.,* (2000). Harvested roots were washed in tap water and allowed to air-dry before weighing. They were then rinsed in double-distilled water, peeled with a stainless steel knife, and rinsed in double distilled water again. The roots were then sectioned, weighed, and dried at 80°C for 48 hours, after which they were weighed again. Dry matter was based on the differential between the results of the two weighings, then the dry samples were pulverized using an IKA A10 Basic Analytical Mill (IKA Works, Inc, Wilmington, NC), bottled in Corning Snap-Seal tubes (product no. 1730, Corning, New York); and stored at 60°C until assayed for iron and zinc concentration.

Analysis for various minerals was based on the methods of Huang and Schulte (1980); and Havlin and Soltanpour (1980). In short, 1 g samples were digested in 5 ml of nitric acid added. The samples were placed on a Magnum 120 Plant/Soil Digester (Ivesdale, Il). After 45 minutes, a 3 ml aliquot of H_2O_2 was added to each sample, prior to the block reaching 90°C. The samples were heated until the volume was reduced to 0.5 ml, then diluted to 12.5 ml and filtered using Whatman #2 paper. The samples were then quantified for the various minerals via inductively coupled plasma mass spectrometry using a Spectro Ciros CCD (Kleve, Germany). For every twenty samples a National Institute for Standards and Technology (Gaithersburg, MD) 1547 peach sample was used for repeatability measurements.

In all cases, samples that showed aluminum levels above 3 ppm dwb, were considered to be contaminated and were discarded. A generic threshold of >5-6 ppm dwb (dry weight basis) was suggested by Pfeiffer and McClafferty (in press), but recent perspectives suggest >3 ppm to be more appropriate (Wolfgang Pfeiffer, 2006 personal communication.) Additional research is needed in the area of contamination thresholds.

The dry weight calculations and the results of the assay were analyzed using an ANOVA model in PROC GLM (SAS 9.1, SAS Institute Inc, Cary, N.C.) to test variation in iron and zinc concentration, and in dry matter. Means and letter groupings were produced using the PDMIX.SAS macro (SAS 9.1, SAS Institute Inc, Cary, N.C.) Sample code for this experiment is presented in Appendix A.

3.3 Results and Discussion

Due to significant year effects in the model for iron, zinc, and dry matter, results for 2004 and 2005 were presented separately (Tables 3.1 and 3.2). In 2004, iron concentration ranged from a high of 7.27 ppm in 'Pata de Oso' to a low of 2.48 ppm in 'Pung-mi' (fwb). In 2005 iron concentration ranged from a high of 7.92 ppm in 'Kyukei No. 63' to a low of 3.20 ppm in 'Chuquimanco.' In both years there was a difference in iron concentration of between 2.5 and three fold among the genotypic mean estimates. In 2004, the top two genotypes differed significantly from roughly the lower three-quarters in mean iron concentration. Similarly in 2005, two of the three top ranking genotypes separated statistically from the others. The higher standard deviation of the second-ranking genotype, 'Kawogo', caused it to be statistically indistinguishable from the bulk of the other genotypes. The important point is that in both years there were two genotypes which have consistently exhibited higher iron concentration than most others. It is also worth noting that in both years, the top ranking genotypes were the same, 'Pata de Oso' and 'Kyukei No. 63.' Furthermore, despite the significant year effect for iron, a perusal of the rankings for the two years will show that while the relative rankings were not identical, they were broadly similar: genotypes tended to repeat their general positions in the rankings in the two years of the study. By raw mean iron estimates, the bulk of the genotypes fell into the range of three to four ppm in 2004 and four to five ppm in 2005. Germplasm screenings in wheat and maize showed differences of two-fold and six-fold, respectively, demonstrating similar patterns to our current study (Gregorio, 2002 and Brkić, 2004). No data has been published to date demonstrating genotypic variability; these disclosures represent summaries communicated in perspective-based publications.

Data documenting iron concentration in plants is predicated on estimates with concomitantly low aluminum concentration. Aluminum is considered an artifact of soil contamination and residual soil iron included in the estimate (Gabriela Burgos, 2004 personal communication). Our preliminary study (Chapter 2) showed evidence of treatments with elevated iron in association with higher aluminum concentration. One would expect a significant positive correlation between iron and aluminum in the samples if there were widespread soil contamination. While

there is no strong threshold for how much aluminum concentration indicates soil contamination, 5 to 6 ppm on a dry weight basis is recommended (Pfeiffer and McClafferty, in press), but recent perspectives suggest that samples with aluminum concentration >3ppm should not be used (Wolfgang Pfeiffer, 2006 personal communication.). Mean aluminum concentration by genotype ranged from 0 to 0.6190 ppm in 2004 and from 0 to 0.7694 in 2005, far below the proposed threshold of 3 ppm; it is also noteworthy that while 'Kawogo', one of the genotypes with high iron in 2005, also had relatively higher mean aluminum concentration for that year, at 0.76 ppm, it still ranked highly in mean iron concentration in 2004 despite having low mean aluminum concentration (0.23 ppm). The rest of the top five genotypes for iron across both years had, at highest, 0.38 ppm aluminum ('Kyukei No. 63' in 2005) and ranged as low as 0.03 ppm ('Kamala Sundari' in 2004.).

Our data was further analyzed through a correlation between iron and aluminum concentration. We found a Pearson Correlation Coefficient of -0.00741 with a p-value of 0.8818 for the 2004 samples, indicating that there was no significant relationship between iron and aluminum concentration; however, for the 2005 data set, we found a positive correlation of 0.19569 with $p<0.0001$. We consider the iron concentration for genotypes presented herein as reflective of genetic potential for iron uptake. Correlations do not infer cause and effect. More research is needed in estimating threshold levels in all crops for aluminum. No published data is extant on this topic.

In 2004 mean zinc concentration by genotype ranged from 3.43 ppm for 'Kyukei No. 63' to 1.03 ppm for 'Kalmegh S-3'; in 2005. it ranged from 4.13 ppm for 'Pata de Oso' to 1.29 ppm for 'Norin No. 4.' This is more than a three-fold difference in both cases and as such, is an even greater difference than that found among the genotypes for iron. There was also greater differentiation of the top genotypes by zinc concentration than by iron concentration. In 2005, the top two genotypes were statistically higher than about nine-tenths of the other genotypes. In 2004, the differentiation was less clear. The top genotype in that year, 'Kyukei No. 63', can indeed be differentiated from about nine-tenths of the other genotypes, but the next few high ranking genotypes had relatively large standard deviations and were not statistically different from half the other genotypes. The overall rankings by mean zinc concentration followed a similar pattern to the rankings by iron concentration. Relative rankings were not identical from year to year, but were broadly similar, i.e., high-ranking genotypes in one year tended to rank highly in the other. For zinc, most genotypes fell between 1.5 and 2.5 ppm in both years. These results in total suggest there is a potential positive relationship between iron and zinc; significantly, the Pearson Correlation Coefficient (0.76206, $p<0.0001$ in 2004 and 0.67796, $p<0.0001$ in 2005) for both years was significant.

In 2004, dry matter ranged from 35.91%, for 'Kyukei No. 63' to 18.14% for 'Hung Loc 4', whereas it ranged from 39.89%, again for 'Kyukei No. 63,' down to 17.48% for 'IPS 163' in 2005. In both years, dry matter was fairly consistent within genotype; in both cases, the top two genotypes could be distinguished by dry matter from about four-fifths of the remainder. Similarity from year to year in dry matter rankings was less obvious than with iron and zinc rankings; however, the trend persists. Most of the genotypes in 2004 were between 25 % and 35% dry matter; in 2005, most of them fell between 20 % and 30%. Dry matter was significantly correlated ($p<0.0001$) with both iron and zinc (corrected for fresh weight) across both years; positive Pearson correlation coefficients ranged from 0.2472 to 0.4437 with iron by dry matter and zinc by fresh weight in both years; that is, genotypes with high dry matter concentration tended to have higher iron and zinc concentration, on a fresh-weight basis. Dry-down of the samples tends to concentrate the micronutrient concentration in roots with high dry matter, so perhaps this accounts for part of the correlation. For example, in 2004 the Australian genotype 'IPS 163' had the highest iron concentration by dry matter of all the genotypes. However, this genotype also had the lowest dry matter concentration of all the genotypes that year, so when iron concentration was corrected for dry matter concentration, this genotype fell to 24th. When correlations were run between dry matter concentration and raw (uncorrected for dry matter concentration) iron and zinc concentration, the correlation between dry matter and iron in 2005 was found not to be significant; in the other three data sets (iron in 2004 and zinc in both years) significant ($p<0.0001$) negative Pearson Correlation coefficients (-0.2513 to -0.2617) were found.

The underlying purpose of this research is to identify the genotypic range of iron and zinc in sweetpotato for human nutrition. To attempt to put this data into perspective, we have estimated how much of a general daily requirement for iron and zinc will be provided by eating one root of each of these genotypes. We have based this on a daily requirement of 8 μg/day (http://www.iom.edu/Object.File/Master/7/294/Webtableminerals.pdf/) and a typical root, weighing 300 g. This represents a mid-range level of iron required for most demographic groups, e.g., children, adult men, non-pregnant adult women. Our genotypes range from providing 25% to 30% of the daily allowance of iron ['Kyukei No. 63' (Japan), 'Pata de Oso' (Peru), 'Kawogo' (East Africa)] and 12% to 15% of the daily allowance for zinc ['Kyukei No. 63' (Japan), 'Pata de Oso' (Peru)] on the high end, down to 9% to 12% for iron ['Chuquimanco' (Peru), 'Pung-mi' (Korea)] and 4% to 6% for zinc ['Pung-mi' (Korea)].

In addition to providing a significant proportion of the daily requirements of iron and zinc, sweetpotatoes were low in phytates (undetectable levels of phytate in 'Beauregard' and 'L01-29' based on six samples assayed by

Kevin Peterson and Victor Raboy, personal communication, 2005) and high in ascorbic acid (23.6 mg/100 g, Woolfe, 1992). As noted in Chapter 1, phytates were compounds which were used for the storage of phosphorus in seed, and which interfere with the availability of iron and zinc in the human diet (Raboy, 2002). Crops such as common bean (*Phaseolus vulgaris*) and other Leguminaceæ do have high iron concentration in seeds, but the high phytate concentration interferes with proper absorption of iron and zinc, among other micronutrient cations (Raboy, 2002). Ascorbic acid, in contrast, helps aid efficient iron uptake. Orange-fleshed sweetpotato is estimated to have high levels of ascorbic acid. Sweetpotato is unique among crops in its innate iron concentration and low phytate and high ascorbic acid concentration. Improvement made in iron and zinc levels in sweetpotatoes should translate fairly directly to improved nutrition for the consumer.

While this genotypic variation provides a good basis for breeding for increased iron and zinc concentration, we realize other varieties may exist which have higher levels of iron and zinc and which would be suitable as parents in a breeding program to further raise iron and zinc concentration. Results might also differ tremendously if the study were conducted in soil with low levels of available iron and zinc; future studies should explore the range of iron and zinc levels in genotypes in such soils.

Table 3.1. Means, standard deviations and statistical groupings for iron, zinc, and dry matter by genotype for sweetpotato roots in 2004.

Genotype Information			Iron (ppm)[Z]			Zinc (ppm)[Z]			Dry Matter		
PI	Name	Origin	Mean	STD	Statistical Grouping	Mean	STD	Statistical Grouping	Mean	STD	Statistical Grouping
599384	Pata de Oso	Peru	7.27	0.43	A	3.15	0.25	ABC	34.81%	1.27%	ABCDLM
633965	Kyukei no. 63	Japan	6.64	0.30	AB	3.43	0.17	A	39.89%	0.89%	A
612675	Kawogo	East Africa	6.40	0.61	ABCD	1.94	0.35	ABCDEFG	28.31%	1.79%	DEFGHIJKNOPQRS
585100	Tinto	Mexico	5.65	0.35	ABCE	2.09	0.20	BCDEFG	37.95%	1.03%	ABC
538295	Yuca de Calango	Peru	5.60	0.29	ABC	2.94	0.16	AB	33.71%	0.84%	BCDL
531135	7044	Costa Rica	5.32	0.30	ABCDF	2.32	0.17	BCDEF	38.65%	0.90%	AB
check	Wagabulige	East Africa	5.29	0.86	ABCDFGHIJ	3.19	0.49	ABCDEFG	36.15%	2.54%	ABCDEFGHIJK
508508	Kogane-sengan	Japan	5.28	0.35	ABCDFG	2.49	0.20	ABCDE	33.93%	1.03%	BCDE
633964	AVRDC-CN 1732-4	Peru	5.28	0.61	ABCDFGHIJ	1.97	0.35	ABCDEFG	28.75%	1.79%	DEFGHIJKNOPQRS
531127	Pampa Culevra	Peru	5.23	0.33	ABCDFG	2.06	0.19	BCDEFG	38.58%	0.96%	AB
599391	Yanshu 1	China	5.08	0.39	ABCDFGHK	1.85	0.22	BCDEFG	21.10%	1.13%	RSW
556934	Cuitzeo	Honduras	4.83	0.43	ABCDFGHI	1.93	0.24	BCDEFG	24.28%	1.27%	JKNOPQRSW
538349	Bugsbunny	Puerto Rico	4.82	0.35	BCDFGHI	2.14	0.20	BCDEFG	23.80%	1.04%	NOPQRSW
595873	Xushu 18	China	4.81	0.38	BCDFGHI	1.36	0.22	EFG	33.67%	1.13%	BCDEF
573299	Chin Mi	Korea	4.79	0.35	BCDFGHI	2.02	0.20	BCDEFG	31.10%	1.03%	DEFGHIJ
606273	Xiangnonghuangpi	China	4.75	0.35	BCDFGHI	2.06	0.20	BCDEFG	32.42%	1.04%	CDEFGH
573316	Hawaii	Tonga	4.71	0.38	BCDFGHI	2.09	0.22	BCDEFG	27.62%	1.13%	EFGHIJKMNOPQR
Z: Iron and zinc concentrations were corrected for dry matter.											

(table continued)

Genotype Information			Iron (ppm)[Z]			Zinc (ppm)[Z]			Dry Matter		
PI	Name	Origin	Mean	STD	Statistical Grouping	Mean	STD	Statistical Grouping	Mean	STD	Statistical Grouping
585085	Baracutey	Mexico	4.68	0.43	BCDFGHIJ	1.71	0.25	BCDEFG	33.06%	1.27%	BCDEFGH
633442	Los Cerrillas	Uruguay	4.64	0.31	CDFGHI	2.45	0.17	ABCDE	24.05%	0.90%	NOPQRS
573329	Wart	Puerto Rico	4.54	0.29	CDFGHI	2.40	0.16	BCDE	31.32%	0.85%	DEFGHI
208029	Camaguey	Cuba	4.49	0.61	ABCDFGHIJ	2.11	0.35	ABCDEFG	28.71%	1.79%	DEFGHIJKNOPQRS
599377	IPS 163	Australia	4.47	0.39	CDFGHIJ	2.18	0.22	BCDEFG	17.48%	1.13%	W
564124	IB01	Samoa	4.45	0.50	BCDFGHIJ	1.80	0.28	BCDEFG	28.24%	1.47%	DEFGHIJKNOPQRS
531121	Ihuanco	Peru	4.38	0.35	CDFGHIJ	2.38	0.20	ABCDEF	30.12%	1.04%	DEFGHIJKU
599366	Q 23722	Peru	4.30	0.35	CDFGHIJ	2.01	0.20	BCDEFG	31.95%	1.04%	CDEFGHI
614800	Kokei no. 14	Japan	4.29	0.50	BCDFGHIJ	1.80	0.28	BCDEFG	27.87%	1.47%	DEFGHIJKNOPQRS
153655	Tinian	Northern Mariana Islands	4.22	0.50	BCDFGHIJ	2.19	0.28	ABCDEFG	29.99%	1.47%	DEFGHIJKNOP
606252	Duanyanghong	China	4.21	0.35	CDFGHIJ	1.51	0.20	DEFG	33.42%	1.04%	BCDEF
599389	Qunli 1	China	4.17	0.50	CDFGHIJ	1.53	0.28	DEFG	36.34%	1.47%	ABCD
508514	74-637	China	4.16	0.30	CDFGHIJ	2.18	0.17	BCDEFH	33.02%	0.90%	CDEF
585086	Regional de Tehuantepec	Mexico	4.14	0.32	CDFGHIJ	1.56	0.18	DEFG	28.98%	0.96%	DEFGHIJKNV
595891	Kemb 37	East Africa	4.11	0.30	CDFGHIJ	1.79	0.17	DEFG	28.26%	0.89%	EFGHIJKNO
599369	Haiti	Cuba	4.00	0.29	CDFGHIJ	1.79	0.16	DEFG	32.28%	0.84%	DEFG
308200	459	New Zealand	3.97	0.33	CDFGHIJ	1.88	0.18	CDEFG	27.53%	0.96%	FGHIJKNOPQ
595869	Honiara	Fiji	3.96	0.38	CDFGHIJ	1.58	0.22	DEFG	25.86%	1.13%	IJKNOPQRS
308198	19	New Zealand	3.95	0.30	CDFGHIJ	1.47	0.17	EFG	31.14%	0.89%	DEFGHIT
538291	Porfirio	Peru	3.93	0.35	CDFGHIJ	2.26	0.20	BCDEFH	22.46%	1.04%	PQRSW
508532	Wiripipi, Itawa	Venezuela	3.88	0.50	CDFGHIJ	2.17	0.28	ABCDEFG	31.97%	1.47%	BCDEFGHIJK
595882	IITA-TIS 2544	Nigeria	3.88	0.33	CDFGHIJ	1.62	0.18	DEFG	25.00%	0.96%	KNOPQRS
564134	L 13	Papua-New Guinea	3.87	0.39	CDFGHIJ	1.73	0.22	DEFG	28.10%	1.13%	EFGHIJKLNOPQ
585059	Hung Loc 4	Vietnam	3.81	0.33	CDFGHIJ	1.50	0.18	EFG	26.85%	0.96%	HIJKNOPQRS
585065	Kamala Sundari	Bangladesh	3.80	0.35	CDFGHIJ	1.89	0.20	BCDEFG	28.96%	1.04%	DEFGHIJKNO
573319	Siale	Tonga	3.76	0.35	CDFGHIJ	1.38	0.20	EFG	27.27%	1.03%	FGHIJKNOPQR
564127	IB14	Samoa	3.75	0.39	CDFGHIJ	1.59	0.22	DEFG	28.88%	1.13%	DEFGHIJKNOP
564130	ACC 206	Solomon Islands	3.67	0.30	DFGHIJ	1.85	0.17	DEFG	25.01%	0.89%	KNOPQRS
599368	Cuba 2	Cuba	3.64	0.39	CDFGHIJ	1.92	0.22	BCDEFG	28.90%	1.13%	DEFGHIJKNOP
564159	VSP 4	Philippines	3.63	0.43	CDFGHIJ	1.77	0.24	BCDEFG	22.30%	1.27%	OPQRSVW
531125	Chuquimanco	Peru	3.61	0.39	CDFGHIJ	2.08	0.22	BCDEFG	24.72%	1.14%	JKNOPQRS
564129	ACC 309	Solomon Islands	3.61	0.32	DFGHIJ	1.91	0.18	BCDEFG	32.13%	0.96%	CDEFGH
508506	Koto-puki	Japan	3.57	0.33	DFGHIJ	1.41	0.19	EFG	33.99%	0.96%	BCDL
573334	Reddish Gold	Myanmar	3.44	0.50	CDFGHIJ	1.60	0.28	BCDEFG	24.61%	1.46%	IJKNOPQRSW
286621	Kanifuta	New Zealand	3.42	0.43	CDFGHIJ	1.49	0.24	DEFG	28.51%	1.27%	DEFGHIJKNOPQ
585051	TIS 2532	Nigeria	3.40	0.43	DEFGHIJ	1.27	0.24	EFG	21.55%	1.27%	QRSW
564157	VSP 1	Philippines	3.32	0.32	GHIJ	1.64	0.18	DEFG	23.12%	0.96%	OPQRSW
531128	Boca de Chisco	Peru	3.30	0.50	CDFGHIJ	2.27	0.28	ABCDEFG	23.97%	1.47%	JKNOPQRSTW
318848	Kamula Belep	New Caledonia	3.23	0.32	HIJ	1.86	0.18	CDEFG	30.11%	0.96%	DEFGHIJKU
585077	Camote Amarillo	Guatemala	3.15	0.43	FGHIJ	1.32	0.24	EFG	26.30%	1.27%	GHIJKNOPQRS
606280	Guangshu 70-9	China	3.01	0.29	IJ	1.51	0.16	EFG	27.39%	0.84%	GHIJKNOPQ
531134	Lamote	Costa Rica	2.98	0.35	HIJ	1.26	0.20	FG	26.56%	1.04%	HIJKNOPQRS
286623	CLPR9A	New Zealand	2.95	0.61	CDFGHIJ	1.69	0.35	BCDEFG	31.29%	1.79%	BCDEFGHIJKNO

Z: Iron and zinc concentrations were corrected for dry matter.

(table continued)

Genotype Information			Iron (ppm)[Z]			Zinc (ppm)[Z]			Dry Matter		
PI	Name	Origin	Mean	STD	Statistical Grouping	Mean	STD	Statistical Grouping	Mean	STD	Statistical Grouping
614795	Guangshu 7	China	2.93	0.35	IJK	1.22	0.20	FG	26.25%	1.03%	HIJKNOPQRS
585061	Kalmegh S-30	India	2.91	0.39	HIJ	1.03	0.22	GH	25.68%	1.13%	IJKNOPQRS
344134	ACIC	Papua-New Guinea	2.90	0.38	HIJ	1.19	0.22	FG	26.63%	1.13%	GHIJKNOPQRS
585071	Won-Mi	Korea	2.80	0.61	CDFGHIJ	1.93	0.35	ABCDEFG	29.19%	1.79%	DEFGHIJKNOPQRS
564132	L 3	Papua-New Guinea	2.78	0.43	HIJ	1.22	0.25	EFG	23.58%	1.27%	NOPQRSUW
585073	Pung-mi	Korea	2.48	0.32	J	1.11	0.18	G	25.26%	0.96%	JKNOPQRS
Z: Iron and zinc concentrations were corrected for dry matter.											

Table 3.2. Means, standard deviations and statistical groupings for iron, zinc, and dry matter by genotype for sweetpotato roots in 2005.

Genotype Information			Iron (ppm)[Z]			Zinc (ppm)[Z]			Dry Matter		
PI	Name	Origin	Mean	STD	Statistical Grouping	Mean	STD	Statistical Grouping	Mean	STD	Statistical Grouping
633965	Kyukei no. 63	Japan	7.92	0.43	AB	4.02	0.20	AB	35.91%	1.27%	A
612675	Kawogo	East Africa	7.87	0.86	ABCDEFG	2.83	0.40	ABCDEFGHIJKM	26.04%	2.54%	ABCDEFGHIJKMNO
599384	Pata de Oso	Peru	7.80	0.38	A	4.13	0.18	A	29.19%	1.14%	ABCDEFGH
585065	Kamala Sundari	Bangladesh	7.50	0.86	ABCDEFGHI	3.32	0.40	ABCDEFGHIJK	25.04%	2.54%	ABCDEFGHIJKMNO
508508	Kogane-sengan	Japan	7.43	0.39	ABCD	2.52	0.18	CDEFGHI	29.24%	1.14%	ABCDEFGH
573300	L9	Papua-New Guinea	7.27	0.29	ABC	3.16	0.13	BCD	27.60%	0.85%	DEFGHI
538295	Yuca de Calango	Peru	7.11	0.32	ABCDJ	3.17	0.15	ABCDL	32.04%	0.96%	ABCD
585100	Tinto	Mexico	6.70	0.35	ABCDE	2.59	0.16	CDEF	32.25%	1.04%	ABCD
531121	Ihuanco	Peru	6.38	0.50	ABCDEFGH	2.59	0.23	CDEFGHIJKM	27.20%	1.48%	CDEFGHIJK
531127	Pampa Culevra	Peru	6.05	0.61	ABCDEFGHIK	2.41	0.28	CDEFGHIJKM	29.30%	1.80%	ABCDEFGHIJK
633442	Los Cerrillas	Uruguay	5.91	0.25	ABCDEF	2.49	0.11	DEF	34.61%	0.73%	AB
286621	Kanifuta	New Zealand	5.88	0.43	ABCDEFGHIL	1.63	0.20	FGHIJKM	31.04%	1.27%	ABCDEF
595891	Kemb 37	East Africa	5.78	0.30	ABCDEFGN	3.26	0.14	ABC	22.60%	0.90%	IJKMNO
508506	Koto-puki	Japan	5.57	0.35	CDEFGHIL	2.30	0.16	DEFGHIJKM	33.71%	1.04%	ABC
595869	Honiara	Fiji	5.52	0.26	DEFGHLO	2.22	0.12	EFGHIJKM	24.28%	0.76%	GHIJK
566657	Sumor	South Carolina	5.36	0.44	BCDEFGHIK	2.22	0.20	DEFGHIJKM	26.12%	1.29%	DEFGHIJK
556934	Cuitzeo	Honduras	5.33	0.27	EFGHIL	2.13	0.12	EFGHIJKM	23.79%	0.80%	HIJKMN
585059	Hung Loc 4	Vietnam	5.17	0.39	DEFGHIK	2.31	0.18	DEFGHIJKM	18.14%	1.14%	NO
595882	IITA-TIS 2544	Nigeria	5.07	0.30	EFGHIK	2.52	0.14	CDEF	23.95%	0.90%	GHIJKMNP
308198	19	New Zealand	5.05	0.26	EFGHIK	2.18	0.12	EFGHIJKM	25.10%	0.76%	FGHIJK
599369	Haiti	Cuba	4.97	0.30	EFGHIK	2.26	0.14	EFGHIJKM	26.24%	0.89%	EFGHIJK
573329	Wart	Puerto Rico	4.96	0.61	ABCDEFGHIK	2.89	0.28	ABCDEFGHIJ	27.80%	1.80%	ABCDEFGHIJKM
538349	Bugsbunny	Puerto Rico	4.92	0.26	EFGHIK	2.61	0.12	CDEN	23.00%	0.76%	IJKMNO
585085	Baracutey	Mexico	4.82	0.61	ABCDEFGHIK	1.97	0.28	CDEFGHIJKM	22.54%	1.80%	FGHIJKMNO
633964	AVRDC-CN 1732-4	Peru	4.81	0.50	DEFGHIKP	2.10	0.23	DEFGHIJKM	25.20%	1.48%	DEFGHIJKMNO
573299	Chin Mi	Korea	4.79	0.50	DEFGHIKP	1.92	0.23	EFGHIJKM	23.71%	1.48%	FGHIJKMNO
585086	Regional de Tehuantepec	Mexico	4.69	0.35	EFGHIKP	2.43	0.16	CDEFGHIJKM	26.75%	1.04%	DEFGHIJ
599366	Q 23722	Peru	4.64	0.39	EFGHIKP	2.62	0.18	CDEFG	22.04%	1.14%	IJKMNO
531134	Lamote	Costa Rica	4.64	0.43	EFGHIKP	1.81	0.20	EFGHIJKM	26.27%	1.27%	DEFGHIJK
208029	Camaguey	Cuba	4.62	0.29	FGHIKP	2.30	0.13	EFGHIJKM	25.85%	0.85%	FGHIJK
595873	Xushu 18	China	4.61	0.29	FGHIKP	1.71	0.13	GHIJKM	23.61%	0.84%	HIJKMNO
Z: Iron and zinc concentrations were corrected for dry matter.											

(table continued)

Genotype Information			Iron (ppm)[Z]			Zinc (ppm)[Z]			Dry Matter		
PI	Name	Origin	Mean	STD	Statistical Grouping	Mean	STD	Statistical Grouping	Mean	STD	Statistical Grouping
286623	CLPR9A	New Zealand	4.56	0.30	FGHIKP	2.47	0.14	CDEFG	28.14%	0.90%	CDEFGHL
153905	Norin No. 2	Japan	4.53	0.35	FGHIKP	2.33	0.16	DEFGHIJKM	29.29%	1.04%	ABCDEFG
564159	VSP 4	Philippines	4.53	0.39	EFGHIKP	2.63	0.18	CDEFG	21.69%	1.14%	IJKMNO
531146	Tainung 65	Taiwan	4.52	0.39	EFGHIKP	1.72	0.18	FGHIJKMN	27.29%	1.14%	CDEFGHIJ
614800	Kokei no. 14	Japan	4.47	0.61	CDEFGHIKP	1.90	0.28	DEFGHIJKM	26.54%	1.80%	BCDEFGHIJKMNO
564127	IB14	Samoa	4.44	0.61	CDEFGHIKP	2.66	0.28	BCDEFGHIJKM	25.87%	1.80%	CDEFGHIJKMNO
564134	L 13	Papua-New Guinea	4.43	0.35	FGHIKP	1.89	0.16	EFGHIJKM	24.75%	1.04%	FGHIJKM
606273	Xiangnonghuangpi	China	4.41	0.35	FGHIKP	2.09	0.16	EFGHIJKM	24.84%	1.04%	FGHIJKM
153907	Norin no. 4	Japan	4.39	0.61	DEFGHIKP	1.29	0.28	FGHIJKM	25.80%	1.80%	CDEFGHIJKMNO
585077	Camote Amarillo	Guatemala	4.38	0.35	FGHIKP	1.92	0.16	EFGHIJKM	24.59%	1.04%	FGHIJKMN
531135	7044	Costa Rica	4.31	0.39	FGHIKP	2.52	0.18	CDEFGHIM	25.24%	1.14%	FGHIJKM
606252	Duanyanghong	China	4.30	0.25	GHIKP	1.68	0.11	HIJKM	25.33%	0.73%	FGHIJK
564161	Margarita	Puerto Rico	4.25	0.29	GHIKP	1.90	0.13	EFGHIJKM	22.46%	0.85%	JKMNO
531128	Boca de Chisco	Peru	4.24	0.50	EFGHIKP	2.40	0.23	CDEFGHIJKM	21.97%	1.48%	GHIJKMNO
614795	Guangshu 7	China	4.15	0.50	EFGHIKP	2.21	0.23	CDEFGHIJKM	21.63%	1.48%	HIJKMNO
585061	Kalmegh S-30	India	4.06	0.27	HIKP	1.90	0.13	EFGHIJKM	21.94%	0.80%	JKMNO
564132	L 3	Papua-New Guinea	3.99	0.43	FGHIKP	2.14	0.20	EFGHIJKLM	20.91%	1.27%	JKMNO
573334	Reddish Gold	Myanmar	3.98	0.39	GHIKMP	2.00	0.18	EFGHIJKM	25.22%	1.14%	FGHIJKM
599391	Yanshu 1	China	3.94	0.24	IKP	1.65	0.11	IJKO	19.91%	0.70%	MNO
573319	Siale	Tonga	3.89	0.26	IKP	1.90	0.12	FGHIJKM	25.10%	0.76%	FGHIJK
564124	IB01	Samoa	3.77	0.35	IKOP	1.83	0.16	EFGHIJKM	25.71%	1.04%	FGHIJK
599377	IPS 163	Australia	3.73	0.23	KP	1.99	0.11	EFGHIJKM	19.21%	0.68%	O
318848	Kamula Belep	New Caledonia	3.67	0.26	KP	2.24	0.12	EFGHIJKM	26.11%	0.76%	FGHIJ
585071	Won-Mi	Korea	3.66	0.27	KP	2.54	0.12	CDEF	24.03%	0.80%	GHIJKM
153909	Taihaku Saitama no. 1	Japan	3.64	0.29	KP	1.63	0.13	HIJKM	25.57%	0.85%	FGHIJK
564129	ACC 309	Solomon Islands	3.56	0.86	CDEFGHIKP	2.04	0.40	CDEFGHIJKM	27.04%	2.54%	ABCDEFGHIJKMNO
564130	ACC 206	Solomon Islands	3.50	0.50	HIKNP	1.81	0.23	EFGHIJKM	21.04%	1.48%	IJKLMNO
606280	Guangshu 70-9	China	3.32	0.50	KLP	1.97	0.23	EFGHIJKM	25.20%	1.48%	DEFGHIJKMNO
585073	Pung-mi	Korea	3.22	0.24	P	1.61	0.11	KO	21.50%	0.70%	KMNO
531125	Chuquimanco	Peru	3.20	0.50	KLP	1.29	0.23	MO	20.38%	1.48%	JKMNO
Z: Iron and zinc concentrations were corrected for dry matter.											

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CHAPTER 4: HERITABILITY

4.1 Introduction

Sweetpotato possess both genetic complexity and opportunity. Hexaploidy (2n=6x=90) and self-incompatibilty has spawned a large number of land races from which to develop new and valuable cultivars. There is evidence that sweetpotato functions as a tetraploid (Buteler *et al*., 1999) in microsatellite segregation inferring allopolyploidy. Ukoskit and Thompson (1997) examined the ratio of simplex to non-simplex DNA markers and reported that sweetpotato is an autopolyploid, while Kriegner *et al*., (2001) showed partial homology in linkage map data. Irrespective, no trait exists which has been documented as a case of simple Mendelian inheritance. Jones (1986) describes trait inheritance in quantitative terms and mass selection as an opportune vehicle to coalesce alleles from disparate genes to improve traits.

Breeding cycles in sweetpotato take two years to complete and a great deal of time can be spent working toward improvement in a given trait before any tangible data supports the effort. Heritability estimates represent an efficient means of determining the feasibility of improving the trait (Jones, 1986). Heritability studies were typically based on regression of offspring on parents using parent-progeny populations and covariances of relatives (Jones, 1986; Long, *et al.,* 2004; Diers and Fehr, 1989; Spehar, 1994; Kim *et al.,* 1996). In short, heritability is the observable fraction of variation due to genotypic differences in relationship to the total phenotypic variation (Jones, 1986). The total genetic variance can be partitioned to estimate variance due to the environment, epistasis (dominant genetic variance), and additive genetic variance. The later is most important and supports the possibility of trait improvement (genetic gain); a strong additive variance positively affects narrow-sense heritability (h^2). A rating of .5 (h^2) or higher is often recognized as an indicator that a given trait can be improved. Another common estimate is broad-sense heritability (H); however, no estimate of additive genetic variance is made (nor genetic gain) but a similar feasibility measure is given. Heritability estimates vary for a given trait depending on the methodology used.

Parental inclusion in heritability studies is normally a given, but in this study it was desirable to estimate heritability of iron and zinc concentration using germplasm from outside the United States. Quarantine restrictions which allow the importation of seed but not plant matter without a lengthy quarantine period, prevented the use of routine parent/progeny populations. Therefore, we estimated broad-sense heritability of the traits of interest using progeny only using imported seed. No USDA/APHIS import restriction is in effect for seed; international restrictions

were also minimal. A technique adapted from use in sorghum (Cisse and Ejeta, 2003) which estimates broad-sense heritability using analysis of variance among half-sibling families descended from one pollen and multiple egg parents was used.

Neither narrow sense heritability nor genetic gain can be estimated using this method, but in sweetpotato, it represents a novel approach which allows the breeder to quickly estimate heritability with readily imported germplasm. Our specific research objective was to determine broad sense heritability of important micronutrients, iron and zinc, and dry matter in sweetpotato.

3.2 Materials and Methods

Field research for estimating heritability was done at the Sweetpotato Research Center at Chase, Louisiana. The soil was a Gilbert silt loam (fine-silty, mixed, thermic) with a pH of 5.43. Soil in each replication within the study was tested and found to vary minimally (see Appendix B). In the spring of 2004, fifteen full-sibling families of genotypes, all from the same male parent (PC03_1) obtained from the International Potato Center, Lima Peru, were replicated four times in a completely randomized design. The fifteen female parents were 103001 (HY3.4), 103003 (YARDA), 103004 (192096.3), 103005 (193067.3), 103006 (195306.8), 103009 (195605.54), 103014 (199009.7), 103015 (199014.2), 103018 (199020.2), 103024 (100027.3), 103031 (199035.7), 103032 (199047.10), 103033 (199062.1), 103036 (100056.14) and 103072 (199076.1). There were seven plants per plot with one foot (0.3 m) between plants and five feet (1.5 m) between plots; the five middle plants of each plot were considered for analysis. This experiment was repeated in the spring of 2005. In the second year, missing plants were replaced with plants of the cultivar 'Beauregard', to ensure that all plots contained seven plants.

Where possible, similarly-sized, marketable roots were harvested for analysis from the five middle plants; no more than one root per plant was used. Some of the genotypes in this trial produced small roots or no roots at all, thereby making it impossible, in some cases, to harvest five roots per plot or to harvest roots of the ideal size and shape.

Processing of tissue samples for zinc and iron analysis was based on the methods of Norbotten *et al.,* (2000). Harvested roots were washed in tap water and allowed to air-dry before weighing. They were then rinsed in double-distilled water, peeled with a stainless steel knife, and rinsed in double distilled water again. The roots were then sectioned, weighed, and dried at 80°C for 48 hours, after which they were weighed again. Dry matter was based on

the differential between the results of the two weighings, then the dry samples were pulverized using an IKA A10 Basic Analytical Mill (IKA Works, Inc, Wilmington, NC), bottled in Corning Snap-Seal tubes (product no. 1730, Corning, New York); and stored at 60°C until assayed for iron and zinc concentration.

Analysis for various minerals was based on the methods of Huang and Schulte (1980); and Havlin and Soltanpour (1980). In short, 1 g samples were digested in 5 ml of nitric acid added. The samples were placed on a Magnum 120 Plant/Soil Digester (Ivesdale, Il). After 45 minutes, a 3 ml aliquot of H_2O_2 was added to each sample, prior to the block reaching 90°C. The samples were heated until the volume was reduced to 0.5 ml, then diluted to 12.5 ml and filtered using Whatman #2 paper. The samples were then quantified for the various minerals via inductively coupled plasma mass spectrometry using a Spectro Ciros CCD (Kleve, Germany). For every twenty samples a National Institute for Standards and Technology (Gaithersburg, MD) 1547 peach sample was used for repeatability measurements.

In all cases, samples that showed aluminum levels above 3 ppm dwb, were considered to be contaminated and were discarded. A generic threshold of >5-6 ppm dwb (dry weight basis) was suggested by Pfeiffer and McClafferty (in press), but recent perspectives suggest >3 ppm to be more appropriate (Wolfgang Pfeiffer, 2006 personal communication.) Additional research is needed in the area of contamination thresholds.

The data resulting from the assay were analyzed using a technique adapted from one used to estimate heritability in sorghum (Cisse & Ejeta, 2003). Broad-sense heritability based on family means was calculated using the formula: H^2=[MS(among families) – MS(year*family)]/MS(among families). Analyses of variance (see Appendix A for SAS code) were used to examine differences in iron and zinc concentration by fresh matter, and in dry matter concentration.

3.3 Results and Discussion

For iron in fresh matter, family mean estimates (Table 4.1) ranged from 6.81 ppm for '103022' to 3.99 ppm for '103014.' The family with the highest mean iron estimate had a large variation and therefore could not be confidently separated from any other family; however, the second- through sixth-ranking families had mean iron estimates that were significantly higher than the family with the lowest. On an individual basis, iron levels ranged from 9.97 ppm for a progeny of '103072' to 2.22 ppm for a progeny of '103033.' Both estimates suggest a reasonable range in variability. As in the genotypic variability study, no sample was included in the data set if more than 3 ppm of aluminum was

detected on a dry weight basis. Mean aluminum estimates by family ranged from 1.61 ppm for '103032' to 2.22 ppm for '103009'; the family with the highest mean iron estimate, '103022', had a mean aluminum estimate of 1.30 ppm. On an individual basis, there was one sample whose aluminum level rounded to 3.00 ppm; it is worth noting, however, that it had iron concentration of only 4.89 ppm, around the middle of the range. The sample with the highest iron concentration, at 9.97 ppm, had aluminum concentration of 2.26 ppm; the sample with the next-highest level of iron concentration, with 9.23 ppm iron, had aluminum concentration of only 1.37 ppm. Taken together, this leads us to be confident that soil contamination was not a confounding factor in our heritability estimates. Finally, the heritability estimate for iron concentration was 0.74. This is an estimate of broad-sense heritability and so does not partition out additive genetic effects from dominance and interaction effects; nonetheless, we find it a very encouraging estimate for the purposes of breeding for increased iron concentration in sweetpotatoes.

For zinc in fresh matter, the numbers were even more encouraging. This is important, since zinc concentration in sweetpotatoes is more in need of improvement than is iron concentration. Our data in chapter 3 shows zinc daily requirements were minimally met (~15% based on a 300 g root) in the highest ranking germplasm lines versus those for iron (~30%). Mean zinc estimates by family (Table 1) ranged from 3.12 ppm for '103001' to 2.27 ppm for '103031'. There was better separation by mean zinc concentration among the families than by mean iron concentration. The top ranking estimate was significantly different from the bottom five ranking estimates, while the bottom ranking genotype was significantly different from the top ten. Zinc concentration of individual samples ranged from 6.40 ppm for a progeny of '103009' to 1.04 ppm for a progeny of '103009'. Our estimate of broad-sense heritability was 0.82. This is a higher estimate than that for iron, which, as was mentioned before, we find encouraging since zinc in sweetpotatoes is generally lower than iron while human diets, generally speaking, require as much zinc as they do iron.

Family mean estimates for dry matter concentration were separated even more strongly than iron or zinc. Estimates ranged from 34.86% dry matter for '103022' to 25.56% for '103009'. As with iron concentration, the highest estimate had a large standard deviation and could not be reliably segregated from any other estimate; however, the second-highest ranking family was significantly different from the five lowest ranking families; the lowest ranking family was significantly different from the eight highest-ranking families. Dry matter concentration of individual progenies ranged from 45.39% for a progeny of '103024' to 16.35% for a progeny of '103009'. The broad-sense

heritability estimate for dry matter concentration was 0.92. Our results were higher, but consistent with previous broad sense heritability values (.48 and .65) estimated by Jones (1977) and Liang (1982), respectively. In the current study, dry matter heritability was higher than estimates for iron and zinc. Consumers in developing countries who were targeted by this study mostly prefer sweetpotatoes with high dry matter concentration; the high heritability estimate should mean that while breeding new varieties with higher iron and zinc concentration, it will be fairly easy to maintain the desirable high dry matter trait.

Taken in concert, these results suggest that improvements in iron and zinc concentration in sweetpotato were possible using traditional mass-selection breeding techniques. A caveat to our work is that heritability estimates may differ for other parental-progeny populations. Secondly, the soil environment in which the study is conducted most likely will affect micronutrient uptake and thus alter heritability estimates. We were also unable to produce estimates of narrow sense heritability because restrictions on the import of plant material did not allow us to use a traditional parent-progeny regression model. Yet this novel research shows the utility of the adapted sorghum model (Cisse & Ejeta, 2003) to find broad-sense heritability estimates from families of half-siblings. Research is needed to estimate narrow sense heritability in tropical soils.

Table 4.1. Mean estimates by family for iron, zinc, and dry matter in sweetpotato roots.

Family	Iron[z]			Zinc[z]			Dry Matter		
	Mean	Standard Deviation	Statistical Grouping	Mean	Standard Deviation	Statistical Grouping	Mean	Standard Deviation	Statistical Grouping
103001	5.13	0.23	A	3.12	0.14	A	32.05%	0.81%	AB
103003	4.78	0.25	AB	2.74	0.15	ABC	30.62%	0.88%	ABC
103004	4.76	0.31	AB	2.47	0.18	ABC	31.43%	1.10%	ABC
103005	4.80	0.26	AB	2.52	0.16	ABC	29.27%	0.93%	ABCD
103006	5.33	0.27	A	3.03	0.16	AB	30.24%	0.96%	ABC
103009	5.15	0.21	A	2.40	0.13	BC	25.56%	0.76%	D
103014	3.99	0.23	B	2.34	0.14	BC	28.28%	0.81%	BCD
103015	5.35	0.27	A	2.80	0.16	ABC	31.99%	0.98%	AB
103018	4.20	0.26	AB	2.34	0.15	BC	30.03%	0.91%	ABC
103024	4.93	0.21	AB	2.72	0.12	ABC	28.84%	0.74%	BCD
103031	4.66	0.21	AB	2.27	0.12	C	27.01%	0.74%	CD
103032	5.05	0.25	AB	2.79	0.15	ABC	29.17%	0.89%	ABCD
103033	4.59	0.23	AB	2.43	0.14	BC	29.23%	0.82%	ABCD
103036	5.12	0.22	A	2.75	0.13	ABC	29.78%	0.77%	ABC
103072	4.75	0.28	AB	2.36	0.17	BC	33.28%	1.01%	A

[z]: Iron and zinc concentrations were corrected for dry matter.

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CONCLUSION

All of the research described in this study relates to the HarvestPlus program to biofortify staple crops like sweetpotato with iron and zinc. The main objective was to determine if we could improve sweetpotato through traditional breeding methods for these micronutrients; to that end we have estimated the heritability of iron and zinc concentration in sweetpotato and screened a number of genotypes from around the world for high concentration of iron and zinc, potentially for use in breeding a new, biofortified variety.

Since there were no published protocols for sampling of iron and zinc concentration in sweetpotato, it was first necessary find out how much variation exists within sweetpotato genotypes-that is, from plant to plant, from root to root, and within roots, in order to know what sort of sampling we had to perform in order to get a reliable estimate of iron and zinc concentration for a given genotype.

In this preliminary part of the study, we found significant variation in both iron and zinc concentration among genotypes and less, but still significant, variation among plots within genotypes and among plants from the same plot for at least one or the other of the micronutrients, in each case. We did not find significant variation in either micronutrient among roots from the same plant, nor from one part of a root to another. Our data suggests that meaningful data based on one root per replication is possible; a combined sample including two or more roots per replication extends the rigorousness of the assay. It is also straightforward and sound to dissect roots for sampling representative of the entire root.

Genotypic variability studies revealed a three fold difference between the low- and high-performing genotypes in both iron and zinc concentration; in short, exploitable genetic diversity does exist. Importantly, we also discovered a highly significant correlation between iron and zinc content; the same genotypes that ranked highly in concentration of one mineral also tended to rank highly in concentration of the other. Genotypes also tended to repeat their general positions in the rankings from year to year. 'Pata de Oso', from Peru, ranked first or second in iron concentration in both years and ranked within the top three in zinc concentration in both years as well; in fact, it ranked highest in zinc content in 2004. It would be an excellent genotype to examine as a potential parent in the sweetpotato biofortification program. A second genotype, 'Kyukei No. 63', from Japan, also ranked highly in both minerals in both years. A third genotype, 'Kawogo', from East Africa, ranked in the top three genotypes in both years for iron concentration, but was an exception to our trend in that unlike other high-iron genotypes, it did not rank

highly in zinc concentration. While it is not obvious from these top three performers, an examination of the overal rankings reveals an interesting geographic trend. In general, the genotypes from Latin America are clustered at the top of the list, and those from the Far East and Pacific have a tendency to rank towards the bottom. It would be interesting to examine whether there are traits held in common among these large groups of genotypes, and see if any of them could be correlated with iron and zinc content.

Finally, high broad-sense heritability estimates for iron and zinc concentration infer that traditional breeding methods can be used to improve the nutritional value of the crop. Heritability and meaningful genetic diversity leads us to believe that sweetpotato can contribute to the alleviation of iron and zinc malnutrition in sub-Saharan Africa and other underdeveloped areas around the world.

APPENDIX A: STATISTICAL ANALYSES

Root Variation

The following code is used in the proximal-distal variation experiment. It is also representative of the code used in the cambium-cortex, root to root, and plant to plant experiments, with the exception that in the root to root study, the variables:

```
year variety root zone
```

were changed to:

```
year variety plant root
```

and in the plant to plant experiment, they were changed to:

```
year variety plot plant
```

```
dm'log;clear;output;clear';
title1 'proximal/distal';

data sweet;
input year variety root zone dryWeight ironDry ironFresh zincDry zincFresh;
datalines;

(partial data set)
1 101 1 1 0.32 11.78 3.88 5.58 1.84
1 101 1 2 0.31 12.43 3.94 7.31 2.31
1 101 1 3 0.30 13.05 4.00 7.41 2.27
1 101 1 4 0.31 15.33 4.77 8.00 2.48
1 101 1 5 0.34 24.48 8.56 10.10 3.53
2 111 3 4 0.30 13.28 4.08 10.00 3.07
2 111 3 5 0.32 11.98 3.88 9.26 3.00
2 111 4 1 0.30 25.56 7.89 16.66 5.14
2 111 4 2 0.31 18.28 5.71 12.60 3.93
2 111 4 3 0.31 18.35 5.74 12.57 3.93
2 111 4 4 0.32 14.56 4.73 11.52 3.74
2 111 4 5 0.34 15.58 5.33 11.49 3.93
run;

title2 'analyze entire data set to determine whether there is a year effect';
proc glm data=sweet;
class year variety root zone;
model dryWeight ironFresh zincFresh = year variety root(variety) zone(root);
run;

title2 'generate separate data sets by year';
data y1;
set sweet;
where year=1;
run;

data y2;
set sweet;
where year=2;
run;

title2 'run analysis on year 1 subset';
proc glm data=y1;
class variety root zone;
model ironFresh zincFresh dryWeight= variety root(variety) zone(root);
```

```
random root(variety);
run;

title2 ‘run analysis on year 2 subset’;
proc glm data=y2;
class variety root zone;
model ironFresh zincFresh dryWeight= variety root(variety) zone(root);
random root(variety);
run;
```

Root Contamination

```
dm’log;clear;output;clear’;
title1 ‘roots by condition’;

data roots;
  input labcode $ condition $ rootweight dishweight dryweight freshweight drymatter
aldry alfresh fedry fefresh zndry znfresh;
datalines;
U-1      1normal 57.4   2.24 12.97 4.07 0.1705  38.77  6.61  35.48  6.05 16.42 2.80
U-2      1normal 81.9   2.22  9.47 3.69 0.2028   6.34  1.29  26.98  5.47  9.92 2.01
U-3      1normal 57.9   2.22 14.39 4.84 0.2153   2.83  0.61  23.40  5.04 15.24 3.28
U-4      2tap    97.73  2.24  9.19 3.63 0.2000   3.18  0.64  23.69  4.74  9.22 1.84
U-5      2tap   104.39  2.23 15.36 4.56 0.1775   2.34  0.41  38.36  6.81 15.35 2.72
U-6      2tap    91.89  2.22 11.62 4.03 0.1926   2.15  0.41  23.23  4.47 11.90 2.29
U-7      3dirty 103.61  2.22 15.67 4.79 0.1911  22.33  4.27  48.85  9.33 13.74 2.63
U-8      3dirty  98.89  2.22 19.7  5.74 0.2014  38.09  7.67  57.14 11.51 10.14 2.04
U-9      3dirty  75.91  2.22 13.96 4.75 0.2155  74.64 16.08 101.12 21.79 12.92 2.78
U-10     4skin  103.61  2.26 12.95 4.26 0.1871 155.69 29.13 185.34 34.68 15.12 2.83
U-11     4skin   98.89  2.22 12.72 4.3  0.1981 178.87 35.43 203.74 40.36 10.89 2.16
U-12     4skin  103.61  2.2  11.13 3.96 0.1971 191.82 37.81 197.06 38.84 13.29 2.62
run;

proc glm data=roots;
class condition;
model drymatter aldry alfresh fedry fefresh zndry znfresh = condition;
means condition;
run;
```

Tips Contamination

```
dm “output;clear;log;clear”;

title1 ‘Tips Analysis’;
title2 ‘analyze both sets of data to determine whether there is a
batch effect’;

data main;
input  batch rep genotype condition drymatter ironDry ironFresh
zincDry zincFresh aldry alfresh;
datalines;

(partial data set)
2 1 101 2 0.18 34.07 6.43 14.04 2.65 2.70 0.50
2 1 102 1 0.06 50.08 3.03 12.49 0.75 14.42 0.87
2 1 102 2 0.05 69.83 4.17 25.96 1.55 19.50 1.16
1 3 66 1 0.10 39.45 4.19 30.11 3.20 3.47 0.37
1 3 66 2 0.11 12.01 1.33 26.16 2.90 11.43 1.27
1 3 67 1 0.16 31.02 5.17 29.14 4.85 2.55 0.42
1 3 67 2 0.13 26.22 3.54 30.50 4.12 4.02 0.54
1 3 101 1 0.15 40.70 6.40 20.36 3.20 7.15 1.12
1 3 101 2 0.13 37.83 5.20 23.64 3.25 6.05 0.83
1 3 102 1 0.12 36.28 4.64 33.79 4.32 9.71 1.24
1 3 102 2 0.09 38.91 3.79 31.87 3.10 8.45 0.82
1 4 49 1 0.15 32.69 4.92 24.10 3.62 6.36 0.95
run;
```

```
proc glm data=main;
class batch rep genotype condition;
model drymatter ironDry ironFresh zincDry zincFresh aldry alfresh=batch condition
  genotype batch*genotype condition*genotype
batch*condition*genotype;
test h=genotype condition e=condition*genotype;
means batch;
run;

title2 'run analysis on batch 1 subset';
data batch1;
set main;
where batch=1;
run;

proc glm data=batch1;
class rep genotype condition;
model drymatter ironDry ironFresh zincDry zincFresh aldry alfresh=
condition genotype condition*genotype;
test h=genotype condition e=condition*genotype;
means genotype condition;
run;

title2 'run analysis on batch 2 subset';
data batch2;
set main;
where batch=2;
run;

proc glm data=batch2;
class rep genotype condition;
model drymatter ironDry ironFresh zincDry zincFresh aldry alfresh=
condition genotype condition*genotype;
test h=genotype condition e=condition*genotype;
means genotype condition;
run;
```

Genotypic Variation

```
dm "output;clear;log;clear";
title1 'Genotype Study';

data main;
infile 'z:\research\data\genotype\dataSets\qryGtype3.txt';
input  year rep genotype root drymatter iron zinc aluminum ironDry zincDry;
run;

(partial data set)
1.00 4.00 105.00 2.00 0.19 3.38 1.72 0.00 16.91 8.60
1.00 4.00 106.00 2.00 0.24 4.15 2.06 0.00 16.80 8.38
1.00 4.00 106.00 3.00 0.21 4.37 2.09 0.41 20.16 9.66
1.00 4.00 107.00 2.00 0.21 3.91 1.89 0.00 17.78 8.63
1.00 4.00 107.00 3.00 0.21 3.14 1.77 0.00 14.97 8.45
1.00 4.00 108.00 1.00 0.30 4.92 2.98 0.00 16.31 9.88
1.00 4.00 108.00 2.00 0.30 5.95 3.13 0.00 19.30 10.15
2.00 1.00 5.00 1.00 0.33 6.04 1.46 0.00 17.99 4.37
2.00 1.00 5.00 2.00 0.30 5.71 1.93 0.00 18.76 6.33
2.00 1.00 6.00 1.00 0.30 4.87 3.38 0.40 16.06 11.13
2.00 1.00 6.00 2.00 0.28 3.08 2.12 0.00 10.68 7.37
2.00 1.00 7.00 1.00 0.27 5.52 2.60 0.35 20.01 9.45
2.00 1.00 7.00 2.00 0.26 5.03 2.52 0.27 19.11 9.57
2.00 1.00 7.00 3.00 0.26 4.87 2.35 0.34 18.13 8.74
2.00 1.00 9.00 1.00 0.28 4.00 2.86 0.35 13.96 10.00
2.00 1.00 9.00 2.00 0.28 3.29 2.19 0.00 11.42 7.60
2.00 1.00 11.00 1.00 0.34 5.78 2.52 0.00 16.64 7.26
2.00 1.00 11.00 2.00 0.34 4.87 2.15 0.00 14.27 6.31
```

```
title2 ‘Model Both Years for all Variables to See if there is a Year Effect’;
proc glm data=main;
class year rep genotype;
model drymatter iron zinc=year genotype year*genotype rep(year);
run;

title2 ‘generate separate data sets by year’;
data y1;
set main;
where year=1;
run;

data y2;
set main;
where year=2;
run;

title2 ‘2004 ANOVA’;
proc glm data=y1;
class year rep genotype;
model iron zinc drymatter aluminum = genotype rep;
run;

title2 ‘2005 ANOVA’;
proc glm data=y2;
class year rep genotype;
model iron zinc drymatter aluminum = genotype rep;
run;
```

Heritability

```
dm “output;clear;log;clear”;
title1 ‘Heritability’;

data three;
infile ‘z:\research\data\heritability\three.txt’;
(partial data set)
1.00 103001 4 4 0.32 3.54 2.14 0.00
1.00 103001 5 5 0.32 3.51 2.13 1.17
2.00 103001 6 6 0.33 5.55 4.36 1.44
2.00 103001 8 8 0.28 5.23 3.99 1.07
1.00 103001 9 9 0.22 4.16 2.29 1.29
2.00 103001 9 9 0.27 5.42 4.66 0.00
1.00 103003 1 21 0.34 7.22 3.13 1.50
2.00 103003 1 21 0.30 3.06 1.97 2.32
1.00 103003 10 30 0.30 4.10 1.78 1.30
2.00 103003 11 31 0.25 4.65 3.26 0.00
1.00 103003 11 31 0.29 3.36 1.37 1.16
2.00 103003 12 32 0.27 3.41 2.93 0.00
input  year mother genotype individual drymatter iron zinc;
run;

Proc glm data=three;
Class year individual mother genotype;
Model drymatter iron zinc = year mother year*mother
genotype(mother) year*genotype(mother);
Run;
```

APPENDIX B: SOIL CHARACTERISTICS AT RESEARCH PLOTS

Location	Ca, ppm	Cu, ppm	Mg, ppm	pH (1:1 Water)	P, ppm	K, ppm	Na, ppm	S, ppm	Zn, ppm
Burden	849.64	0.00	133.21	5.59	89.79	67.35	47.37	0.00	0.00
Chase	794.69	1.14	126.70	5.44	41.48	60.60	67.60	9.64	1.45

VITA

Michael W. Courtney was born in Baton Rouge, Louisiana, on November 4, 1965. He attended Louisiana State University, receiving a Bachelor of Fine Arts in 1995, whereupon he worked in the graphic design industry for a number of years. He returned to LSU in 2004 to pursue a matster's degree in horticulture and is a candidate for the degree of Master of Science during Spring Commencement 2007.