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Iron and zinc physiology in sweetpotato

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IRON AND ZINC PHYSIOLOGY IN SWEETPOTATO

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the
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Master of Science

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by
Mary Catherine Singleton
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ABSTRACT

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is an important provider of nutrition in developing countries. High productivity in the form of starch and beta carotene richness underlies its potential. A sweetpotato with adequate levels of iron and zinc would greatly aid efforts to reduce dietary deficiencies of iron and zinc. The objective of this work is to document the physiological accumulation of iron and zinc in sweetpotato during development and the mechanisms responsible for iron uptake.

Six varieties ('IPS 163, 'Beauregard', 'Koto-puki', 'Pung-mi', 'Duanyanghon', and 'Xushu 18') were compared to understand iron and zinc accumulation during development. Results showed that micronutrient content in developing roots varied minimally during storage root development (13-16 weeks after planting). Varieties tend to accumulate these micronutrients similarly throughout development. These results suggest that varieties can be assayed for iron and zinc concentration anytime roots are of sufficient size for analysis.

Hydroponic studies were conducted to determine how high and low iron concentration genotypes respond to the absence of iron in nutrient solution. Variables included iron reductase, pH, and root mass development. Results from the nutrient solution study showed that the pH of nutrient solution increased. A low pH environment facilitates the transition from an unavailable form of Fe (III) to an available form Fe (II). Sweetpotato may respond differentially than other species.

Iron reductase activity differed among varieties. High iron accumulating 'Pata de Oso' showed reduced iron reductase activity in plants grown in an iron deficient environment. In contrast, 'Pung-mi', a low iron accumulating variety, significantly increased iron reductase activity when grown in an iron deficient environment. These results suggest that high iron accumulating varieties did not increase iron reductase activity. Varieties poor in iron

accumulation either did not vary or seemed to increase iron reductase activity in an iron deficient environment. A greater root mass is also a means by which plants can uptake greater amounts of iron. High iron concentration varieties had the lowest root mass in comparison to low concentration varieties. When Fe-sufficient and Fe-deficient treatments were compared, only 'Pung-mi' showed a significantly smaller root mass when grown in an Fe-deficient environment.

CHAPTER 1: INTRODUCTION

1.1 Iron Nutrition in Humans and Iron Deficiency

Iron deficiency may affect three billion people worldwide (Long *et al.*, 2004). In developing countries like Africa, iron deficiency is widespread because of poor nutrition and iron loss by parasitic infection (Hercberg *et al.*, 1987). Iron is an important component in human diets because it regulates enzyme activity and plays a role in the immune system (Lynch, 2003). It is also an important component of human blood because iron is the central atom of hemoglobin (Tuman and Doisy, 1978). Humans require 10-15 milligrams of iron per day; if iron levels are not regulated, the deficiency can lead to mental and psychomotor impairment in children, and an increase in both morbidity and mortality of mother and child at childbirth (Frossard *et al.*, 2000). Most adults ages 20-50 years old require 14 μg iron/ kg of body weight for males and 22 μg / kg of body weight for females (Herbert, 1987).

Anemia occurs when an individual does not absorb the necessary amount of iron from the bloodstream. When the hemoglobin level of an individual falls below a cut-off point defined according to sex, age and other physiological considerations, the cause is usually anemia (Hercberg *et al.*, 1987). Anemia affects over 80 million African children and over 60 million African men and women (Hercberg *et al.*, 1987).

Iron losses from the body occur from the shedding of cells internally and externally; most of which occurs in the gastrointestinal tract (Hercberg *et al.*, 1987). Men have a mean iron loss of 14 $\mu\text{g}/\text{kg}/\text{day}$ (Finch, 1959). The average total iron losses in menstruating women are approximately 1.4 mg/day (Hercberg *et al.*, 1987). The amount of iron absorbed is impacted by three variables: the amount of iron ingested, its bioavailability, and the iron status of the

individual (Herberg *et al.*, 1987). Iron in food is present in two forms: heme iron and non-heme iron; of the two, heme iron is easily absorbed into the body (Herberg *et al.*, 1987).

1.2 Zinc in Human Physiology and the Symptoms of Zinc Deficiency

Malnutrition is the most common cause of zinc deficiency (Ronaghy, 1987). Over three billion people worldwide suffer from malnutrition (Welch and Graham, 2004). Health problems caused by zinc deficiency include anorexia, dwarfism, weak immune system (Solomons, 2003) skin lesions, hypogonadism, and diarrhea (McClain *et al.*, 1985).

Zinc is found primarily in bone and skeletal muscles of humans (Frossard *et al.*, 2000). Zinc plays an important role in the immune system; it is necessary for T lymphocyte development (Ronaghy, 1987). Alcohol dehydrogenase, an enzyme that breaks down toxins in the human body, also depend on an adequate zinc supply to function properly (Ronaghy, 1987). In Africa, it is estimated that 500-600 million people are at risk for low zinc intake (HarvestPlus, 2007). Males, ages 15-74, need between 12-15 milligrams of zinc daily, while females, ages 12-74, need between 6-8 milligrams of zinc daily (Sandstead, 1985).

1.3. Iron's Function in Plants

Iron is one of the 16 essential elements needed for plant growth. Iron is used for the synthesis of chlorophyll and is essential for the function of chloroplasts (Abadia, 1992). Without sufficient iron levels, plants show apical leaf chlorosis and slower root growth.

Despite the usually high abundance of iron in soils, the low solubility of iron bearing minerals limits the iron available for uptake by higher plants (Schmidt, 1999). Although abundant in soil, iron is one of the most common nutrients limiting plant growth in the world (Guerinot, 2001).

Higher plants are divided into two categories by the way plants convert unavailable iron, Fe (III), into available iron, Fe (II). Strategy I plants (non-graminaceous plants) convert Fe (III) into Fe (II) by proton extrusion through ATPases and iron reduction by Fe (III) reductases located in the plasma membrane of root cells (Mok *et al.*, 2000). The reduction of Fe (III) to Fe (II) by ferric chelate reductase (iron reductase) is thought to be an obligatory step in iron uptake for Strategy I plants (Frossard *et al.*, 2000). Sweetpotato is a Strategy I plant. Strategy II plants (graminaceous plants) uptake Fe (II) by releasing high affinity chelates, called phytosiderophores, that form Fe (III) complexes and are absorbed into the roots (Zaharieva *et al.*, 2000).

1.4 Iron Reductase

Iron is prevalent in many types of soils in the form of Fe (III), which is unavailable to plants (Schmidt, 1999). To capture iron from the soil, plants use an enzyme called iron reductase, which converts Fe (III) to Fe (II) (Romera *et al.*, 2003). Strategy I and Strategy II plants contain iron reductases in the plasma membrane of the root cells, but in Strategy I plants, iron reductase activity is regulated by the availability of iron (Mok *et al.*, 2000). Reduction of iron from ferric to ferrous on the root surface is a necessary process for iron uptake in Strategy I plants (Lihua *et al.*, 2004). Sweetpotato is a Strategy I plant and likely regulates iron uptake by iron reductase. Plants have several responses to iron deficiency, including lowering the pH to make iron more available and increasing the root area to mine for iron and other micronutrients (Romera *et al.*, 2003).

1.5 Zinc's Function in Plants

As one of the essential elements needed for plant growth, zinc deficiencies can cause many problems within the plant. Visual symptoms of zinc deficiency in plants are leaf mottling,

interveinal chlorosis, and reduced plant growth. Zinc is involved in membrane integrity, enzyme activation, and gene expression (Kim *et al*, 2002). Despite the importance of zinc as a micronutrient for plant growth, there have been relatively few studies of the mechanism of zinc uptake (Reid *et al*, 1995). 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 Romheld, 1999).

1.6 About Sweetpotatoes

By weight, sweetpotato [*Ipomoea batatas* (L.) Lam.] is the seventh most important food crop worldwide, after wheat, rice, maize, potato, barley, and cassava (Woolfe, 1992). It is the only member of the *Convolvulaceae* family that is of major economic importance (Woolfe, 1992). The extensive acreage dedicated to sweetpotato is due to a number of environmental and economic factors. Known as a food security crop, sweetpotato is able to yield substantial tonnage in environments that poorly accommodate rice, wheat, or corn. Sweetpotato is a hardy tropical crop that grows with ease on marginal land endemic to the region with the added plus of having good nutritional benefits. In Africa, sweetpotato is regarded as the highest biomass-producing crop, critical given the small acreage producers have (Woolfe, 1992).

In East Africa, white flesh sweetpotatoes are most commonly grown. This is counter to what is found in the United States where beta-carotene rich, orange-fleshed sweetpotato is universally grown. There is a concerted effort to introduce orange-flesh sweetpotatoes to the East African region to enhance nutrition.

In poor countries like Africa, vitamin A deficiency is widespread, especially in children (Low *et al.*, 2007). It is estimated that 127 million children worldwide suffer from vitamin A

deficiency (West, 2002), which can cause a weak immune system and potentially cause a condition that leads to blindness (Sommer and West, 1996). Vitamin A is a precursor to retinol A which is essential for normal ocular development (Biofortified Sweetpotato, 2006). Sweetpotato is a promising food security crop for underdeveloped countries like Africa because sweetpotatoes are high in beta-carotene, easily grown, produce high amounts of biomass, and are drought resistant (Low *et al.*, 2007). Combining beta-carotene, iron, and zinc in a well adapted sweetpotato variety would be valuable in combating micronutrient deficiencies in the region.

1.7 Heritability Estimates for Iron and Zinc Uptake

Heritability is a measure of the extent to which observed phenotypic differences for a trait are due to genetic differences (Klug and Cummings, 2005). Heritability estimates represent an efficient means of determining the feasibility of improving traits (Jones, 1986). The two measurements of heritability are broad-sense heritability (H^2) and narrow-sense heritability (h^2). Broad-sense heritability measures the proportion of phenotypic variance (V_P) that is due to genetic variation (V_G) for a single population under the limits of the environment during the experiment (Klug and Cummings, 2005). Broad-sense heritability can be calculated $H^2 = V_G/V_P$ (Klug and Cummings, 2005). 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). Narrow-sense heritability (h^2) is the proportion of phenotypic variance (V_P) due to additive genotypic variance (V_A) (Klug and Cummings, 2005). Narrow-sense heritability estimates are useful for predicting the phenotypes of offspring during selection; 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 knowledge of parental phenotypes (Klug and Cummings, 2005). Narrow-sense heritability can be calculated $h^2 = V_A/V_P$ (Klug and Cummings, 2005).

Heritability estimates showed high broad-sense heritability for iron ($H^2=0.73$), zinc ($H^2=0.81$), and dry matter ($H^2=0.93$) among sweetpotato half-sib families. There was also a positive correlation between iron and zinc content in sweetpotato storage roots (Courtney, 2007). Previous research (Courtney, 2007) showed that roots on the same plant do not vary from one another; roots of the same genotype from different plants in the same plot do not vary significantly from one another. Thus, no significant variation found in roots from the same plant; meaning that only one root per replication is necessary for sampling. This indicates that genotype variability among roots from the same line is minimal.

A main goal of sweetpotato breeding is to develop a sweetpotato root high in iron and zinc content. Data suggests traditional breeding strategies can be used to improve micronutrient content given high and meaningful genotypic variability and high heritability. The focus is now on developing quick and efficient selection techniques for high iron and zinc content.

Mass selection approaches in sweetpotato require a two year cycle. In the first year, the crosses are made; the following summer, the true seed is planted and mature roots are harvested after the growing season. Roots are assayed over winter, and not until the following summer are lines selected for inclusion in the next breeding cycle nursery. If selection could be made earlier during development, (e.g., small, immature fleshy roots), then the cycle time could be cut in half.

Courtney (2007) found that genotypic variation of iron and zinc concentration exists in sweetpotato storage roots. There is a 2.5 to three fold difference in genotypic iron concentration. There is more than a three fold difference in genotypic zinc concentration. Courtney (2007) found a highly significant correlation between iron and zinc content; genotypes that ranked high

for iron content also ranked high for zinc content. Genotypes remained consistent in rankings in both years. A Peruvian line, 'Pata de Oso', ranked high in both years for iron and zinc content and would make a good parent in a crossing nursery.

Broad-sense heritability estimates for iron and zinc were 0.74 and 0.82, respectively. These numbers are encouraging because iron and zinc content can be improved using traditional mass-selection techniques. Courtney (2007) found that dry matter heritability estimates were 0.92; these results were also encouraging since consumers in third world countries prefer sweetpotatoes with high dry matter content. Since dry matter had a high heritability estimate, iron and zinc can be improved and still produce a sweetpotato that is high in dry matter.

1.8 Objectives

There are three main objectives to this research. The first objective is a study on the accumulation of iron and zinc in sweetpotato storage roots during development. The ability to select genotypes early in the growth cycle permits early selection and genetic recombination. The second objective is to study how sweetpotato genotypes, both high and low in iron accumulation extract iron from their environment. The third objective is a study on the iron and zinc concentrations in greenhouse grown sweetpotato leaves. The objectives are to develop screening methods to select for iron and zinc as early as possible in the breeding cycle and understand how sweetpotato accumulates iron and zinc during development.

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CHAPTER 2: IRON AND ZINC ACCUMULATION DURING STORAGE ROOT DEVELOPMENT

2.1 Introduction

Sweetpotato is a vital commodity to small-scale farmers with limited land, labor, and capital (Courtney *et al.*, 2008). Sweetpotato performs well in poor soils that lack sufficient nutrients and water as well as thrives in fertile environments where yields exceed those of cereal crops (Woolfe, 1992). Sweetpotato is known as a food security crop because of its ability to thrive in the tropic's harsh conditions and infertile soils, unlike corn or maize. Although sweetpotato is invaluable in combating chronic food shortages, the crop could contribute further to dietary micronutrition in regions of the tropics and subtropics where micronutrient deficiencies are pronounced (Reddy *et al.*, 2005). Current strategies to overcome these shortages abound, from fortified, processed foods to vitamin supplements; therefore, a complement to these approaches would be to fortify existing staple crops (HarvestPlus, 2007). Notable among these efforts is the development of carotene-enriched rice (golden rice) through genetic transformation (Datta *et al.*, 2007) and β -carotene-rich sweetpotato germplasm. The use of this approach has been demonstrated with sweetpotato through the improved vitamin A status of children (van Jaarsveld *et al.*, 2005). Our present interest is to complement the known caloric and carotenoid contributions of sweetpotato to the diet by improving iron and zinc concentrations.

Previous research identified the genotypic range of iron and zinc in sweetpotato for human nutrition (Courtney, 2007). Courtney (2007) estimated the general daily requirement for iron and zinc at 8 $\mu\text{g}/\text{day}$ (<http://www.iom.edu/Object.File/Master/7/294/Webtableminerals.pdf>) with a typical root, weighing 300 g. This concentration represents a mid-range level of iron required for most demographic groups, e.g., children, adult men, non-pregnant adult women. Courtney (2007) showed that various sweetpotato genotypes ranged from provided 25% to 30%

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)]. The high concentration of iron and zinc could be enhanced further. Courtney *et al.*, (2008) showed that inheritance levels on a broad-sense basis were relatively high (0.74 for iron; 0.82 for zinc) and traditional breeding approaches like mass selection could be used.

Mass selection in sweetpotato requires a two-year cycle. In the first year, the crosses are made and the following summer the true seed is planted and mature roots are harvested after the growing season. Roots are assayed over winter and not until the next summer are lines selected for inclusion in the next breeding cycle nursery. If selection could be made earlier during development, e.g., small, immature fleshy roots, then the cycle time could be reduced. Therefore, the objective of this research was to study the physiological accumulation of iron and zinc of sweetpotato during storage root development. The ability to select genotypes early in the growth cycle permits identification of superior micronutrient rich parents for recombination in crossing nurseries.

2.2 Materials and Methods

Field research was conducted at Hammond Research Station at Hammond, Louisiana in 2006 and 2007. Before planting, soil was fertilized with 280 kg ha⁻¹ of 13-13-13. The soil was is a cabana fine sandy loam with a pH range of 5.0-5.5. The plot was a complete randomized design. Genotypes included in the study were ‘Pung-mi’ (Korea), ‘IPS 163’ (Australia), ‘Los Cerrillas’ (Uruguay), ‘Duanyanghon’ (China), ‘Pata de Oso’ (Peru), ‘Kamula Belep (New Caledonia), ‘Koto-puki’ (Japan), ‘Yanshu 1’ (China), ‘Xushu 18’ (China), ‘L3’ (Papua-New

Guinea), 'Kalmegh S-30' (India), '19' (New Zealand), 'Guangshu 70-9' (China), 'Bugsbunny' (Puerto Rico), and 'ACC 309' (Solomon Islands). Genotypes were replicated three times. There were 20 plants per plot, spaced 0.3 m apart and 1.5 m between plots. Ten weeks after planting, (September 19, 2006 and August 31, 2007, respectively) roots were collected weekly for a period of six weeks. One root from each genotype was considered for analysis. Previous research indicated that one root per replication was sufficient for sampling (Courtney, 2007).

Processing methods 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. Roots were then rinsed in double distilled water, peeled with a stainless steel knife, and rinsed in double distilled water a second time. The roots were sectioned, weighed, and dried at 80°C for 48 hours, after which they were weighed again. Dry samples were pulverized using an IKA A10 Basic Analytical Mill (IKA Works, Inc, Wilmington, NC), then bottled in Corning Snap-Seal tubes (product no. 1730, Corning, New York), and stored at ambient temperature until assayed for iron and zinc concentration.

Analysis for aluminum, iron, and zinc was based on the methods developed by Huang and Schulte (1985), and Havlin and Soltanpour (1980). 1 gram samples were digested in a 5ml of nitric acid. The samples were placed on a Magnum 120 Plant/Soil Digester (Ivesdale, IL). After 45 minutes, a 3ml aliquot of H₂O₂ was added to each sample, prior to the block reaching 90°C. The samples were heated until the volume was reduced to 0.5ml, then diluted to 12.5ml using distilled water and filtered using Whatman #2 paper. The samples were then quantified for the minerals via inductively coupled plasma mass spectrometry using a Spectro Ciros CCD (Kleve, Germany).

For every 20 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 3ppm dwb, were considered to be contaminated and were discarded. A generic threshold of >5-6ppm dwb (dry weight basis) was suggested by Pfeiffer and McClafferty (2007), but recent perspectives suggest >3ppm to be more appropriate (Pfeiffer and McClafferty, 2007). Additional research is needed in the area of contamination thresholds.

The iron, zinc, and dry matter calculations were analyzed using PROC MIXED. Means and letter groupings were produced using PDMIX800.SAS macro.

2.3 Results and Discussion

2.3.1 Iron Accumulation in Storage Roots Over Harvest Intervals

The six genotypes chosen for iron and zinc analysis were ‘Beauregard’, ‘Koto-puki’, ‘Pung-mi’, ‘Xushu 18’, ‘IPS 163’, and ‘Duanyanghon’. Data was collected from week thirteen to sixteen. Roots prior to thirteen weeks were too small and roots at the termination of the study were at marketable size. Growing conditions and the period in which this research was conducted extended through the root enlargement period. Statistical analysis showed no significant difference in week and genotype and week interactions, indicating that no significant change in iron and zinc occurred over time. Furthermore, no difference was observed for year (as a random effect); therefore, data was combined and presented in Table 2.1. Results showed that genotypes on a dwb differed significantly at $p=0.05$ (Table 2.1). Mean iron concentration for ‘IPS 163’ was significantly higher in comparison to all other genotypes (Table 2.1). ‘Xushu 18’ and ‘Beauregard’ were the next highest ranking genotypes differing only from ‘Koto-puki’ and ‘Pung-mi’. ‘IPS 163’ had 90% higher iron concentrations in comparison to ‘Pung-mi’. This demonstrates that the genotypes included in the study had broad diversity in iron uptake

potential. These results are consistent with Courtney (2007). He found ‘IPS 163’ and ‘Beauregard’ had high iron concentrations in comparison to ‘Koto-puki’ and ‘Pung-mi’.

Table 2.1 Iron and zinc concentration of sweetpotato cultivars (2008).

| Genotype Information | | Iron (mg/kg dwb) | Zinc (mg/kg dwb) |
|----------------------|--------------------|------------------|------------------|
| Cultivar | Country (Origin)** | Mean±SD* | Mean±SD* |
| Beauregard | United States | 17.18±1.46bc | 6.96±1.51b |
| Koto-puki | Japan | 13.88±1.57de | 4.27±1.55d |
| Pung-mi | Korea | 12.58±1.52e | 5.74±1.53c |
| Xushu 18 | China | 17.87±1.48b | 5.99±1.51bc |
| IPS 163 | Australia | 23.85±1.49a | 11.39±1.52a |
| Duanyanghon | China | 15.48±1.51cd | 5.88±1.53bc |

**Country of origin <http://www.ars-grin.gov/npsg/>

*Results within a column followed by common letter do not differ significantly at 0.05% level.

Low week to week variability is demonstrated by ‘IPS 163’ (Fig 1). The difference between iron concentrations in week thirteen verses week sixteen, 26.6 ppm and 22.0 ppm, respectively, was less than 17%. Some genotypes had moderate levels of iron concentrations, ‘Koto-puki’, ‘Xushu 18’, and Duanyanghon’. ‘Koto-puki’ varied minimally in iron concentrations over the harvest intervals (Fig 1). The highest iron concentration was observed at week fourteen, 14.7 ppm, and the lowest iron concentration was at week sixteen, 13.1 ppm, respectively, a difference of 11%. ‘Xushu 18’ iron concentrations did decline somewhat over the harvest intervals. The highest iron concentration was at week fourteen, 19.2 ppm, and lowest at week sixteen, 15.6 ppm, respectively, a difference of 19%. ‘Duanyanghon’ varied from 17.8 ppm at week fourteen to 14.2 ppm at week sixteen, respectively, a difference of 20% (Fig 1). Low week to week variability is demonstrated by the lowest ranking genotype, ‘Pung-mi’ which varied from 12.9 ppm at week sixteen to 12.2 ppm at week fourteen, a difference of only 6% (Fig 1). Variability of iron concentration was less than expected and ranks changed only modestly from week to week. This in combination with a lack of statistical significance between weeks

suggests that roots maintain a relatively consistent level of iron concentration throughout storage root development.

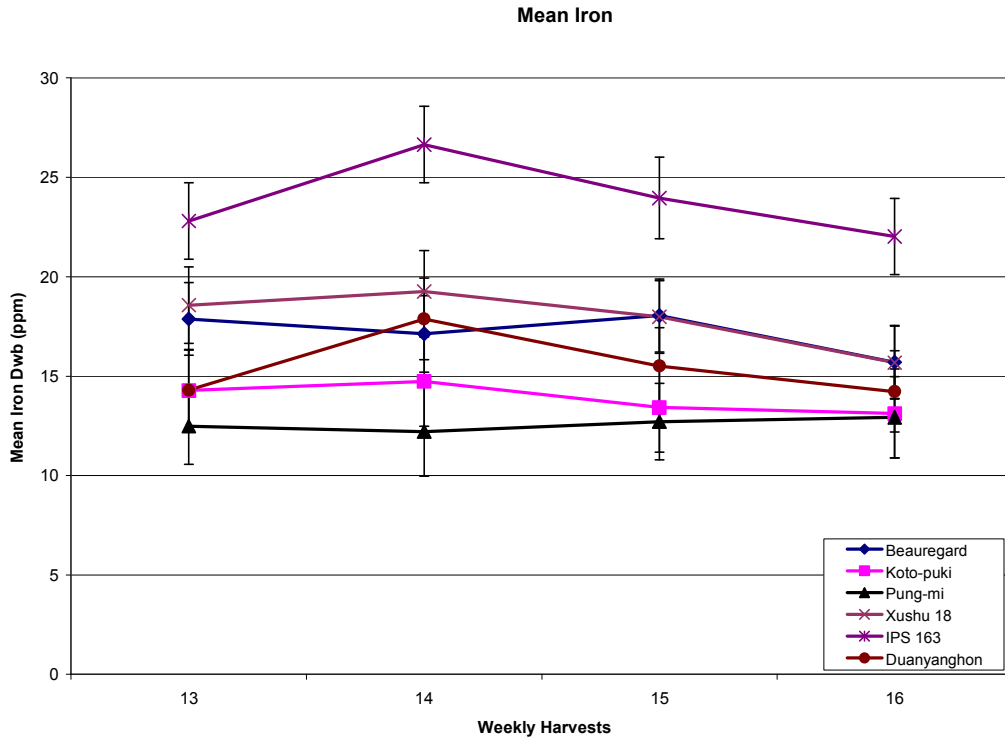


Fig 2.1 Mean iron (dwb) concentrations and standard deviations for sweetpotato genotypes after 13-16 weeks of growth.

Iron concentration from soil could skew results; however, aluminum concentrations never exceeded 3 ppm, indicating that contamination was minimal at best (Pfeiffer and McClafferty, 2007). Previous research (Courtney, 2007) showed no significant correlation between genotypes with high iron and high aluminum concentrations. Levels of aluminum in this previous study were consistent with the present work.

The overall rankings by mean zinc concentration on a dwb followed a similar pattern to the rankings by mean iron concentration. Results for zinc showed that genotypes differed significantly at $p=0.05$ (Table 2.1). Mean zinc concentration for ‘IPS 163’ was significantly higher in comparison to all other genotypes. ‘Beauregard’, ‘Xushu 18’, and ‘Duanyanghon’ were

the next highest ranking genotypes. The lowest ranking genotypes were ‘Pung-mi’ and ‘Koto-puki’.

2.3.2 Zinc Accumulation in Storage Roots Over Harvest Intervals

‘IPS 163’ had 63% higher zinc concentration than ‘Koto-puki’. These results are consistent with Courtney (2007). He found ‘IPS 163’ and ‘Beauregard’ had higher zinc concentrations in comparison to ‘Pung-mi’ and ‘Koto-puki’.

Low week to week variability is demonstrated by ‘IPS 163’ (Fig 2). The highest zinc concentration was at week fifteen with 12.7 ppm and lowest at week sixteen with 10.2 ppm, respectively, a difference of 19%. Another sweetpotato genotype known to have high zinc concentration in storage roots is ‘Beauregard’. The highest zinc concentration for ‘Beauregard’ is at week thirteen with 7.6 ppm and lowest at week sixteen with 6.3 ppm, respectively, a difference of 17%.

There were several sweetpotato genotypes that have moderate zinc concentrations in storage roots (Courtney, 2007). These genotypes are ‘Pung-mi’, ‘Xushu 18’, and ‘Duanyanghon’. ‘Pung-mi’ remained fairly consistent in zinc concentration over harvest intervals. The highest zinc concentration was at week thirteen with 6.3 ppm and lowest at week fourteen with 5.1 ppm, respectively, a difference of 18%. ‘Xushu 18’ had the highest zinc concentration at week thirteen with 6.9 ppm and lowest at week sixteen with 5.0 ppm, respectively, a difference of 27%. ‘Duanyanghon’ had the highest zinc concentration at week fourteen with 7.3 ppm and lowest at week sixteen with 4.8 ppm, respectively, a difference of 33%.

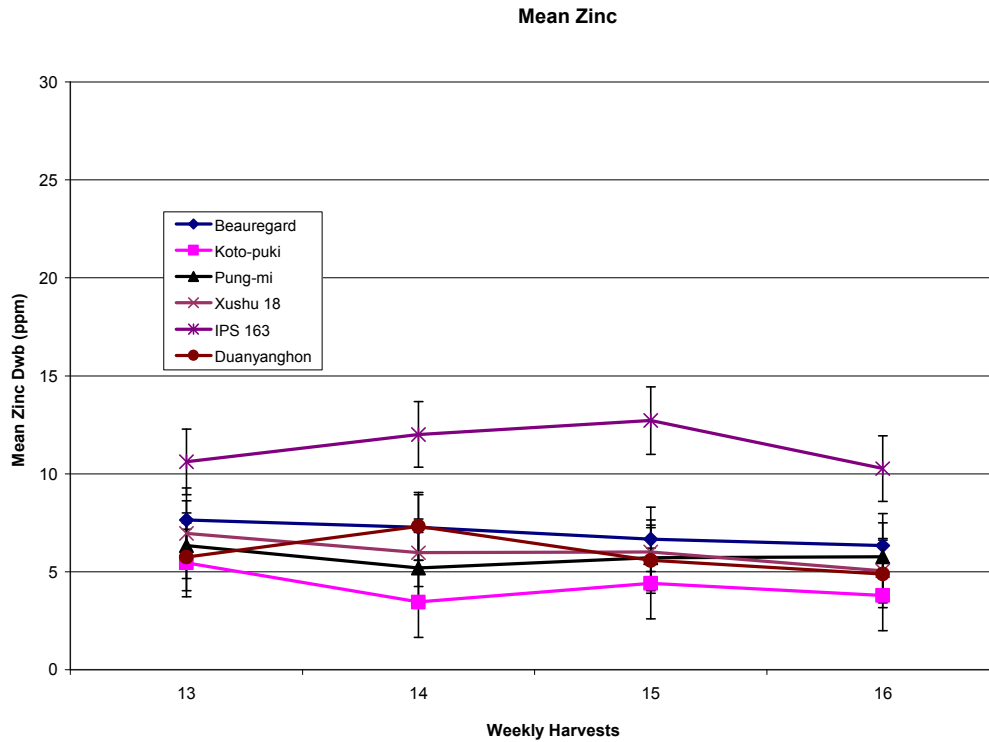


Fig 2.2 Mean zinc (dwb) concentrations and standard deviations for sweetpotato genotypes.

‘Koto-puki’ was the lowest ranking genotype for zinc concentration (Fig 2). The highest zinc concentration was at week thirteen with 5.4 ppm and lowest at week fourteen with 3.4 ppm, respectively, a difference of 37%.

Data was also analyzed to determine the total iron and zinc accumulation in a sweetpotato root. Iron and zinc were both significant at the $p \leq 0.05$ significance level. For iron, replication, variety, week, and year were all significant; only replication and variety were significant for zinc. Data presented in Table 2.3.

Table 2.2 Total iron and zinc accumulation in sweetpotato roots (2008).

| Genotype Information | Iron (mg/kg dwb) | Zinc (mg/kg dwb) |
|----------------------|------------------|------------------|
| Cultivar | Mean* | Mean* |
| 595873 | 1039a | 320bc |
| 599377 | 937ab | 443a |
| 606252 | 791abc | 260bc |
| 508506 | 689bc | 354ab |
| Bx | 572c | 230c |
| 585073 | 523c | 222c |

*Results within a column followed by common letter do not differ significantly at 0.05%.

2.3.3 Iron and Zinc Concentration Evaluated on a Fresh Weight Basis and Dry Matter

The data was also analyzed on a fresh weight basis (fwb) taking dry matter concentrations into account. No significant week effect was found for iron and zinc concentration, indicating that no change in iron (Fig 3) and zinc (data not presented) occurred over time. These results were somewhat consistent with the previous results analyzed on a dry weight basis.

The highest ranking genotype for iron concentration on a fresh weight basis was 'Xushu 18' (Fig 3). The highest iron concentration was at week fourteen with 5.95 ppm and lowest at week thirteen with 4.89 ppm, respectively, a difference of 18%. Sweetpotato genotypes 'Koto-puki' and 'IPS 163' ranked second and third in iron concentration on a fresh weight basis. The highest iron concentration for 'Koto-puki' was at week fourteen with 6.28 ppm and lowest at week sixteen with 4.30 ppm, respectively, a difference of 32%. The highest iron concentration for 'IPS 163' was at week fifteen with 5.3 ppm and lowest at week thirteen with 4.8 ppm, respectively, a difference of 8%. Minimal variability existed in iron concentration on a fwb for 'Duanyanghon'. Iron concentrations ranged from 4.9 ppm at week fourteen to 4.7 ppm at week thirteen, a difference less than 1%. 'Beauregard' had the highest iron concentration at week fifteen with 4.5 ppm and lowest at week sixteen with 3.9 ppm, respectively, a difference of 14%. 'Pung-mi' was the lowest ranking genotype for iron concentration on a fresh weight basis. The highest iron concentration for 'Pung-mi' was at week sixteen with 4.1 ppm and lowest at week thirteen with 3.2 ppm, respectively, a difference of 21%.

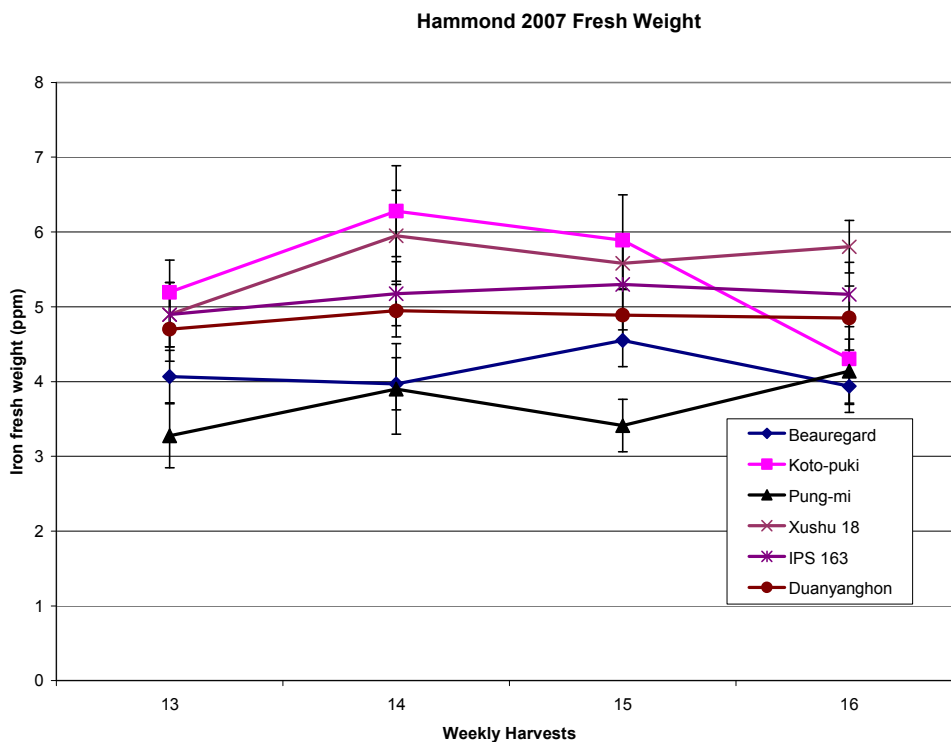


Fig 2.3 Mean iron (fwb) concentrations and standard deviations for sweetpotato genotypes.

Dry matter ranged from 33.16% for ‘Koto-puki’, the highest ranking genotype, to 22.28% for ‘IPS 163’, the lowest ranking genotype (Table 2.2). When ‘Koto-puki’ was evaluated for iron concentration on a dry weight basis, it was one of the lowest ranking genotypes; however, when the same genotype was evaluated by fresh weight, it ranked highest. ‘IPS 163’ was the top ranking genotype when evaluated for iron concentration on a dry weight basis, but fell to the lowest ranking genotype when evaluated by fresh weight.

Previous research (Courtney, 2007) showed dry matter was significantly correlated ($p \leq 0.0001$) with both iron and zinc (corrected for fresh weight). He also showed that positive Pearson correlation coefficients ranged from 0.2472 to 0.4437 with iron by dry matter and zinc by fresh weight; 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 low dry matter, i.e. more fresh matter is needed from a low dry matter line to equal similar amounts to a line with high dry matter.

Table 2.3 Iron concentration on a fresh weight basis and dry matter of sweetpotato cultivars (2008).

| Genotype Information Cultivar | Iron (ppm) ^{z***} | Dry Matter | |
|----------------------------------|----------------------------|-------------|--------------|
| | Country (Origin)** | Mean±SD* | Mean±SD* |
| Beauregard | United States | 4.13±0.17c | 21.41±0.86d |
| Koto-puki | Japan | 5.41±0.28ab | 33.15±1.40a |
| Pung-mi | Korea | 3.62±0.22c | 22.80±1.10cd |
| Xushu 18 | China | 5.55±0.22a | 25.62±1.10bc |
| IPS 163 | Australia | 5.13±0.23ab | 22.27±1.18d |
| Duanyanghon | China | 4.84±0.19b | 27.70±0.96b |

***Data presented on a fresh weight basis.

**Country of origin <http://www.ars-grin.gov/npgs/>.

*Results within a column followed by common letter do not differ significantly at 0.05% level.

Taken in concert, these results indicate that genotypes do differ from one another and there is no difference in storage root iron and zinc concentration between 13-16 weeks. This data suggests that a sweetpotato storage root can be harvested at any time and the micronutrient concentrations measured. Evaluation of genotypes could be made early on during the maturation process, thus speeding the selection procedure. Storage roots can be harvested and analyzed early during maturation and combined in a crossing nursery quickly. Researchers interested in assessing iron and zinc can base an accurate assay at anytime time there is sufficient storage root matter to assess. However, the smaller the root, the more difficult it is in eliminating soil as a contaminant.

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CHAPTER 3: IRON REDUCTASE ACTIVITY

3.1 Introduction

Despite the usually high abundance of iron in soils, the low solubility of iron bearing minerals limits iron concentrations available for plant uptake (Schmidt, 1999). The most common form of iron in aerobic soils is Fe (III) (Schmidt, 1999). Because Fe (III) is not available to the plant, Fe (III) is one of the most common nutrients limiting plant growth in the world (Guerinot, 2001). Higher plants are divided into two categories based on the mechanisms used to convert Fe (III) to Fe (II): Strategy I plants (non-graminaceous) and Strategy II plants (graminaceous) (Romera and Alcantara, 2004).

Strategy I plants have several means of capturing iron from their environment such as altering pH, enhanced iron reductase activity, and mining (i.e., extensive root growth) (Romera, personal communication 2006). The most obvious phenotypic change is the appearance of subapical root hairs. Extensive root hair development is a way to increase surface area and enable more uptake potential. This is particularly so when the rhizosphere is acidified (Romera, personal communication, 2006; Romera and Alcantara, 2004). Acidification is a process that is mediated by plasma membrane bound reductases (Mok *et al.*, 2000). The end result is that protons extruded into the rhizosphere lowers the pH of the soil solution around the root mass and increases solubility of Fe (III) by converting it to Fe (II) (Kim and Guerinot, 2007).

Strategy I plants, like sweetpotato, reduce ferric iron before uptake, a process that is mediated by a plasma membrane-bound redox system (Schmidt, 2003). Strategy I plants convert Fe(III) into Fe(II) by proton extrusion through ATPases and iron reduction by Fe (III) reductases located in the plasma membrane of root cells (Mok *et al.*, 2000). ATPase is an enzyme that transfers protons through the cell membrane to the outside using ATP (Staiger, 2002). Under

iron deficiency, the protons in the rhizosphere lower the pH and reduce Fe (III) to Fe (II) and thus increase solubility of iron (Kim and Guerinot, 2007).

Sweetpotato has the potential of altering the rhizosphere to uptake iron to meet physiological needs. Previous research showed that sweetpotato genotypes vary by 66% in their uptake of iron (Courtney, 2007). In the absence of soluble iron, sweetpotato may alter pH, making the soil solution around the rhizosphere more acidic in order to mine iron out of the soil, relying on iron reductase, or simply producing an expansive root mass to mine iron from the soil (Romera *et al.*, 2003). No previous work has examined how sweetpotato accumulates iron. The objective of this research is to investigate differential expression of iron reductase, root mass, and changes in pH for high and low iron accumulating genotypes.

3.2 Materials and Methods

Based on Courtney (2007) estimates for high and low iron uptake genotypes, four sweetpotato genotypes were selected for this study: ‘Pata de Oso’ (Peru), ‘Pung-mi’ (Korea), ‘Guangshu 70-9’ (China), and ‘Los Cerrillas’ (Uruguay). The genotype with high iron concentration in storage roots, ~7.5 ppm, is ‘Pata de Oso’ (Courtney, 2007). ‘Los Cerrillas’ is moderate in iron concentration, ~5.2 ppm (Courtney, 2007) and genotypes with lower iron concentration are ‘Guangshu 70-9’, ~3.1 ppm, and ‘Pung-mi’, ~2.8 ppm (Courtney, 2007). Stem cuttings taken from vines of each genotype were approximately 18-20 cm long. Each genotype was replicated 4 times in each of two treatments (with and without iron) in nutrient solution.

Each stem cutting was suspended in 500 mL Erlenmeyer flasks filled with one liter of a complete nutrient solution. The nutrient solution contained 9.4463 g/L of calcium nitrate, 3.2042 g/L of Magnesium sulfate, 2.6139 g/L of potassium sulfate, 1.7418 g/L of potassium phosphate, 0.0745 g/L of potassium chloride, 0.0123 g/L of boric acid, 0.0033 g/L of manganese sulfate,

0.0015 g/L of copper sulfate, 0.0028 g/L of zinc sulfate, 0.0012 g/L of ammonium molybdenum, and 0.1468 g/L of iron ethylenediaminetetraacetic acid iron (III) sodium salt (Romera personal communication, 2007). Foam plugs were used to hold the cuttings in the opening of the flask which was covered with aluminum foil. The nutrient solution in flasks was maintained at a full level throughout the study. pH of nutrient solution in flasks was checked every five days to monitor potential pH changes. Air was continually pumped into the flasks using aquarium pumps (Aqua Culture 5-15 gallon/Single Outlet Aquarium Air Pump, Bentonville, Arkansas) to ensure the roots were aerated. Stem cuttings were allowed to grow for five days in a complete nutrient solution, and then half of the stem cuttings were transferred to iron deficient solutions. Stem cuttings were allowed to grow in the two different iron solutions an additional 10-14 days until the iron deficient plants displayed apical leaf yellowing, a common symptom of iron deficiency.

Once the stem cuttings displayed iron deficiency symptoms, the nutrient solution and iron deficient solution were decanted and replaced with a solution containing 0.0367 g/L of iron EDTA [ethylenediaminetetraacetic acid iron (III) sodium salt] and 0.1477 g/L of ferrizine [3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt]. The stem cuttings were immediately placed in the EDTA ferrizine solution. The clear ferrizine solution becomes purple as an indicator of iron changing from (III) to (II) (Ellsworth *et al.*, 1997). The flasks were wrapped in aluminum foil and placed in full sun for 5 hours to facilitate iron uptake. After 5 h, each sample of solution was pipetted into a cuvette and the absorbance read using a Perkin Elmer Lambda 35 UV/VIS Spectrometer (St. Louis, Missouri) at 562.0 nm.

A standard curve solution was made using a stock solution of 300 μM ferrozine and 0.0278g FeSO_4 . A serial dilution was made using both solutions (0, 10, 20, 30, 40, 50 μM Fe). The spectrophotometer was autozeroed using 300 μM ferrozine.

3.3 Results and Discussion

3.3.1 pH Changes of Hydroponic Solution

Data documenting the pH changes of the nutrient solution was analyzed using PROC MIXED (9.1, SAS Institute Inc, Cary, N.C.). Treatment and genotype were the only significant factors at $P=0.05$ significance level. The two treatments were growing plants in a complete nutrient solution and growing plants in an iron deficient nutrient solution. The statistical analysis indicated no significant difference in genotype by treatment.

In Trial 1, all the genotypes grown in Fe-deficient conditions raised the pH of solution. ‘Pata de Oso’ raised the pH from 5.5 to 6.86, an increase of 20%. ‘Pung-mi’ raised the pH from 5.5 to 6.77, an increase of 19%. ‘Los Cerrillas’ raised the pH from 5.5 to 6.28, an increase of 12%. ‘Guangshu 70-9’ raised the pH from 5.5 to 6.89, an increase of 20%.

Trial 2 produced similar results. All genotypes grown in Fe-deficient solution raised the pH of the solution. ‘Pata de Oso’ raised the pH from 5.5 to 6.59, an increase of 17%. ‘Pung-mi’ raised the pH from 5.5 to 6.65, an increase of 17%. ‘Los Cerrillas’ raised the pH from 5.5 to 6.12, an increase of 10%. ‘Guangshu 70-9’ raised the pH from 5.5 to 6.58, an increase of 16%. When the trials were combined, the statistical analysis indicated that ‘Los Cerrillas’ was significantly different from the others.

The pH of the nutrient solution of plants grown in the Fe-sufficient treatment did not change significantly (data not shown). This contrasts with significant changes in the pH of the nutrient solution of plants grown in Fe-deficient treatment. The expectation was for the pH to

decrease rather than increase. A decrease in pH is associated with an environment conducive to reducing unavailable Fe (III) to available Fe (II) (Romera, personal communication, 2006). The implications are that sweetpotato stressed in an Fe-deficient environment may not be able to access iron well. A caveat to our work is that sweetpotato roots may behave differently when grown in actual soil versus a nutrient solution. The present study also did not assess pH levels at the root surface which may have differed from the general pH of the nutrient solution.

Susin *et al.*, (1996) found that Fe-deficient sugar beets, *Beta vulgaris*, exhibited an increase in iron reductase activity of 10-20 fold over control plants when assayed at a pH of 6.0 or below. Iron reductase activity increased 2-4 times when assayed at a pH of 6.5 or above. These results suggest that iron reductase is more active at a pH of 6.0 or below. Moog *et al.*, (1995) found the optimum iron reductase activity for *Arabidopsis thaliana* roots was a pH of 5.0-5.5.

3.3.2 Iron Reductase and Reducing Capacity of Sweetpotato Genotypes

The reducing capacity for each genotype was expressed in nanomoles of Fe (III)·g⁻¹ root fresh weight·hr⁻¹. Mean reducing capacity of the two combined hydroponic trials was analyzed using LSD for mean genotype separation (Fig 4). The statistical analysis indicated that the independent variables genotype, replication, and trial were not significant. The dependent variable, nanomoles of Fe (III)·g⁻¹ root fresh weight·hr⁻¹, was significant.

The original reducing solution, consisted of 0.0367g/L of iron EDTA [ethylenediaminetetraacetic acid iron (III) sodium salt] and 0.1477g/L of ferrizine [3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt] contained 873.78 nm Fe. The reducing capacity for each genotype can be calculated as the differential between the nanomoles of iron in the original reducing solution and the nanomoles of iron in the final

reducing solution (Fig 4). Results were consistent with findings from Romera and his work with the ferric reducing capacity of cucumbers and *Arabidopsis* (Romera *et al.*, 2003; Romera and Alcantara, 2004).

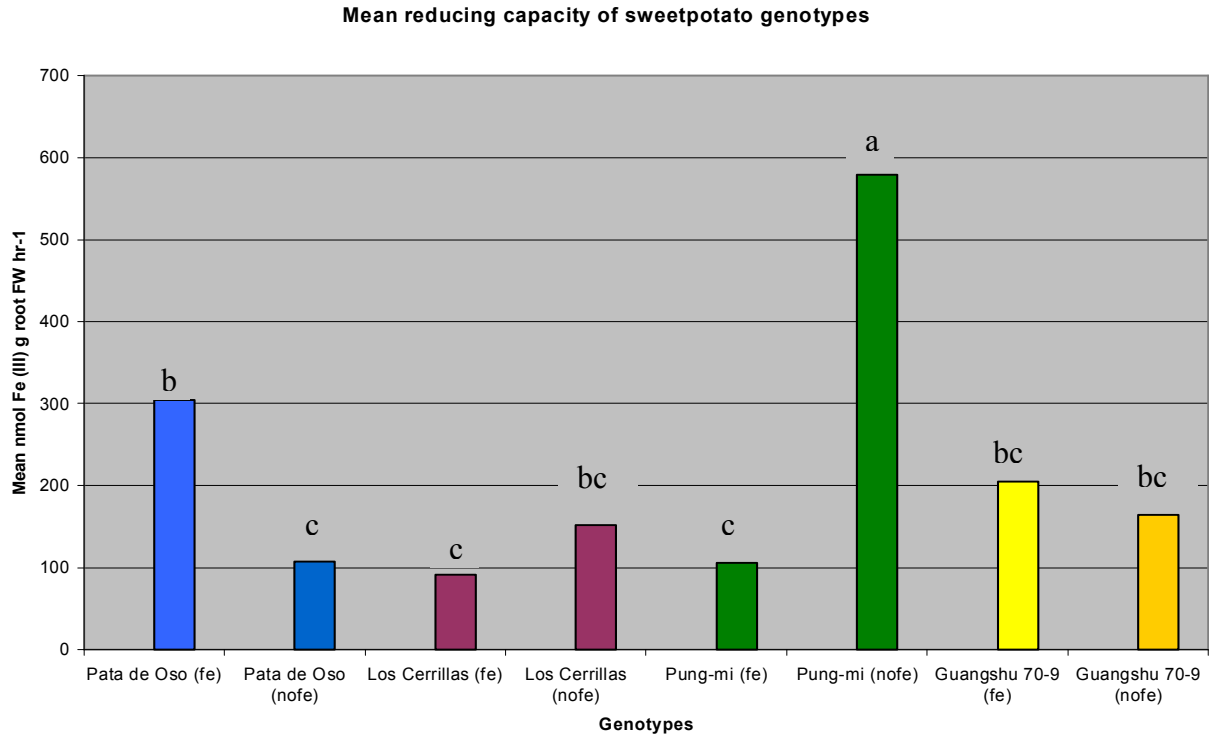


Fig 3.1 Mean reducing capacity of sweetpotato genotypes in nutrient solution, Fe (III) after 10 days with (fe) and without (nofe) iron using ferrozine and EDTA.

The sweetpotato genotype that reduced the greatest amount of iron from solution was ‘Pung-mi’ ($p \leq 0.05$). This genotype, grown in Fe-sufficient conditions, reduced 12% of available iron from the solution. The same genotype, grown in Fe-deficient conditions, reduced 66% of available iron. ‘Guangshu 70-9’, grown in Fe-sufficient conditions, reduced 23% of the iron. ‘Guangshu 70-9’, another low iron concentration variety, did not increase iron reductase activity when grown in an Fe-deficient environment. ‘Los Cerrillas’, grown in Fe-sufficient conditions, reduced 10% of the iron. The same genotype, grown in Fe-deficient conditions, reduced 17% of the iron. Results for ‘Pata de Oso’ run counter to our expectations. ‘Pata de

Oso', grown in Fe-sufficient conditions, reduced 35% of the iron. When grown in Fe-deficient conditions, 'Pata de Oso' reduced 12% of the available iron.

High iron accumulating 'Pata de Oso' showed significantly reduced iron reductase activity in plants grown in an iron deficient environment. In contrast, 'Pung-mi', a low iron accumulating variety, significantly increased iron reductase activity when grown in an iron deficient environment. These results suggest that high a iron accumulating variety did not increase iron reductase activity. Varieties poor at accumulating iron did increase iron reductase ability ('Pung-mi') or did not show any difference ('Guangshu 70-9').

Romera *et al.*, (1992) found that the reducing capacity for sunflower (*Helianthus annuus*) and cucumber (*Cucumis sativus*) was highest at pH of 5.5-6.0. The reducing capacity for sunflower at pH 5.5 and 6.0 was approximately 300 nanomoles of Fe (II)·g⁻¹ root fresh weight·hr⁻¹. The reducing capacity of cucumber at pH 5.5 and 6.0 was approximately 1250 nanomoles of Fe (II)·g⁻¹ root fresh weight·hr⁻¹. Romera and Alcantara (2003) found that the reducing capacity of Fe-deficient *Arabidopsis thaliana* was approximately 1200 nanomoles of Fe (II)·g⁻¹ root fresh weight·hr⁻¹. These results coincide with the results from the nutrient solution study.

3.3.3 Fresh Weight of Hydroponic Stem Cutting

Analysis of each genotype's fresh root weight was done using PROC GLM. Means were separated using LSD (p≤0.05). The statistical analysis indicated that trial, genotype, and treatment were significant at the p≤0.05 significance level. The dependent variable, root weight, was also significant. Root weights of genotypes grown in Fe-sufficient conditions for trial one differed significantly at p≤0.05 (Fig 5). The two genotypes with the greatest root mass were 'Los Cerrillas' and 'Guangshu 70-9', with root mass of 12.7 grams and 11.51 grams, respectively. The third highest ranking genotype was 'Pung-mi', with 10.29 grams of root mass

(Fig 5). The lowest ranking genotype was ‘Pata de Oso’, with 3.35 grams of root mass. ‘Los Cerrillas’ had 74% more root mass than ‘Pata de Oso’.

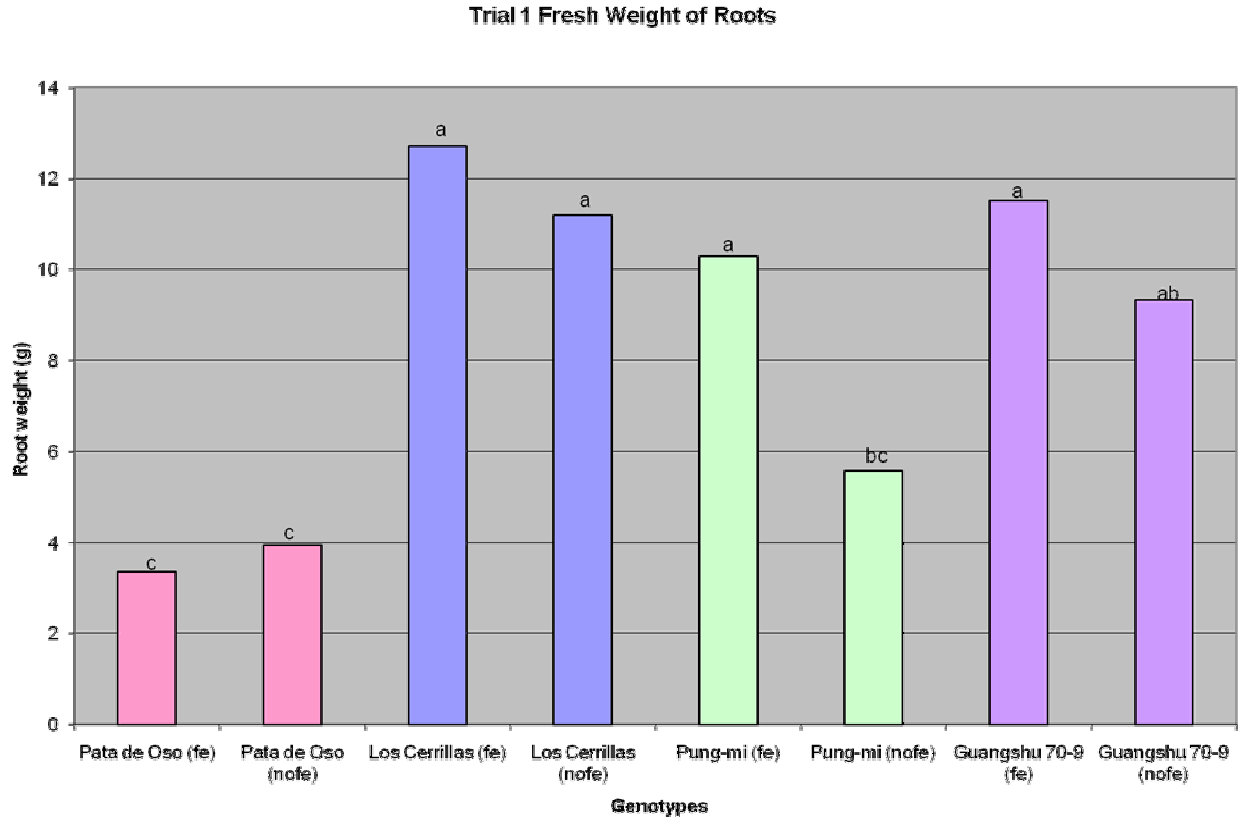


Fig 3.2 Trial 1 fresh weights of hydroponic stem cutting roots (g) after 10 days grown with (fe) and without (nofe) iron

Root weights of genotypes grown in Fe-deficient conditions for trial one differed significantly at $p \leq 0.05$ (Fig 5). The highest ranking genotypes were ‘Los Cerrillas’ and ‘Guangshu 70-9’, with root masses of 11.19 grams and 9.324 grams, respectively (Fig 5). The third highest ranking genotype was ‘Pung-mi’, with a root mass of 5.56 grams. The lowest ranking genotype was ‘Pata de Oso’, with a root mass of 3.93 grams. ‘Los Cerrillas’ had 65% more root mass than ‘Pata de Oso’. Root weight of all the genotypes except ‘Pung-mi’ did not differ significantly regardless of treatment (Fig 5). ‘Pata de Oso’, ‘Los Cerrillas’, and

‘Guangshu 70-9’ produced approximately the same root mass in Fe-sufficient and Fe-deficient conditions.

Root weights of genotypes grown in Fe-sufficient conditions for trial two differed significantly at the $p \leq 0.05$ level (Fig 6). The highest ranking genotypes were ‘Los Cerrillas’ and ‘Guangshu 70-9’, with root masses of 5.07 grams and 4.75 grams, respectively (Fig 6). Similar to results in trial one, the statistical analysis indicated no significant difference between them. The third ranking genotype was ‘Pung-mi’, with a root mass of 3.87 grams (Fig 6). The lowest ranking genotype was ‘Pata de Oso’, with a root mass of 1.75 grams (Fig 6). ‘Los Cerrillas’ had 66% more root mass than ‘Pata de Oso’.

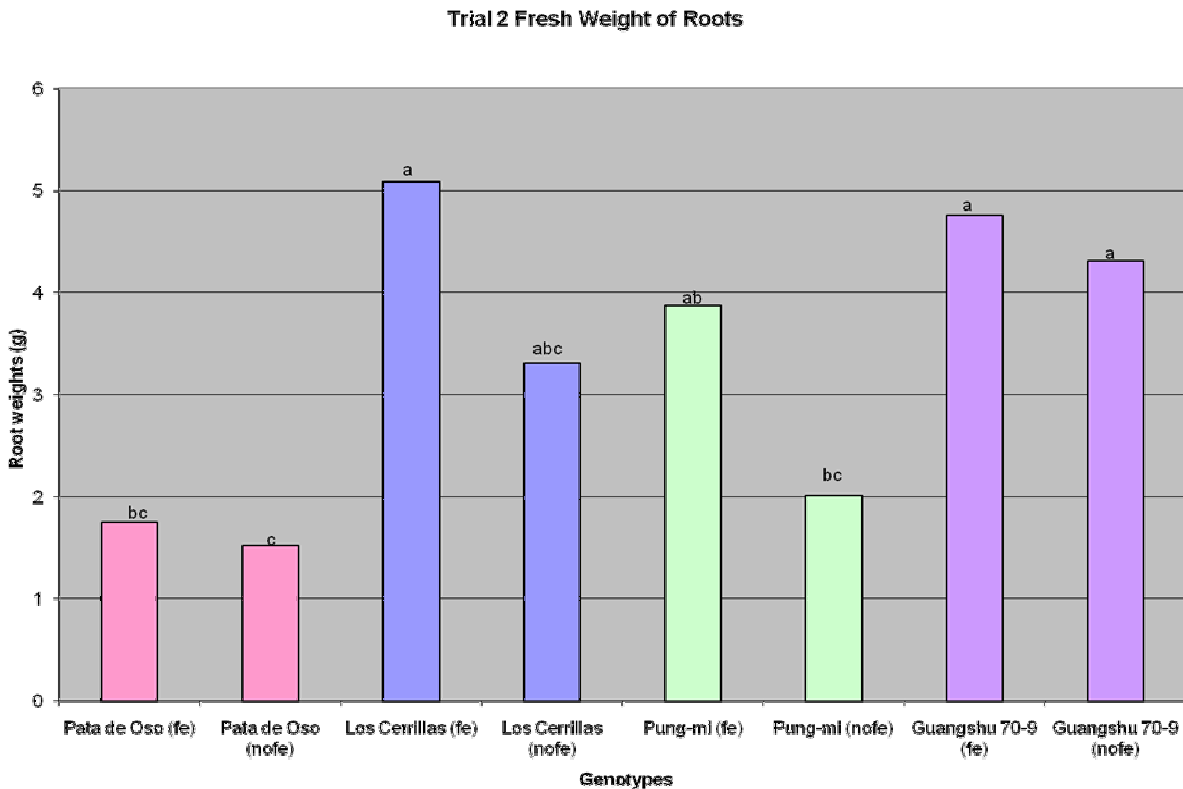


Fig 3.3 Trial 2 fresh weights of hydroponic stem cutting roots (g) after 10 days grown with (fe) and without (nofe) iron.

Root weights of genotypes grown in Fe-deficient conditions for trial two differed significantly at the $p \leq 0.05$ level (Fig 6). The highest ranking genotype was ‘Guangshu 70-9’, with a root mass of 4.3 grams. The second highest ranking genotype was ‘Los Cerrillas’, with a root mass of 3.3 grams. The third ranking genotype was ‘Pung-mi’, with a root mass of 2.01 grams. The lowest ranking genotype was ‘Pata de Oso’, with a root mass of 1.51 grams. ‘Guangshu 70-9’ had 65% more root mass than ‘Pata de Oso’.

Root weight of all genotypes except ‘Los Cerrillas’ did not differ significantly regardless of treatment (Fig 6). ‘Pata de Oso’, ‘Pung-mi’, and ‘Guangshu 70-9’ produced approximately the same root mass in Fe-sufficient and Fe-deficient conditions. There was minimal difference in fresh root weights of high and low iron accumulating genotypes. The Fe-deficient genotypes seemed to produce less roots than Fe-sufficient genotypes.

The root mass measured only represents the root mass that the plant has after 10-14 days of growth. Since this is an early measurement of root mass, it does not represent the root mass that a marketable size sweetpotato storage roots might have. Mature root mass may differ substantially from a 14 day old plant.

Moog *et al.*, (1995) found that the root mass of Fe-deficient *Arabidopsis thaliana* was lower than the root mass of plants grown in an Fe- sufficient environment. Landsberg (1996) showed the iron-stress response in sunflower (*Helianthus annuus*) roots. He did not report a decrease in the root mass from an Fe-deficient sunflower, but did report that the roots developed root tip swelling and an increase in root hairs (Landsberg, 1996). These results coincide with our findings that Fe-deficient roots will generally have less root mass than an Fe-sufficient root mass.

Taken in concert, these results indicate that the pH of solution actually increased instead of decreased, root mass is not an indicator of greater mining ability, and the ability of a sweetpotato to uptake iron out of solution via iron reductase is not dependent on the sweetpotato being categorized as a high or low iron accumulating variety. A decrease in pH is associated with an environment conducive to reducing unavailable Fe (III) to available Fe (II) (Romera personal communication, 2006). We believe the reason the pH did not decrease was because plants may locally acidify the area surrounding the rhizosphere (Romheld *et al.*, 1984). This acidification cannot be detected while testing the pH of the entire nutrient solution. It is possible that after an initial increase in pH, which was unexpected, roots may lower the pH of the solution; however, our study may have ended prior to this occurring. These results showed that sweetpotato may not effectively mine iron by altering the pH of the solution, counter to the behavior of other species (Romera personal communication, 2007). Iron reductase activity, as measured by the EDTA-ferrozine method, differed significantly among varieties. High iron accumulating variety ‘Pata de Oso’ showed no increase in iron reductase activity in plants grown in an Fe-deficient environment compared to an Fe-sufficient environment. In contrast, ‘Pung-mi’, a low iron accumulating variety, significantly increased iron reductase activity when grown in an Fe-deficient environment. ‘Pung-mi’ had the greatest reducing capacity, even though it was one of the varieties that produced the least amount of roots. The fresh weights of roots were charted and the genotypes that produced more roots did not have a greater reducing capacity. High iron concentration varieties had the lowest root mass in comparison to low concentration varieties. When Fe-sufficient and Fe-deficient treatments were compared, only ‘Pung-mi’ showed a significantly greater root mass when grown in an Fe-deficient environment. These variables, pH,

root mass, and iron reductase, do not lend themselves readily for use as indications of high iron concentration varieties.

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CONCLUSION

Previous research showed a three fold difference of iron and zinc in marketable sweetpotato storage roots. Our objective was to characterize how sweetpotato storage roots vary in micronutrient uptake over the development period and how this would help us understand the physiological accumulation of iron and zinc. We used genotypes that varied in iron and zinc uptake.

Results from the iron and zinc accumulation study indicate that the micronutrient content (dry weight basis) in developing roots varied minimally during storage root development. This data suggests that a sweetpotato storage root can be harvested at any time and the micronutrient concentrations measured. Evaluation of genotypes could be made early on during the maturation process, thus speeding the selection procedure. Researchers that are interested in assessing iron and zinc can base an accurate assay at anytime time there is sufficient storage root matter to assess. However, the smaller the root, the more difficult it is in eliminating soil as a contaminant.

Our objective for the nutrient solution study was to look at various plant characteristics that may account for higher iron accumulation in sweetpotato. Results from the nutrient solution study indicate that the pH of the nutrient solution actually increased instead of decreasing, counter to our expectations. Iron reductase activity differed among the varieties. High iron accumulating ‘Pata de Oso’ showed reduced iron reductase activity in plants grown in an Fe-deficient environment. In contrast, ‘Pung-mi’, a low iron accumulating variety, significantly increased iron reductase activity when grown in an Fe-deficient environment. Another low iron accumulating variety, ‘Guangshu 70-9’, did not vary. Varieties poor in iron accumulation either did not vary or seemed to increase iron reductase activity in an Fe-deficient environment.

A greater root mass is also a means by which plants can uptake greater amounts of iron. High iron concentration varieties had the lowest root mass in comparison to low concentration varieties. When Fe-sufficient and Fe-deficient treatments were compared, only 'Pung-mi' showed a significantly greater root mass when grown in an Fe-deficient environment. The low root mass for 'Pung-mi' may have promoted increased iron reductase activity.

Early determination of high iron and zinc accumulating genotypes is needed to speed up the mass selection process. Mass selection for sweetpotato usually takes two years to complete; if selections could be made earlier during development, then the cycle time could be cut in half. These results show that genotypes in a recurring mass selection breeding scheme can be selected at an early development stage, permitting high iron and zinc genotypes to be incorporated into a breeding nursery the same year, thus reducing the cycle time from two years to one year. Results for iron reductase, pH, and root mass were less conclusive. These characteristics are not straightforward to use as selection criteria in a breeding program.

APPENDIX A: SOIL CHARACTERISTICS AND RAINFALL DATA FOR HAMMOND, LOUISIANA

Soil Characteristics for Hammond, Louisiana

| P,ppm | K,ppm | Ca,ppm | Mg,ppm | Na,ppm | S,ppm | Cu,ppm | Zn,ppm | Fe,ppm |
|-------|-------|--------|--------|--------|-------|--------|--------|--------|
| 37.9 | 74.9 | 591 | 106 | 8.62 | 10.8 | 0.84 | 1.2 | 16.7 |

Average Rainfall for Summers 2006 and 2007 in Hammond, Louisiana

| Date | Average Rainfall (cm) |
|-----------------------------------|-----------------------|
| July 2006 | 0.3 |
| August 2006 | 1.0 |
| September 2006 | 0.9 |
| October 2006 | 1.4 |
| June 2007 (end of month) | 0.08 |
| July 2007 | 0.6 |
| August 2007 | 0.6 |
| September 2007 | 0.8 |
| October 2007 (beginning of month) | 0.3 |

APPENDIX B: DIFFERENTIAL UPTAKE OF IRON AND ZINC IN A SOILESS MEDIA

Introduction

Iron is an important component in human diets because it regulates enzyme activity and plays a role in the immune system (Lynch, 2003). It is also an important component of human blood because iron is the central atom of hemoglobin (Tuman and Doisy, 1978). Humans require 10-15 milligrams of iron per day; if iron levels are not regulated, the deficiency can lead to mental and psychomotor impairment in children, and an increase in both morbidity and mortality of mother and child at childbirth (Frossard *et al.*, 2000). Zinc plays an important role in the immune system; it is necessary for T lymphocyte development (Ronaghy, 1987). Alcohol dehydrogenase, an enzyme that breaks down toxins in the human body, also depends on an adequate zinc supply to function properly (Ronaghy, 1987). In Africa, it is estimated that 500-600 million people are at risk for low zinc intake (HarvestPlus, 2007).

Iron is one of the 16 essential elements needed for plant growth. Iron is used for the synthesis of chlorophyll and is essential for the function of chloroplasts (Abadia, 1992). Without sufficient iron levels, plants show apical leaf chlorosis and slower root growth. Despite the usually high abundance of iron in soils, the low solubility of iron bearing minerals limits the iron available for uptake by higher plants (Schmidt, 1999). Although abundant in soil, iron is one of the most common nutrients limiting plant growth in the world (Guerinot, 2001).

Zinc is another essential element needed for plant growth, and deficiencies can cause many problems within the plant. Visual symptoms of zinc deficiency in plants are leaf mottling, interveinal chlorosis, and reduced plant growth. Zinc is involved in membrane integrity, enzyme activation, and gene expression (Kim *et al.*, 2002). Despite the importance of zinc as a

micronutrient for plant growth, there have been relatively few studies of the mechanism of zinc uptake (Reid *et al*, 1995).

Data does not exist for iron and zinc concentrations in sweetpotato leaves. In an effort to understand iron and zinc uptake in sweetpotato leaves, a greenhouse study was conducted to determine the differential uptake of iron and zinc in sweetpotato leaves.

Materials and Methods

Greenhouse research was conducted at the Sweetpotato Research Center at Chase, Louisiana in 2006. Sweetpotato plants were planted in Scotts MetroMix 360, a soil less potting media. The media contained 35-45% medium grade horticultural vermiculite, 31-50% choice cut Canadian sphagnum peat moss, 12-25% processed bark ash, 1-10% pine bark, starter nutrient charge, gypsum, slow release nitrogen, dolomitic limestone, and a long lasting wetting agent. The sweetpotato genotypes planted were '01-29', '103005 B', '103014 A', '103014 B', '103014 C', '103033 A', '103036 A', '103036 B', '103036 C', '103036 D', '103036 E', '103036 F', '91-226 sc B', 'B14', 'Excel weevil', 'Markham', 'Native green A', 'Quanta', 'Cw1985 A', 'Cw1985 B', 'Cw2000 A', 'Cw2000 B', 'Tis 8267 A', 'Tis 8317', 'Tres colores', 'Vap 5 B', 'W119 A', 'W119 B', 'W119 C', 'Waga', and 'Wanmun large'. The sweetpotatoes were planted on a greenhouse bench and allowed to grow for several weeks until they were approximately 8-10 inches tall. Plants were fertilized using a liquid fertilizer, Total Gro Tomato Special 3-13-29. The fertilizer contained 3% nitrogen, 13% phosphoric acid, 29% water soluble potash, 0.1% boron, 0.1% copper, 0.34% iron, 5.4% magnesium, 0.01% molybdenum, 11% sulfur, and 0.045% zinc. The top 4-5 fully opened leaves were harvested and placed into labeled plastic bags and returned to the laboratory for analysis.

Once in the laboratory, leaves were 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. The 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 at ambient temperature until assayed for aluminum, iron, and zinc concentrations. The results of the assay were used to test how much iron and zinc exists in sweetpotato leaves, and if there is a correlation between iron, zinc, and aluminum content. The statistical analysis was done using PROC GLM (SAS 9.1, SAS Institute Inc, Cary, N.C.).

Results and Discussion

The statistical analysis indicated that iron was significant at the $p \leq 0.05$ and aluminum (data not shown) and zinc were not significant at the $p \leq 0.05$ significance level (iron and zinc data presented in Table 4.1). Our data was further analyzed through a correlation between iron and aluminum concentration. We found a Pearson Correlation Coefficient of 0.64704 with a p-value of < 0.0001 for the samples, indicating that there was a significant relationship between iron and aluminum concentration. We found a Pearson Correlation Coefficient of -0.23529 with a p-value of 0.0356 for the samples, indicating that there was no significant relationship between zinc and aluminum. Correlations do not infer cause and effect.

According to the statistical analysis, there are minimal differences in iron and zinc concentrations in sweetpotato leaves. Normally, sweetpotato leaves contain 40-100 ppm iron and 20-50 ppm zinc (Mills and Jones, Jr, 1997). We found that sweetpotato leaves contain 10-82 ppm and 13-25 ppm zinc. Our results are consistent with Bush's book.

Some of the highest ranking genotypes for iron concentration were 'Vap 5 B', 'Native green A', '103036 E', 'Tres colors', '103036 C', and '01-29'. All these genotypes had leaf iron

concentrations above 46 ppm. The highest ranking genotypes for leaf zinc concentration were ‘Qtanta’, ‘Cw1985 A’, ‘Excel weevil’, ‘Waga’, ‘103033 A’, ‘Tres colors’, and ‘Cw1985 B’. All these genotypes had leaf zinc concentration above 19 ppm. Only ‘Tres colors’ had high levels of both iron and zinc. In third world countries sweetpotato leaves are eaten as a food source and it would be beneficial to develop a sweetpotato with high micronutrient levels in the leaves and roots. An extension of this research would be to correlate leaf iron and zinc concentration with levels in the storage root.

Iron and zinc concentrations in sweetpotato leaves (2008)

| Genotype Information Cultivar | Iron (mg/kg dwb) Mean* | Zinc (mg/kg dwb) Mean* |
|----------------------------------|---------------------------|---------------------------|
| Native green A | 64.8 ab | 16.6bcd |
| Tres colores | 49.7abcd | 20.1abcd |
| Vap 5 B | 82.5a | 17.6bcd |
| 91-226 sc B | 23.3de | 16.3bcd |
| 01-29 | 46.9abcde | 17.7bcd |
| Excel weevil | 21.1de | 21.7abc |
| 103036 F | 30bcde | 13.6d |
| Wanmun large | 21.8de | 18.6bcd |
| Cw2000 A | 26.6cde | 15.1cd |
| 103036 C | 47.2abcde | 16.8bcd |
| 103014 C | 32.7bcde | 16.6bcd |
| 103036 A | 44.7bcde | 15.2cd |
| B14 | 45.3bcde | 15.4bcd |
| Cw1985 A | 33.7bcde | 22.4ab |
| 103005 B | 19.5de | 14.8cd |
| W119 A | 23.3de | 18.6bcd |
| 103014 B | 22.3de | 17.6bcd |
| Cw2000 B | 10.3e | 15.5bcd |
| Waga | 28.7bcde | 20.2abcd |
| Markham | 42.1bcde | 18.2bcd |
| Tis 8267 A | 14.8de | 16.7bcd |
| 103036 E | 62.2abc | 17.3bcd |
| W119 B | 13.4de | 17bcd |
| 103036 D | 28.4bcde | 15.8bcd |
| W119 C | 14.1de | 13.7d |
| Qtanta | 25.3cde | 25.5a |
| 103036 B | 17.1de | 18.1bcd |
| Cw1985 B | 23.7de | 19.3abcd |
| 103014 A | 31.7bcde | 17.1bcd |

| | | |
|-----------|---------|----------|
| 103033 A | 17.3bc | 20.2abcd |
| Tis 83/17 | 23.3dec | 18.4bcd |

*Results within a column followed by common letter do not differ significantly at 0.05% level.

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