HERITABILITY OF MINERAL ELEMENTS OF MICRONUTRIENT VALUE IN TWO *SOLANUM SCABRUM* (MILLER) ACCESSIONS FROM KENYA AND CAMEROON

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

To my father and my mother Mr and Mrs Wamalwa for their financial and emotional support

LIST OF TERMS AND ACRONYMS USED AND THEIR DEFINITION

EDXRF- Energy Dispersive X-ray Reflective Fluorescence

K- Potassium

Ca- Calcium

Fe- Iron

Zn- Zinc

Cu- Copper

Mn- Manganese

MIE- Micro elements (Zn, Fe, Cu, Mn)

MAE- Macro elements (Ca, K)

µg- microgram $= 10^{-3}$ mg

ng- nanograms $= 10^{-3}$ µg

µg/g– microgram per gram

ppm– parts per million = µg g^{-1} = mg kg^{-1}

MS- Mean square

df- Degrees of freedom

s.e.- Standard error

HIMA- High macro-elements

HIMI- High micro-elements

TOLOMA- Too low macro-elements

KENRIK- Kenya Resource Center for Indigenous Knowledge

IPGRI- International Plant Genetic Resources Institute

ALVs- African leafy vegetables

F1- Filial generation one

F2- Filial generation two

AVRDC- Asian Vegetable Research and Development Center

IFPRI- International Food Policy Research Institute

ABN- African Biosciences Network

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Indigenous leafy vegetables account for 75.3% of all vegetables consumed in Africa and eight families account for 50% of the indigenous African leafy vegetables with Amaranthaceae and Solanaceae ranking at the top. *Solanum scabrum* belongs to Solanaceae family and is popularly consumed as a vegetable soup in Kenya. Two *S. scabrum* accessions were used for this study GPA62 (an accession from Cameroon) and GPA111 (an accession from Maseno-Kenya). GPA62 and GPA111 were crossed from which F1 plants were generated. F2 generation plants were generated by selfing F1. Energy Dispersive X-ray Fluorescence (EDXRF) analysis was undertaken on GPA62, GPA111, F1 and F2 leaves to determine mineral elements of micronutrient value. The micro and macro element concentration in leaves of GPA111 was compared to GPA62 and F2 progeny and heritability determined. Heritability was estimated based on 2 criteria namely: single elements criterion 'a', and Macro elements (MAE) concentration (potassium and calcium summed) and microelements (MIE) (manganese, zinc and iron summed) as criterion 'b'. Genstat 5.1 was used to calculate the parent-offspring heritability from linear regression analysis ANOVA. Chi-squares were used to find out the mode of inheritance. The parent-offspring regression and Chi-square results showed that the uptake of single elements had very low heritability levels (<0.1) and that factors were probably inherited independently. However, when classified under MAE and MIE criterion, GPA111 gave a high heritability of 0.24 on MAE while GPA62 gave 0.074. Heritability estimate on criterion 'a' is the most conventional criteria amongst the named above. Heritability estimate on criterion 'b' considers the summation of both K and Ca under MAE in terms of their position on the Periodic Table of elements. MIE has the trace elements summed in the category of heavier metals in the Periodic Table. Therefore, there is a biological merit in the use of single element and MAE-MIE heritability criteria. In fact the MAE-MIE heritability criterion appears to have improved the values obtained compared to the single element criterion. These heritability values, however, have limitations that ought to be recognized as thus heritability (h^2) is generally population and environment specific; further more, it is a population rather than individual parameter. It was adopted in this study because the two ecotypic strains used as parental lines for crossing were regarded as population based rather than individual variety characterized. Therefore, the estimates thus derived

from this study were probably not indicative of the degree to which the mineral density trait is genetic but rather suggestive of the proportion of phenotypic variance due to possible genotypic factors Chi-square values are suggestive of a monogenic inheritance of mineral density in *S. scabrum*. On the overall, data also suggest that mineral micronutrient density is not entirely genetic and the environmental component in the phenotype may to a large extent be playing a role in the leaf concentration In general, F_2 from the two crossed parents showed higher mineral densities than the parents Further investigation focussing on (Genotype) x Environment (G-E) interaction and studies on specific and general combining abilities (GCA) on selected individual strains or cultivar/ecotypes are warranted This GCA line of inquiry will yield data on average performance of parental lines as characterized by the average amount of heterosis in all hybrid combinations It would also be useful for determining mineral density of parental lines, among the genetic components and a breeding method for high mineral density In mean time, farmers can expect high mineral micronutrient yields by prudently applying appropriate agronomic husbandry interventions such as. (a.) Choosing a strain proven to show high mineral density promise and (b.) Choosing the right site and soil fertility conditions

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

In the year 2002, the Kenyan population growth rate was 4% per annum compared to the 1999 growth rate of 1 59% per annum (Chadha *et al.*, 2001), a doubling in a period of hardly three years This high population growth rate was not matched by an equally high economic growth, which stood at 1 4 % for 1999 and 1 1 % for 2002 (Economic surveys, 1999, 2002). To meet expectations of a rapidly growing population faced with a persistent food deficit, intensive agricultural farming practices that have been adopted have exploited but only a few crop species, and in turn has led to diminished plant diversity including indigenous African leafy vegetables (ALVs)

African leafy vegetables, which are highly regarded for their value as food or medicines, have undergone a high degree of marginalization as emphasis was directed on exotic/introduced vegetables in Kenya. Leafy vegetables account for 75.3% of all vegetables used as cooked 'spinach' in Africa Eight families account for 50 % of the indigenous African leafy vegetables Of the 8 families, Amaranthaceae and Solanaceae are major micronutrient sources (Bosch *et al*., 2005) Amaranths contribute greatly to nutra-health security (Grubben and Slotten, 1981, Teutonico *et al.*, 1985) and as do *Solanum* species (Maundu *et al.*, 1999). This is an indication of their potential to alleviate malnutra-health problems among the vulnerable groups either in food or nutra-health deficit areas

Micronutrient malnutrition is a condition where the micronutrient intake levels recommended is not achieved and this may be as a result of poverty, neglect or lack of knowledge. Micronutrient malnutrition is notably an important issue in developing

countries (Graham *et al.*, 1999; IFPRI, 1996) claiming about 24,000 lives daily (Crop biotech update, 2004). The most affected victims are women and children. HIV/AIDS immuno-compromise condition can be exacerbated further by lack of adequate zinc nutrition among others. Global statistics show that some of the main nutrients lacking or taken in minimal levels include iron, iodine, zinc and vitamin A (Graham and Welch, 1996). It has been shown by IFPRI (1996) that more than two billion people in the world today are iron deficient and a lot has been and is still being done to reduce these numbers.

Micronutrient security is a great threat and a truly hidden hunger as its effects are less immediate as in the case of famine, which can show up in a matter of days. Some attempts have been made in the past to address hidden hunger or micronutrient malnutrition problem in the developing world (IFPRI, 1996). Supplementation and fortification programs as alternatives have not covered wide areas and diets and there is no public investment into them (FAS, 1996). Attempts made in post-harvest handling and preservation strategies to improve produce retention of nutrients (Gomez, 1981; Hagenimana, 1999) were made but sustaining such programs is an enormous challenge. Many rural families in Kenya cannot afford high value foods or animal products for daily nourishment and therefore rely on vegetable or legume based preparations to meet their vitamin and micronutrient needs. Bio-fortification is being recommended by the World Food Programme (WFP) as one amongst many options to address the micronutrient malnutrition problem, which is generally applicable through plant breeding techniques for genetically enhancing staple food germplasm (White and Broadley, 2005).

In many African countries, research and development has concentrated on major staple foods, such as maize, wheat, and sorghum with limited attention to the

complementary local African leafy vegetables (ALVs) with which traditional cereal breads are always eaten. Now major cereals, including polished rice, wheat and maize (IFPRI, 1996) based breads, are hardly compatible menus with African vegetable soups. Where they predominate among urban people in Kenya, hidden hunger may be a problem. This calls for the need to start paying serious attention to micronutrient rich foods that are a direct source for the majority of Kenyans, not only the vulnerable but also the exotic food-dependants (ABN, 1994). International Plant Genetic Resources Institute (IPGRI) and Kenya Resource Centre for Indigenous Knowledge (KENRIK) have undertaken some work on ALVs in Kenya for Amaranth, Cleome, Corchorus, Vigna and Solanum species (Bosch *et al*., 2005).

Solanum species, also known as the African nightshades, are major local vegetables commonly consumed with milled maize flour product called 'ugali' in Kenya. About 30 species of the African nightshades have been identified so far, but only 4 species are commonly cultivated for consumption of the leaves (Schippers, 2002). In addition to that, little or no research has been conducted on these solanum species, which include *S. americanum*, *S. eldoretii*, *S. villosum* and *S. scabrum*. *Solanum scabrum* (Miller) is the most popularly used species in vegetable soup.

1.2 Botany of *Solanum scabrum*

Solanum scabrum usually has laterally branched erect plants that grow in altitudes ranging from sea level to 2000 m above sea level. The leaves are usually large and ovate in shape. The petals are white in colour, the anthers are brown or purple brown and have long styles. The berries are deep purple with opaque cuticles that

remain on the plant and adhere to erect pedicels at maturity (Edmonds and Chweya, 1997; Schippers, 2002).

1.3 Importance of *Solanum scabrum*

Solanum scabrum, also known as garden huckleberries, is an indigenous vegetable grown in Western and Nyanza provinces of Kenya. Although not widely consumed, it is a potential nutritious vegetable that could boost micronutrient nutrition. This vegetable is widely grown and consumed in West Africa (Schipper, 2002). It has also shown to have high seed micronutrient levels as compared to other Solanum species as shown by X-ray Energy Dispersive Fluorescence analysis (EXDRF) technology (Akundahweni, 2004). *S. scabrum*, therefore, has the potential to replace *Solanum nigrum*, a more widely consumed local vegetable in Kenya (Maundu *et al.*, 1999).

1.4 Problem statement

African leafy vegetables (ALVs) are complements to cereal staple breads among the bulk of Africans and yet they are marginal in their status, value and research focus. Donor research funding to cereal staple crop improvement has historically remained significantly higher in crops such as maize, wheat and rice compared to the less known ALVs. Research priorities have in the past ignored the importance of ALVs thus ignored the biblical proverbial saying that 'Man does not live by bread alone' and therefore in some way created a micronutrient deficit problem due to the conventional agronomic thinking of classifying crops as 'major' and 'minor' in the conventional

western agronomic contexts Given the foregoing, renewed research attention on ALVs is warranted

In addition, Solanaceae family is amongst the top ranking widely consumed vegetables in Africa (Schippers, 2002), showing their potential to contribute to nutra-health Although this is the case, there is one commonly consumed leafy Solanum vegetable in Kenya, which is *Solanum nigrum* (Maundu et al 1999) *Solanum scabrum* has the potential to compliment *S nigrum* in areas where it is not grown since it has high elemental levels as revealed by Akundabweni (2004)

"In our Institute of Nuclear Science and Technology laboratory at the University of Nairobi, some strains in a raw leafy form of Solanum, Amaranth and Cleome species have been shown to be higher in mineral elements of micronutrient value" (Akundabweni , Personal Communication)

1.5 Justification

Many poor families in Kenya heavily rely on the staple and native foods such as maize, cassava and leafy vegetables for their daily nourishment These vegetables are also a source of income for the rural populations In cases of food shortages as occurs in most developing countries, the most affected are the women and children (IFPRI, 1996, Fassil *et al.*, 1999) If these vegetables are grown locally, the farm families would have both micronutrient nutrition and food security since they grow all year round while the cereals can be stored for long. This would in turn mean that these households would have more income since they will not buy food

Secondly, solutions to curb malnutrition such as supplementation and fortification programs have proved to be expensive (FAS, 1996, Graham and Welch, 1996) It is therefore necessary to enhance the nucronutrient density in vegetable crops

through genetic improvement for quality and to offer sustainable use. Genetic improvement may not be possible unless the underlying basic genotypic differences are elucidated. Besides, only then will there be a possibility of conserving a genetic pool for posterity. The need for preservation and conservation comes in the wake of industrialization as many exotic foods are replacing indigenous foods of both the wild and weedy species, meaning there could be irreversible genetic erosion (Ayad *et al.*, 1997, Guarino *et al.*, 1997).

Thirdly, *Solanum scabrum* was found to have high micronutrient content (Akundabweni, 2004) and could therefore, contribute greatly to improved health. EDXRF analysis on the nutrient mineral density (NMD) of this species showed that there are considerable differences even within species in calcium of up to 26000 µg/g (Akundabweni, 2004). Since the inheritance of the nutrients in *S. scabrum* was not known, it could have been either genetic or environmental. The use of X-ray for nutrient analysis and Simple Sequence Repeats (SSR) for genetic screening in this study was employed.

Fourthly, *S. scabrum* has been documented to be an important vegetable species but breeding and selection was highlighted as a development gap by Bosch *et al* (2005).

1.6 Questions arising

- What is the inheritance mechanism (genetic or environment) of the nutrient density factor?
- Which methods can be used to analyze for such variation?
- Of what value is that information as a basis for further improvement?

1.7 Reaction to the questions arising

Initial work of Akundabweni using X-ray Fluorescence analysis (Pers Comm) showed that some accessions of *Solanum* (e.g GPA 111) from Maseno, where the vegetable is most popular, was extremely low in its raw micronutrient density while GPA62 from Cameroon (Ex Manife) was significantly richer GPA62 also has a much larger leaf Such variation offers opportunities to transfer the genes conditioning desirable trace mineral density levels from poor parental lines to rich ones for purposes of improving both agronomic quantity and quality in the African leafy solanum vegetable It is in this respect that for the proposed research, the selected *Solanum scabrum* accessions was identified for this study GPA111 and GPA62 were crossed and EDXRF analysis was done on the parents, F1 and F2 leafy fractions to determine their micronutrient and heritability status

1.8 Objectives

This research study had the following objectives: -

- To evaluate the micronutrient density in the parental and F2 generations using EDXRF analysis in *Solanum scabrum;*
- To determine the extent of genetic variation between parents and F2 generations of *Solanum scabrum,*

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Introduction of *Solanum scabrum*

The Solanaceae family is ranked among the top ten indigenous vegetables in Africa (Schippers, 2002) showing its importance in many African countries. *Solanum scabrum*, on the other hand, has been prioritised in various international and regional workshops held in various countries of Africa (Aphane, *et al.*, 2003). The species has been known to grow in a wide range of environments from sea level to 2000 m above sea level (Bosch *et al.*, 2005). In addition to that, preliminary results of EDXRF analysis done on *S. scabrum* (Akundabweni, 2004) showed that seed from GPA62 had high mineral elements of macro and micronutrient levels (High MAE-MIE) as compared to GPA111 (Low MAE-MIE).

Genetically, the differences between HI-MAE/MIE and low MAE/MIE should segregate in the Mendelian fashion when crossed depending on the genes controlling these factors. In principle, successful genetic crosses are heritability-dependant. By heritability-dependant it is meant that passing of a trait from parents to progeny will depend on the extent of its heritability (Watson, 1970). The nutrient concentration in *S. scabrum* could either be affected by genotype, environment or the interaction.

2.2 Genetic factors that would affect growth and expression of *Solanum scabrum*

Genetic factors that may have affected the trait expression in *S. scabrum* would have been polyploidy and recombinants due to the 20% out-crossing that occur within the species (Edmonds and Chweya, 1997). *S. scabrum* is a hexaploid with 2n=6x=72, which explains the high variability in its phenotypic expressions (Edmond and Chweya,

1997, Gbile, 1986). Recombination occurs at meiosis and it involves many combinations of chromosomes to give different offspring (Strickberger, 1990) Other processes that affect the trait expression include selection and breeding

2.2.1 Natural selection in *Solanum scabrum*

Solanum scabrum is a self-fertilizing species with known out-crossing of about twenty percent (Edmonds and Chweya, 1997) Natural selection in this species possibly occurs in the wild since it is not completely domesticated in Kenya but a lot of farmer/grower selection has been done in other countries, such as Cameroon, Nigeria and Cote d'Ivoire, given its popularity (Schippers, 2002) This seems to explain the micro-richness in GPA62 and low apparent micronutrient concentration in GPA111 (Akundabweni, 2004)

2.2.2 Breeding methods in *Solanum scabrum*

Successful breeding methods in self pollinated species include pure-line selection, mass selection and hybridization Pure-line selection involves identifying a single line and developing a new variety out of the progeny whilst with mass selection the new variety is a composite of many pure lines Hybridization is made up of pedigree, bulk and backcross selection In pedigree method records are kept for each of the progenies, which contrasts to bulk method where no attempt is made to keep track of ancestry, backcross method involves transfer of specific genes to a good variety, which is deficient in some characteristic (Allard, 1960)

Under hybridization, *Solanum* species are 80 % self-fertilizing and facultatively autogamous, which favours the rapid increase of the populations Artificial crossing for

S. scabrum has been done and it involves the deliberate transfer of pollen from stamen to a stigma and eventually to an ovule for fertilization purpose. Pollen growth begins when ripe pollen lands onto a compatible stigma (Hartman *et al*., 2002) where the pollen grain rapidly takes in the water and extends within two hours. In most plants, growth of pollen tube lasts between twelve to forty-eight hours from germination to fertilization (Richards, 1986).

Anthesis in *Solanum* species occurs at sunrise and pollen is released two days later. The maternal parents should have their young flowering buds emasculated one to three days before pollination to allow for the full development and reflection of the petals. The stigmas remain receptive for three and a half days after opening of the flower buds. After crossing is complete and successful, the petals fall off, leaving the protruding ovary (Edmonds and Chweya, 1997).

Bagging of the artificially pollinated flowers often result in reduced fruit set due to unfavourable temperature and humidity conditions. Pollination is enhanced by tapping pollen from the dehiscing anther onto a thumbnail followed by wiping it across the stigma. Maturation of the berries takes six to eight weeks after pollination. The ripe berries are harvested and then stored at 4°C until required for seed extraction (Edmonds and Chweya, 1997).

2.2.3 Effects of genetic crosses on *Solanum*

Solanum species have different ploidy levels and this has increased and/or decreased chances of making crosses from parents successfully. Edmonds and Chweya (1997) found success in crosses involving the same species (intraspecific crosses) although variably as opposed to interspecific crosses, which were more successful when same floral sizes were used. *S. scabrum* hybrids have been reported to yield

higher vegetative propagation and higher berry-yielding plants than selfed progeny (Edmond and Chweya, 1997), which was also observed for GPA62 and GPA111

There is documentation on successful interspecific crosses made on *S. scabrum* (Ganapathi and Rao, 1987) with other *Solanum* species like *S. nigrum*, *S. americanum*, *S. villosum* and *S. douglasii*. Intraspecific and/or interspecific crosses have been made with these latter species (Edmonds and Chweya, 1997; Jacoby and Labuschagne, 2006). Although the *S. scabrum* interspecific crosses were successful, it has been no documentation on any intraspecific crosses and no heritability studies in micronutrient levels have been documented for *S. scabrum* so far.

2.3 Environmental factors affecting *Solanum*

The environmental aspects that may have affected the phenotypic expression of *S. scabrum* in terms of nutrient composition of the analysed seed from GPA62 and GPA111 were both edaphic and agronomic factors.

2.3.1 Effect of soil environment on *Solanum scabrum*

Trace mineral content of plant foods reflects the trace mineral concentration in the soil in which they are grown. Soil type and composition can modify the mineral content in a given crop. For example, soil rich in zinc ions enables the plant to have more zinc compared to the Zn-deficient soil depending on the species and plant genotype (Grusak and Eduardo, 1999) and also depends on whether the crop/plant is a dicot or a monocot since they take up ions from the soil in different forms.

The micronutrient phyto-uptake factor affects the nutrient mineral density in crops. Dicotyledonous plants and non-grass monocotyledons take in iron in ferrous

form [Fe (II)] while grasses absorb iron from the soil in ferric form [Fe (III)], with the former predominating in well-aerated soils. This means that a soil might be rich in either iron forms but the availability to dicot or plants is almost negligible. As for other micronutrients such as copper, zinc and manganese, root influx is thought to occur during absorption into the plant.

Fertilizer application is another factor affecting nutrient uptake by crops and it may or may not play an important role in the plant's micronutrient nutrition. Inorganic Fe fertilizer added to Fe-deficient soils is normally ineffective because iron is quickly converted to plant unavailable forms. The predominant form is normally the ferric (III) form, which is unavailable to the dicots. For Zn-deficient soils, if inorganic Zn added to the soil, especially for the sandy soil with a poor cation exchange capacity (CEC), poor absorption of the micronutrient is encountered (Kabata and Pendias, 1984). Since GPA62, GPA111 and their offspring had no Fe-fertilizers they were dependent on the history of the Nitisol potting medium.

Nitisols have been known to have high iron content since they are characterized by low pH enhancing solubility. These soils should, therefore, enhance the solubility of iron into ferrous form (Fe II) (Grusak and Eduardo, 1999) unless they are highly weathered.

2.3.2 Agronomic practice effects on quantity and quality of *Solanum*

Agronomic practices and crop husbandry are important aspects in crop nutrition, growth and development. These include timely planting, weeding, manure/fertilizer applications and watering. Timely planting and weeding reduces the chances of weeds from competing for soil micronutrients available for plant nutrition. Some studies were done for rate of fertilizer application on *S. nigrum* and it was found

that if nitrogen application as fertilizer is high, there were cases of decline in leaf contents of Ca, Mg, P and K at harvest (Fawusi, 1983). This study showed that *S. scabrum* would also be affected by the practices given to it during growth

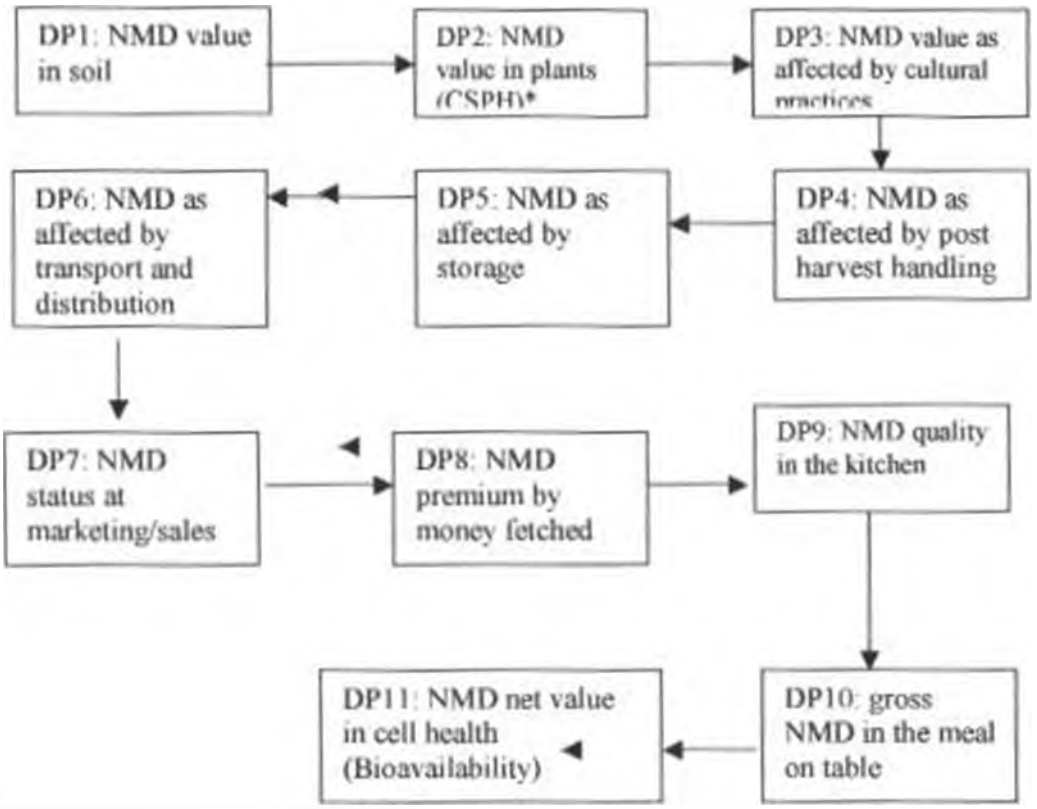
2.3.3 Produce from the *Solanum* grown under Nitisol

Plants grown on Nitisols are expected to have high Fe content due to the fact that these soils have a mechanism of enhancing its solubility. One of the characteristics of these soils is that they are well aerated so that the iron available for plant use is in ferrous form (Fe II), which is suited for dicots (Grusak and Eduardo, 1999) such as *S. scabrum* varieties GPA62 and GPA111. The final nutrient concentrations at time of collection were used to calculate heritability

There are many levels at which the sample could have been analyzed and the results would vary due to the treatment given at that time. For example, when *S. nigrum* leaf samples were harvested and consumed immediately, they were shown to have more vitamins as compared to samples harvested and transported on open trucks exposed to sunlight to market places (Fawusi, 1983). The concentrations in *S. scabrum* were analysed at fresh leaf stage from the greenhouse

2.3.3.1 Levels at which nutrient mineral densities can be calculated for *Solanum scabrum*

Solanum scabrum nutrient levels are expected to vary according to the treatment given to it after harvest. The density points (DP) will vary due to the fact that the vegetable will be exposed to different environments before consumption of the produce. The nutrient mineral density points, which may be analyzed for the Solanum leaf samples were outlined in a flow chart (Figure 1)



(Adopted from Akundabweni, unpublished)

Figure 1: Flow chart of Conveyer Belt Cascade of events that could affect nutrient concentration in *S. scabrum* from sowing to consumption

* CSPH = Concentration Stage of Produce Handling at fresh leaf stage
NMD = Nutrient Mineral Density
DP = Density Points

Concentrations for *S. scabrum* plants may vary due to the treatments given to them as in the case of DP2 compared to DP9 in the kitchen when they are about to be prepared. It was assumed that *S. scabrum* had the highest nutrient concentration at DP2 since they had been oven-dried immediately after harvest. Other studies have shown that the quality of vegetables can be prolonged if respiration and transpiration can be reduced (Tan *et al.*, 2005) along the produce chain. If quality of the vegetables were maintained, the nutrient concentrations in the vegetables at DP2 at harvest would reflect the concentrations at DP9 in the kitchen. Further assuming that these elements

are not destroyed by cooking methods, same concentrations of the elements would be at DPH in the cell

2.3.4 Post-harvest handling effects on nutrient mineral density of *Solanum*

Post-harvest handling affects the nutrient quality of *Solanum* species finally consumed by family households (Panhwar, 2006). The post-harvest practices include drying, curing and preservation depending on the family's choice. Although experiments have not been done specifically for *S. scabrum*, these practices most likely affect the nutrient concentrations of leafy vegetables. A study done on *S. nigrum* on post harvest handling when the vegetable was held at 25-28°C for 4 days resulted in a rapid deterioration in quality with regard to ascorbic acid (Fawusi, 1983). Since direct sun has higher temperatures, the drying process has a greater chance of affecting the nutrient quality of the vegetables, before preparing them as accompaniments, thus reducing the mineral elements of nutrient value concentration.

2.3.5 Food preparation effects on *Solanum*

Food preparation after harvest affects the nutrient quality of vegetables as well as fruits, which may affect the concentrations (Tyann, 2005). Different households prepare these vegetables using cooking methods, which highly affect their nutritive quality. The commonly used method for preparing Solanum dishes among the Luhya community of Kenya is boiling them for long hours with the reasons of destroying the anti-nutrients found in them. Although this practice would be beneficial, the vitamins are destroyed by exposure to long hours over the fire, thereby reducing their nutritive

value Although GPA62 had high levels of micronutrients, the method of preparation will affect the consumed concentrations

2.3.6 Distribution and marketing in relation to quantity and quality of *Solanum*

Sale of the *Solanum* vegetable will also widely affect the quality and quantity due to the following reasons When the vegetables are harvested, they are packed in sacks bruising some of the produce, they are then transported to the market places on trucks, which are exposed to the sun s rays that also affect their quality (Wilson *et al.*, 1999) When these vegetables reach their destinations, they are normally withered and have lower concentrations of micronutrients as compared to harvest time. Studies on *S. scabrum* in relation to the effect of exposure of the vegetable to environmental conditions have not been done Research done on *S. nigrum* showed that drying in the sun or in mechanical driers resulted in rapid decline of ascorbic acid content (Fawusi, 1983). GPA62, GPA111 and the offspring would be equally affected if transported to far markets on either open trucks under unfavourable conditions

2.3.7 Home use, nutrient bioavailability and income from *Solanum scabrum*

Different families have various methods of preparing these Solanum dishes and so the finally consumed nutrient concentration is highly variable *Solanum* species, used as food (Guarino, 1997), have medicinal value and are sources of fodder and browse (Edmonds and Chweya, 1997; Guarino *et al.*, 1995; IPGRI, 1997). For food purposes, assuming that households consume GPA62, there would in effect be less spent money on purchase of other accompaniments to substitute for the insufficient nutrient concentrations found in GPA111

The production of Solanum has offered job opportunities to many people from the garden to the final sale in the market place due to the chain of events that take place before the vegetables are consumed (Chweya and Eyzaguirre, 1999). For example, supermarkets like Uchumi and Nakumatt today maintain a regular stocking of nightshade vegetables but can hardly meet the demand. Assuming consumers were aware of the higher nutrient varieties of the African nightshades, faster sales would be made on them before selling the rest.

These vegetables, when bought from market places provide less nutrients than fresh produce. Even then, not all of it is used up or taken up by the body since it may not be available for breakdown (CAZS, 2006; Kannan, unpublished).

In conclusion, given the continuum along which density points are growth and development time-series dependent, this pre-breeding study is only an initial phase of a long journey of the needed scientific inquiry (i.e. at DP2 see Figure 1).

2.4 Genotype by Environment Interaction performance of *Solanum scabrum*

Agricultural production may be increased through improved efficiency in the utilization of resources such as increased productivity per unit of land and of money, and through a better understanding and utilization of the genotype by environment interaction (Allard, 1960; Falconer, 1989). Having singled out the genotypic and environmental factors affecting *S. scabrum*, an interaction of the factors could contribute to the expression of the nutrient concentration. For this study, it was least likely that the genotype by environment interaction could have played a part in the nutrient concentrations of the GPAs since both were planted under the same conditions in the greenhouse for the three generations.

2.5 Nutra-health associated with *S. scabrum* consumption

The word nutra-health brings together nutrition and health for the quality of the food consumed and what the body will gain from it. This is the body health systems at cell level, which dictates the amounts of nutrients needed for the proper body functioning and to a large extent mineral requirements fall in both Type I and Type II nutrient deficiency, which is a classification according to what it at a given mineral dosage affects, for instance for growth (Type II) or for a specific function (Type I) (Akundabweni, pers. comm., Barbara and Michael, 1991). Type I and Type II criterion was used although it was not biologically convincing. The contribution of this study towards Type I and II nutrients is that some of these elements are found in *S. scabrum* but they were considered in MAE-MIE criterion. Responses to essential micronutrient deficiencies fall into 2 categories.

A Type I nutrient deficiency leads to a depletion of body stores and then to a reduction in those metabolic functions dependent on the nutrient (Golden, 1996). Examples of type I nutrient deficiencies are related to the lack of calcium, iron, copper, manganese, iodine and selenium. Ca, Fe and Mn are the principle MAE and MIE elements detectable by EDXRF procedure because of the nutritional deficiency status of many Kenyans especially the vulnerable groups (Imungi and Porter, 1985).

A Type II deficiency because there are no stores other than normal tissue (for example water, oxygen, potassium, sodium, magnesium, zinc, phosphorous, protein, nitrogen, sulphur and some amino acids like threonine, lysine) evokes preservation of plasma and tissue levels even at the expense of growth, repair and immune function (Golden, 1996). Catabolism of normal tissues to release depleted nutrients can lead to the deficits of many or all Type II nutrient deficiency. Treatment requires balanced supplementation of all these, not only of the limiting nutrient. Zinc and Potassium are

the principle MAE and MIE elements detectable by EDXRF procedure because of the nutritional deficiency status of many Kenyans especially the vulnerable groups (Imungi and Porter, 1985) The major interest in a breeding program to address germplasm enhancement methodology or a breeding methodology for higher concentration as a way of contributing to the mitigation the 2 types of deficiencies. Pre-breeding studies such as the one under consideration can demonstrate a germplasm enhancement approach in *S. scabrum* among others for addressing the 2 types of deficiency in Kenya

Type I nutrient deficiencies are function specific since large amounts will result in abnormalities associated with biochemical reactions When there is a decline in the nutrient in question, the body does not respond to the change but the person falls ill thereafter Examples of elements that fall into this category are calcium, iron, copper, manganese, iodine and selenium (Akundabweni manuscript preparation, Barbara and Michael, 1991) Earlier data from Akundabweni's lab recognise mineral elements of nutrient value found in GPA62 and GPA111 from EDXRF results as Fe, Mn and Ca, which are successfully detectable using EDXRF procedure

Type II elements affect growth but not specifically and they tend to have equal concentration ratios in different foods Type II elements respond to decline in body quantities by stopping growth for the body to conserve whatever is available, but a severe decline of the elements leads to breaking down its own tissues to release the nutrient Examples of these elements are potassium, sodium, magnesium, zinc, phosphorous, protein, nitrogen, sulphur and some amino acids like threonine, lysine (Akundabweni manuscript preparation, Barbara and Michael, 1991) The mineral elements of nutrient value found in GPA62 and GPA111 from EDXRF results were potassium and zinc

2.6 Mineral elements of micro and macronutrient value with respect to the development of heritability estimation criteria for *Solanum scabrum*

Potassium (Type II nutrient deficiency) and calcium (Type I nutrient deficiency) follow each other in the Periodic Table having atomic masses of 39.0983 and 40.078 respectively. The mineral elements of micronutrient value have atomic masses of 54.938 for Mn, 55.845 for Fe, 63.546 for Cu and 65.409 for Zn. The trace minerals (with heavier atomic masses) are placed in the Group III or transitional elements category of the Periodic Table. They are required in very small amounts and can easily be toxic if higher amounts are consumed hence the reference to them as micro elements (MIE) while minerals of macronutrient value are required in larger quantities thereby known as macroelements (MAE). The planned MAE (ppm K+Ca) and MIE (ppm Mn+Zn+Fe) heritability estimation criterion is in this particular study an attempt to test its efficacy beside single elements criterion. MIE concentrations are biologically in trace amounts in both plant and animal tissues and therefore there is a possibility that their presence in plant tissues may be influenced by genetic and environmental factors.

Individual elements have been determined to have high heritability in rice and wheat (Garcia *et al*., 1997) thus an interest in this study to adopt single elements heritability estimation criterion.

Iron (Fe) is a Type I nutrient deficiency, which is function-specific and a deficiency of iron in both the young and the old leads to anaemia or the young having low intelligence quotient development (WHO, 1992). It occurs in foods, both plants (such as green leafy vegetables) and in animal meats and fish among others (Imungi and Porter, 1985). In Kenya, it has been noted that iron deficiencies are a concern by the WHO standards (Ministry of Health Demographic Survey, 2000). If GPA62 was to be the main vegetable, in principle, less supplementation should be required to reduce

cases associated with deficiencies as compared to GPA111 (Table 1) assuming that high quality of DPI I is possible

Potassium (K) is a Type II nutrient deficiency that affects the endocrine system, digestive system, cardio-vascular system, respiratory and neurological systems Its deficiency leads to retarded growth and inhibits protein synthesis Since it is found in all living tissues, high quantities of K are required for proper functioning of the body Most foods contain potassium but the best food sources are fruits, vegetables, juices, meats and cereals It is assumed that a diet comprising mainly GPA111 for K (Table 1) will result in retarded growth as compared to GPA62 due to the low K concentration in GPA111

Calcium (Ca) is a Type I nutrient deficiency and is the most abundant mineral in the body since it is important for both structural and metabolic functions and takes about 2 % of body weight It also provides strength to bones and teeth (COMA, 1991) Rich sources of calcium are mainly animal products and seafood although rare for people living far from fishing waters or expensive for the farm poor households The alternatives are plants that are rich in calcium such as indigenous vegetables, cereals (up to 15-398 mg/g) and fruits (47-895 mg/g) (Maundu *et al.*, 1999) *Solanum scabrum* could also be a source of calcium since GPA62 had high amounts of the element

Zinc (Zn) is a Type II nutrient deficiency that is responsible for ageing and its deficiency symptoms were similar to those of HIV/Aids patients (TESCU, 2000) In the body, it is highly concentrated in specialized areas of the brain, pancreas and adrenal gland, but is also present in all cells especially the nucleus since it activates many enzymes It is mostly found in meat products although some plants have little quantities (Imungi and Potter, 1985) *Solanum nigrum* was found to have zinc levels up to 20 times more than indigenous cereals (Deosthale, 1980, Murage, 1990) Both GPA62 and

GPA111 had almost equal concentrations of zinc showing that they contribute equally to the specialised areas of the body

Manganese (Mn) is a Type I nutrient deficiency that acts as an enzyme activator and a constituent of metalloenzyme Manganese is an essential trace mineral concentrated in the bone, liver, pancreas and the brain Sources of Mn include peanuts, pineapple, oatmeal, shredded wheat, raisin bran cereal, beans, rice, spinach, sweet potato and whole wheat bread Dietary components that may adversely affect Mn absorption, retention or excretion include Fe, P, phytates, fibre, calcium, Cu and polyphenolic compounds

Type I and II elements have no bearing on plant tissue concentration since they are categorized on a nutritional basis in respect of the health of the consuming person

2.6.1 Conclusion from foregoing research

The varying concentrations of nutrients (Table 1) found in the seed of *Solanum scabrum* (Akundabweni, 2004) formed the basis of this research Leaf samples were used in this study because it is the leaf that is normally consumed and results were to be compared with seed to find out if there was any relationship The nutrients were classified into minerals of micro and macronutrient value, MIE and MAE respectively

Table 1: Average seed Mineral Density between GPA62 and GPA111 of *Solanum scabrum*

Accession	MAE		MIE		
	Ca (ppm)	K (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)
GPA62	26450 000	39350 000	7800 00	182 5	16 30
GPA111	1 499	0 515	656 05	187 5	24.45
GPA62 minus GPA111	26448 501	39349 485	7143 95	-5 0	-8 15

(Adapted from Akundabweni, 2004)

The nutrients found in the seeds of *S scabrum* of the two accessions varied accordingly as Ca and K had a very high margin (Table 1) Since characters are affected by the genetic make-up and environment or the combination (Falconer, 1989), it was of importance to find out heritability of the nutrients to either eliminate or confirm the influence of the genotype on nutrient content

Plant breeding techniques of selection and hybridisation by the use of artificial crosses are important for developing heterozygous genotypes for self fertilizing species (Allard, 1960) and passing superior genes from parents to offspring (Watson, 1970) EDXRF method was used to analyse the nutrient composition of *S scabrum* and these values were used to calculate heritability.

2.7 Energy Diffusive X-ray Fluorescence Spectroscopy (EDXRF) analyses

EDXRF has been used before for soil analysis (Ashcroft, 1970) and leaf samples to analyse the elemental concentrations from dry plant matter (Akundabweni, 2004) The technique can also be used to analyse all the minerals present in any sample at for determination of elements in large sample sizes (International Atomic Energy Agency, 1997)

2.7.1 Principle behind EDXRF

X-ray spectroscopy is a technique where many elements in a sample are analysed rapidly (IAEA, 1997) A source is excited leading to the emission of X-rays of unique energies specific to the elements making up that sample and their concentrations are given When the primary X-rays emitted have sufficient energy they displace electrons from the innermost orbit creating vacancies that make the atom unstable To

gain stability, the electrons from the outer shells occupy the space in the inner shells, which leads to excitation of highly bound electrons from the inner K shell orbital of the excited atom to the L shell Relaxation of the excited atom in the sample to normal state (back to K shell) is accompanied by emission of fluorescent rays The x-ray fluorescence is brought about by the two binding energies of the two shells as the electrons move from the outer shell to the inner shell The fluorescence analysis leads to the measurement of intensity and concentration of X-ray from the sample (LeamXRF)

Figure 2 depicts that when an external source transmits an x-ray to a sample, an electron from K shell is ejected creating a vacancy and this causes an electron from either L or M shells to jump into the vacancy created by the movement of an electron from K shell In so doing, it emits a characteristic energy unique to the element creating another vacancy in either the L or M shells (Fig 2) The bust of electrons is converted into signals by the pre-amplifier, which go through the cables to the amplifier The amplifier amplifies these signals according to the peak heights and sends them to S-100 Canberra for qualitative analysis Quantitative analysis is performed by AXIL software (Ferrero *et al* , 2001, Kabuye, 2002, Lorber *et al*., 1978, Potts *et al* , 2000)

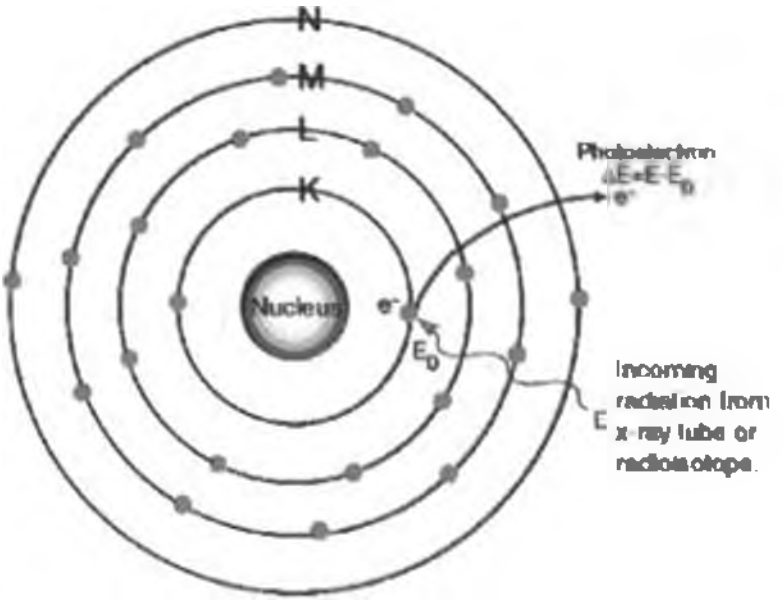


Figure 2: The Principle on which EDXRF works

(Source Images of Nature, 1999, http // ion ess asu edu/descript xrf htm)

2.8 Heritability by parent-offspring regression of traits in plants

Regression of offspring on parent has been applied to relatives for estimation of heritability of traits in plant species as a useful measure of degree of resemblance (Fernando and Gianola, 1988) More still, it has been shown that regression of offspring on parents is unaffected by the number of offspring used since variance of offspring does not enter into calculation of the regression (Appendix 14) Parent-offspring regression has been effectively and widely used for narrow sense heritability studies (Falconer, 1989, Kempthorne and Tandon, 1953) in maize (Smalley *et al.*, 2004), rice and wheat (Garcia *et al.*, 1997), wild birds (Keller *et al.*, 2001), Lucerne (Pecetti and Piano, 2005) asparagus (Cravero *et al* , 2002) nitrogen fixation symbiosis (Fernandez and Miller, 1985) and alcoholism (Marie *et al* , 2005) thus the choice for this study

In contrast to regressions, correlations between offspring (Appendix 11, 12 and 13) and parents do not measure degree of resemblance since the variance component of offspring is included in the equation (Falconer, 1989; Singh and Chaudhary, 1977)

On the other hand, when estimating heritability, the environmental variance is dependent on culture and management conditions with low heritability in variable conditions and high heritability is recorded for uniform conditions. The fitness of the character also affects the heritability values with lowest heritability levels for characters connected with reproductive fitness. This notwithstanding, heritability cannot be estimated with great precision since most estimates have large standard errors (Falconer, 1989)

2.9 Mendelian genetics and Hardy-Weinberg Equilibrium

Gregory Mendel worked on some peas characters (traits) before the 1900 and established a pattern on the inheritance. Two Mendelian laws were derived from this study, which included the first of Independent Segregation and Mendel's second law of Independent Assortment (Watson, 1970). Later studies by Hardy and Weinberg gave rise to Hardy-Weinberg equilibrium to explain Mendelian inheritance (Wigginton *et al*., 2005) with some assumptions made. The Hardy-Weinberg Principle (HWP) stated "In a large, diploid random mating population with no selection, no mutation and no migration, gene frequencies remain constant from one generation to the other" (Kang and Shin, 2004, Watson, 1970, Wikipedia encyclopedia). This was expected for *Solanum scabrum* although there was violation of the HWP since selection and artificial mating had been done for the parents. Stark (2006) did some work on Hardy-Weinberg proportions and showed that these proportions can be maintained by non-random mating provided the populations were discreet and non-overlapping.

Some patterns are expected when two parents are crossed that vary in a trait according to Mendelian ratios such that all the F1 individuals show uniformity or resemble either parent for the dominant type In the second (F2) generation all kinds of gametes produced by the F1 increase the number of genotypes for which the heterozygote increases 3^n times (n is the number of gene pairs for which the hybrid is heterozygote) (Strickberger, 1990) For example, *S. scabrum*, a hexaploid with 2n=6x=72 (Edmonds and Chweya, 1997), the number of possible combinations into genotypes is 3^{72} With this enormous variation, it is expected that numerous combinations of genotypes make up different individuals

Continuous quantitative variation may be produced by a multitude of individual genes, each with a small effect, on the measured character Interaction occurs when genotypes act differently in different environments so that quantitative predictions cannot be made by genotype and environment alone (Falconer, 1989) Breeding of two genetically different plants shows more heterosis in the offspring than either of the two parents considered separately Allard, 1960). Since *S. scabrum* is self-fertilising (Edmonds and Chweya, 1997), it is expected to be homozygous at various loci and inheritance is most likely to be in Mendelian fashion

Some of the descriptors could be redundant in characterizing the *S. scabrum* accessions thereby narrowing down to four main descriptors, i.e. days to flowering, blade width, stem wings and stem colour, were retained for further characterization of the accessions (Focho *et al.*, 2006) Chi-square (χ^2) tests are used to find out if traits are inherited according to Mendelian Second law of Independent Assortment (Watson, 1970)

2.10 Chi-square (χ^2) test

Hardy-Weinberg equilibrium tests are mostly done using chi-squares (Waggnton *et al.*, 2005) These chi-squares test for traits that depart from the Hardy Weinberg Equilibrium in population genetics (Abbiati *et al.*, 1993, Welleck, 2004; Wikipedia encyclopedia) The Chi-square equation consists of observed and expected values for goodness of fit as follows

$$(\chi^2) = \Sigma(\text{Observed} - \text{Expected})^2 / \text{Expected}$$

The observed values are calculated from the number of observations made on a trait in a number of classes while the expected value follows the classic Mendelian ratio, which is calculated The answer is then checked from a table to find out if the Mendelian ratio has been followed or not by getting the degrees of freedom and the level of significance (Strickberger, 1990) The use of chi-square was employed in morphological and nutrient content analyses for inheritance in the Mendelian fashion

For any character conforming to the Mendelian ratios it is expected that the phenotype gives a 1:2:1 ratio, which represents 1 DD 2 Dd, 1 Dd referring to homozygous dorminant, heterozygous and homozygous recessive (Strickberger, 1990) One trait and two trait Chi-squares were used for this experiment for independent assortment (Watson, 1970) The phenotypes in this study, i.e leaf margins, tip shapes and stem colour, which showed variation in the F2 generation, were used to get the values for the Chi-square for the 1:2:1 Mendelian Inheritance ratio.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Plant material

Two *S. scabrum* accessions (GPA62 and GPA111) were used in this study due to noted variations in nutrient variations from previous work by Akundabweni (2004) GPA62 was found to have 10.8% of the micro elemental (MIE) density and 89.2% of the macro element (MAE), and were classified as High Micro elemental (HIMI) and High Macro elemental (HIMA) GPA111 had 99.8% of micro elemental density (HIMI) and 0.2% of macro elemental described as too low macro elements (TOLOMA) The seeds of GPA62 and GPA111 were obtained from ex-manife in Cameroon and Maseno in Kenya (Akundabweni, 2004) respectively

A qualified curator taxonomically confirmed GPA111 and GPA62 at the Herbarium of the National Museums of Kenya (NMK) The species identity was further verified by the fact that the deep purple berries that had formed did not drop off at maturity as confirmed in literature (Edmonds and Chweya, 1997). Morphological tags for *S. scabrum* included some brown anthers for both accessions at flowering, while the petals were white

Fifteen seeds were randomly picked from each accession for crossing to give F1 and F2 offspring The number was chosen based on the fact that *S. scabrum* yields up to 144 seeds per berry (Edmonds and Chweya, 1997) and only about 200 plants were required for the progeny in accordance with Allard (1960) Ten F1 seeds were randomly selected for F1 generation to give rise to F2 progeny It has been noted that in the F1 generation, enough hybrid plants should be grown to produce seed necessary for F2 population of desired size (Allard, 1960) The desired size for the experiment was to

be large enough to allow for calculating Mendelian ratios and small enough to fit in the greenhouse used for the experiments. The seeds were singly sown in soil (Nitisols) packed in plastic pots.

There were six rows of pots with GPA62 and GPA111 in alternating layout (Layout 1; Picture 1); each row with six plants of an accession for ease of making artificial crosses for ease of making reciprocal crosses.

Layout 1 Arrangement of the accessions in the greenhouse

Row 1: *Solanum scabrum* (GPA62)

Row 2: *Solanum scabrum* (GPA111)

Row 3: *Solanum scabrum* (GPA62)

Row 4: *Solanum scabrum* (GPA111)

Row 5: *Solanum scabrum* (GPA62)

Row 6: *Solanum scabrum* (GPA111)

Picture 1: Potted *Solanum scabrum* plants in the greenhouse

The plants were watered once or twice daily during cool and hot days, respectively. However, watering regime also depended on age/size of the plants. Younger plants were watered less frequent relative to mature plants.

3.2 Growth substrate

The soil substrate used in experiments conducted during this study was collected at University of Nairobi, Kabete field Station, classified as Nitisols. The soil was autoclaved, allowed to cool down and then packed in one-kilogram plastic containers in the greenhouse. The seeds were sown into the plastic containers with three-quarter full of soil.

3.3 Energy Dispersive X-ray Fluorescence (EDXRF) analyses

The EDXRF elemental concentration analysis of the leaf samples was conducted at thirteen weeks after planting. Leaf samples were used in this study since they are the ones consumed and not the seed and secondly, EDXRF requires that the samples are crushed and pelleted for analysis, which could have destroyed seed for sowing. The EDXRF system consists of an X-ray spectrometer with Cd-109 radioisotope source, a Canberra Si (Li) detector, an ORTEC spectroscopy shaping amplifier (Model 571), an ORTEC high voltage supply bias (Model 459), an ORTEC liquid nitrogen monitor, a Canberra Multichannel Analyzer or a spectral data processing unit MCA (S-100) linked to a personal computer. The computer was used for data storage and analysis using AXIL and QAES softwares (IAEA, 1997).

Spectral data analysis was conducted as follows. Leaf samples from the parents, F1 and F2 generations were picked separately and put into brown carrier bags and

labelled. The bags with leaf samples were oven-dried for 72 hours at 95° C. The dried crispy leaf samples were finely ground and then sieved with <50 µm mesh and weighed (0.3-0.5 g) using an AT460 DeltaRange balance. The samples were then pelleted prior to elemental concentrations analysis using EDXRF system (IAEA, 1997). During analysis using EDXRF system, individual pellets were irradiated with x-rays in a containment box. The energies emitted from the irradiated sample under the influence of the Cd-109 radioisotope source were amplified and observed on the computer monitor as multiple peaks for different intensities. All samples were first subjected to x-rays from the Cd-109 radioisotope source for 2000 seconds and then for 100 seconds with molybdenum target for absorption correction. The data generated was then analysed using AXIL and QAES computer software to determine the specific nutrient composition of each sample and were computed in parts per million (ppm).

3.4 Breeding

3.4.1 Artificial pollination of GPA62 and GPA111

GPA62 and GPA111 started flowering at eight weeks after sowing. Reciprocal crosses were made between GPA62 and GPA111 to generate the F1 progeny under controlled environment. Pollination was conducted early in the morning to avoid chances of pollination by stray insects in the greenhouse.

At crossing, the flowers were first emasculated followed by artificial transfer of pollen from one flower to another. Emasculation was done just before the petals opened to avoid self-pollination, using small sharp forceps. The process involved delicate removal of five mature yellow stamens surrounding the green pistillate in the middle of the flower. This was followed by either tapping of pollen onto the thumb or onto

toothpicks prior to transferring the pollen to the recipient plant. The thumb and forceps were then wiped using ethanol to avoid contamination. The artificially pollinated flowers were labelled as female, whereas those used as source of pollen were labelled male. No bagging of the crosses was done to reduce the chances of flower abortion created by the microenvironment within the bag as highlighted by Edmonds and Chweya (1997). The crossing exercise was all done under greenhouse conditions to reduce chances of external pollination.

The pollinated flowers were left to mature until the stigma that had turned brown eventually fell off the ovary. The ripe fruits containing F1 seeds (shinny purple in colour) were subsequently harvested five weeks after pollination. Seeds were extracted immediately after harvesting the fruits by squeezing them out of the fruits onto an absorbent paper. F1 seeds were then left to dry for three days under shade before re-sowing to produce F1 plants from which F2 seed was to be later harvested.

3.4.2 Breeding of F1 and F2 generations

The F1 generation seeds were also sown into pots containing autoclaved soil (Nitisols) three days after extraction from the fruits. Two seeds were planted per pot, but only one seedling was allowed to grow to maturity. Ten pots were used for production of F2 generation seeds. The flowers were left to naturally pollinate, since *S. scabrum* is self-pollinating species. In the F1 generation, the fruits were ready in five months from the time of sowing to harvest. F2 generation seed was produced from the selfed F1 flowers. During breeding of F2 generation a total of 211 seeds were sown, two per pot. The flowers were also left to self-pollinate naturally.

3.5 Data collection and analysis

Plant height, leaf width and length data were collected on weekly basis. For leaf length, data was obtained by taking the mean of the three largest leaves on the plant, whereas height measurements were taken from the soil level to the tip of the plant. Plant height and leaf length were assessed in all generations (parents, F1 and F2). Data on stem form, leaf tip shape and stem colour were taken at four and ten weeks after germination. This was undertaken to establish any phenological changes during growth.

EDXRF analysis of leaves was conducted thirteen weeks from after sowing for all generations. The mineral elements of micro and macronutrient value analyzed in EDXRF analysis were potassium, calcium, iron, manganese and zinc. Plant growth (morphological) data were subjected to ANOVA using GENSTAT 5.1 (Genstat, 2000) statistical package against Linear Regression Analysis.

Parent-offspring regression has been effectively used for narrow sense heritability studies (Falconer, 1989, Kempthorne and Tandon, 1953) for maize (Smalley *et al.*, 2004), rice and wheat (Garcia *et al.*, 1997). Hence the preferred choice for this study. In addition, since Parent-offspring regression is not affected by the number of offspring when calculating heritability (Appendix 13; Falconer, 1989) this was the ideal choice for *S. scabrum*. Parent-offspring regression analysis was done using GENSTAT 5.1. Heritability was calculated in 2 criteria namely Single elements criterion and MAE-MIE criterion was done by summing the MAE and MIE separately. Heritability estimates were calculated from Linear Regression Analysis ANOVA using the following formula R^2 = 100 x |1-(RMS/Total Mean Square)| from Genstat statistical package. Mid-parent heritability can only be calculated if the variance were same for both parents (Falconer, 1989), since the variances for this study were not equal, mid-parent values were not calculated.

Correlation has also been used in other experiments to calculate heritability (Falconer, 1989; Smalley *et al*., 2004; Kempthorne and Tandon, 1953) but it is limited by the fact that variance among the offspring is included in the equation (Falconer, 1989)

Chi-square (χ^2) test was done to find out if traits were inherited in a Mendelian fashion (Strickberger, 1990, Watson, 1970) The formula for Chi-square calculations that was used was as follows

$$\chi^2 = \Sigma(\text{Observed} - \text{Expected})^2 / \text{Expected}$$

Chi-square test was based on single elements criterion The two-way Chi-squares were calculated using the formula from SAS software (SAS Institute Inc, 2000) for relationship between qualitative traits

CHAPTER FOUR

4. RESULTS

4.1 Nutrient Mineral Density analyses using EDXRF

4.1.1 Single nutrients classification

The MAE and MIE concentrations among GPA62, GPA111 and their progenies were significantly different (Appendix 7, 8 and 9) (Table 2)

Table 2: Mean concentrations of elements as determined by EDXRF that may correct the Type I and II deficiencies in the leaves of *S. scabrum*

Mineral	GPA62	GPA111	F1	F2
K (µg/g)	43586±1332	32867±1095	39050±1302	49491±714
Ca (µg/g)	24589±963	16153±399	21547±1121	25070±424
Mn (µg/g)	679±49	642±27	627±10	820±35
Zn (µg/g)	55±4	67±2	60±2	72±2
Fe (µg/g)	796±27	487±10	617±24	720±18

NB: Table 2 shows the means and standard errors for each nutrient in all generations

GPA62 had high K and Ca concentrations as compared to GPA111 (Table 2) showing high minerals of macronutrient value and low minerals of micronutrient value concentrations. GPA62, GPA111 and F1 generations had 10 leaf samples each while F2 generation had 211 leaf samples. F2 generation had higher means for all the minerals analysed except Fe when compared to the other generations (Table 2)

However, for F1 generation both K and Ca concentrations had intermediate values as compared to GPA62 and GPA111. Similar trends were observed for Mn, Fe and Zn where GPA62 had higher amounts when compared to GPA111. GPA62 did not show significant differences, as shown in the appendix, ($p<0.05$) than F2 generation for K, Ca, Mn and Fe, but Zn was significantly different (Appendix 1). On the other hand, GPA111 showed significant differences ($p>0.05$ see appendix 2) compared to F2 generation in K, Ca and Fe but no significant differences in Zn and Mn. The

relationship between F1 and F2 generations showed significant differences in K (Appendix 3). For GPA62 and GPA111 compared to F2 generation, there were significant differences in K, Ca and Fe but not for Mn and Zn (Appendix 4, 5).

Table 3: Parent-offspring heritability levels for all mineral elements of nutrient value in the study

Nutrient	GPA62- offspring h²	GPA111- offspring h²
K	0.96% ns	6.4% **
Ca	0.88% ns	1.98% **
Fe	0.04% ns	0.54% *
Zn	1% *	0.46% ns
Mn	0.14% ns	0.4% ns

** Highly significant
* Significant
ns not significant

Heritability for single elements in each of the *S. scabrum* accessions was relatively low (Table 3). The parent-offspring heritability levels were different for both GPA62 and GPA111. The interesting finding from this study is that there seems to be a higher relationship between GPA111 and the offspring as compared to GPA62 for K, Ca and Fe. It would suggest that although GPA62 had high concentrations of most mineral elements (Table 2), the heritable amounts were low and this could be as a result of environmental influence.

Table 4: Nutrient Mineral Densities of *S. scabrum* leaf samples for all generations

Parameter	Plant part	GPA 62	GPA 111	F1	F2
% Macro-element rich	Seed	89 1	0 15	No data	No data
	Leaf	98 5	98 4	98 6	98 6
Mean MIE (µg/g)	Seed	8000 3	1312 9	No data	No data
	Leaf	509.8±60 3	398.6±43.9	434.71 ± 49.9	536 9 ±18 5
				No data	No data
Mean MAE (µg/g)	Seed	65800	2 014		
	Leaf	34091 8±2221 5	24510±1910 3	30298 7±2174 9	36780 3±705 5
		73800 2	1314 9		
Mean MAE + MIE (µg/g)	Seed				
	Leaf	34601 6±670 2	24908 6±560 5	30733 4±1076 3	37317 2±491
	Seed	HIMI	3HIMI		
Micro-element description	Leaf	TOLOMI	TOLOMI	TOLOMI	TOLOMI
Macro-element description	Seed	HIMA	TOLOMA		
	Leaf	HIMA	HIMA	HIMA	HIMA

HIMI - High Microelements; HIMA – High macroelements; TOLOMI – Too low microelements

4.1.2 Macro-elements (MAE) and Micro elements (MIE) Description-based classification

Leaf samples of GPA62 had 34091 µg/g and 509 µg/g for MAE and MIE, respectively (Table 4). A combination of MAE and MIE had a concentration of 34500 µg/g. Further analysis of MAE and MIE for *S. scabrum* in this study indicated that GPA62 and F2 generations were not significantly different ($p<0.05$) in MAE and MIE concentrations (Appendix 7). There were significant differences between GPA111 and the F2 for MAE and MIE (Appendix 8).

Table 5: Parent-offspring description based heritability (%) of MAE and MIE in *Solanum scabrum*

Nutrient	GPA62- offspring h²	GPA111- offspring h²
MAE	0.36 ns	24.8**
MIE	0.74 ns	3.2 *

** Highly significant, * Significant, ns not significant

Heritability was low for the relationship involving GPA62 but high in GPA111 (Table 5). This was the same case for single elements in GPA62 and GPA111 relationships (Table 4). This further shows that more offspring had closer relationships to GPA111 than GPA62 meaning the high concentrations in MAE and MIE for the latter were not genetically linked.

Having considered the heritability levels and variation between the generations, Chi-square tests were done to find out if *S. scabrum* traits are inherited in the Mendelian manner.

4.2 Goodness-of-fit for Chi-square of the elements in F2 generation of *S. scabrum*

Table 6: Chi-square test values for elements in F2 generation of *Solanum scabrum* showing 1:2:1 Mendelian Inheritance

Mineral	Class interval	Observed values	Expected values	Tabulated value	Probability (5% level of significance)
K	20000 - <40000	42	52.75	5.99	0.16
	40000 - <60000	148	105.5		
	>60000	21	52.75		
Ca	10000 - <20000	39	52.75	5.99	0.0676
	20000 - <30000	128	105.5		
	>30000	44	52.75		
Mn	200 - <500	49	52.75	5.99	0.005
	500 - < 1000	139	105.5		
	>1000	49	52.75		
Fe	200 - <500	33	52.75	5.99	0.0064
	500 - <800	114	105.5		
	>800	64	52.75		
Zn	< 60	76	52.75	5.99	0.0676
	60 - < 80	78	105.5		
	> 80	57	52.75		

The F2 generation K content values were used to calculate the Chi-square values Accession GPA62 had about 43000 ppm (µg/g) of K while GPA111 had 32000 µg/g These results show that the intermediate (40000-60000 µg/g) of K had the highest number, which is the same class that accession GPA62 fell into From the Chi-square table at 5% level of significance, the figure of 0.16 was less than the tabulated 5.99 implying that K concentration of *S scabrum* was inherited in the Mendelian 1 2:1 ratio (Table 6)

The values were used for Chi-square for Ca content in the F2 generation GPA62 had about 24000 µg/g of K while GPA111 had 16000 µg/g These results show that the intermediate (20000-30000 µg/g) of Ca had the highest number, which is the same class that GPA62 fell into. From the Chi-square Table at 5% level of significance, X^2 was 0 0676, which fell below the tabulated 5.99 (Table 6) meaning Ca in *S scabrum* is inherited in Mendelian 1:2 1 ratio of independent assortment

These results show that the intermediate class (500-<1000 µg/g) of Mn had the highest observed values (Table 6). These values were used in the Chi-square test for Mn in the F2 generation GPA62 and GPA111 fell in the intermediate class for K with 679 µg/g of K and 642 µg/g, respectively From the Chi-square Table at 5% level of significance, the figure of 0 005 fell below the tabulated 5 99 showing that manganese concentration of *S scabrum* was inherited in Mendelian 1:2:1 ratio of independent assortment

Values used for Chi-square (Table 6) had high frequency between 500 and 800µg/g, which is the same class that GPA62 was categorised From the Chi-square Table at 5% level of significance, the X^2 figure of 0 0064 fell below the tabulated 5.99 showing that Fe in *S scabrum* conformed to Mendelian inheritance

The values (Table 6) were used to get the Chi-square GPA62 had 54.7 µg/g of Zn while GPA111 had 67.2 µg/g These results show that the intermediate class (60-<80 µg/g) of Zn had the highest number, which is the same class that GPA111 fell into From the Chi-square Table at 5% level of significance, the calculated value of 0.0676 fell below the tabulated 5.99 implying that zinc concentration of *S. scabrum* was inherited in a Mendelian 1 2 1 ratio

4.3 Frequency distribution of the nutrient mineral densities of *S. scabrum*

Normality is expected for a large breeding population that follows the Hardy-Weinberg Principle (Allard, 1969), which means that the individuals of a given nutrient should give a normal curve (Figs 3, 4, 5, 6 and 7) These normal distribution pattern shows that only one gene is involved in its segregation (Allard, 1969). Frequency tables of nutrient mineral densities for the F2 generation were drawn to find out if there was normality as expected for large populations

4.3.1 Potassium

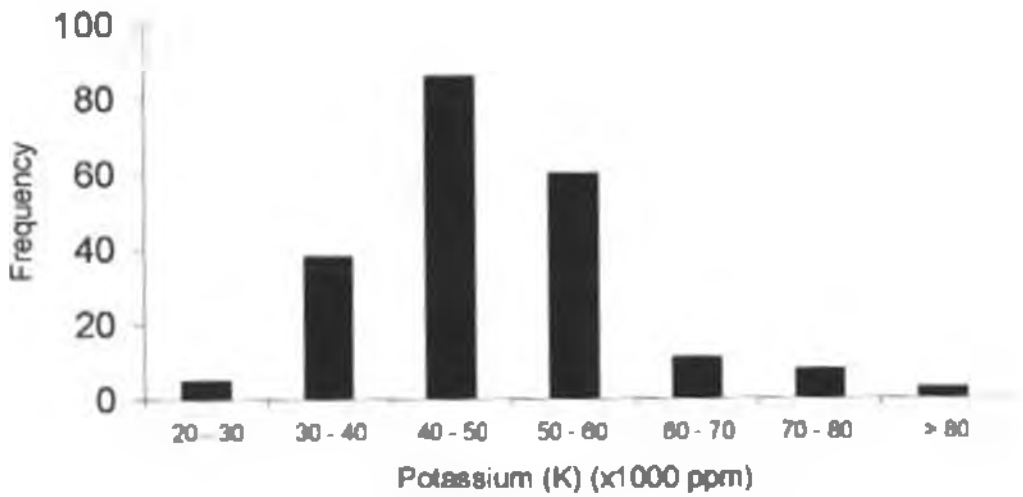


Figure 3: Distribution of Potassium in the F2 generation of *Solanum scabrum*

4.3.2 Calcium

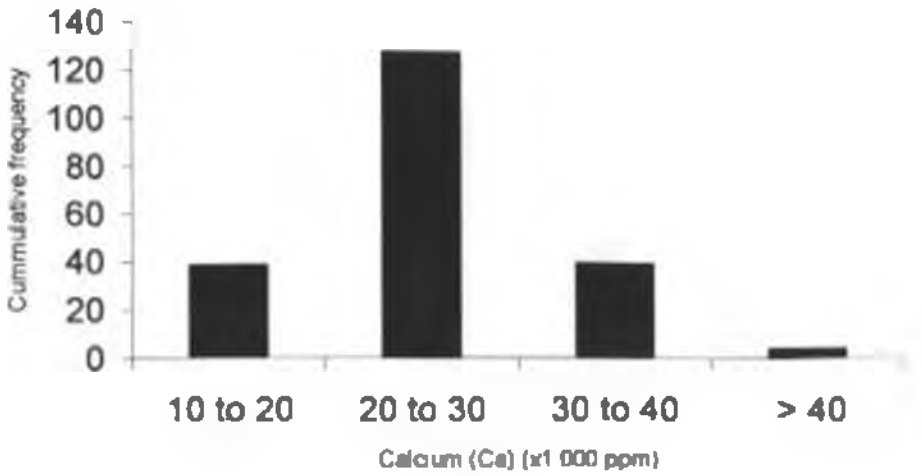


Figure 4: Distribution of Calcium in F2 generation of *Solanum scabrum*

4.3.3 Iron

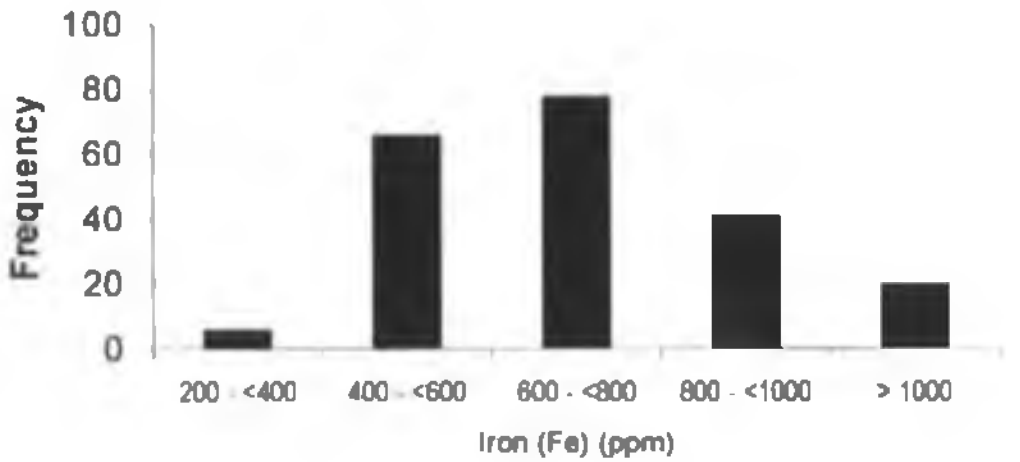


Figure 5: Distribution of iron in F2 generation of *Solanum scabrum*

4.3.4 Manganese

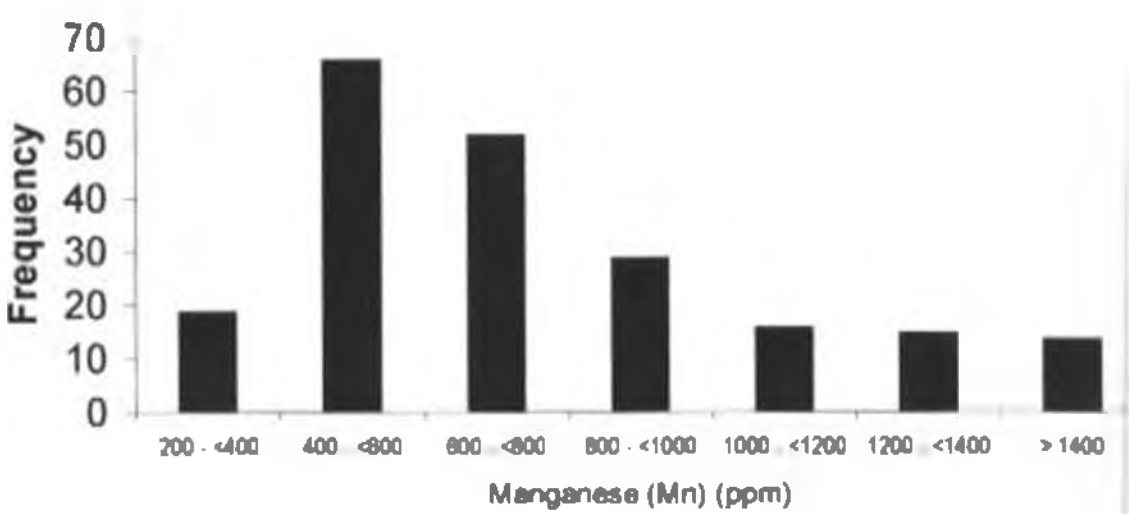


Figure 6: Distribution of Manganese in F2 generation

4.3.5 Zinc

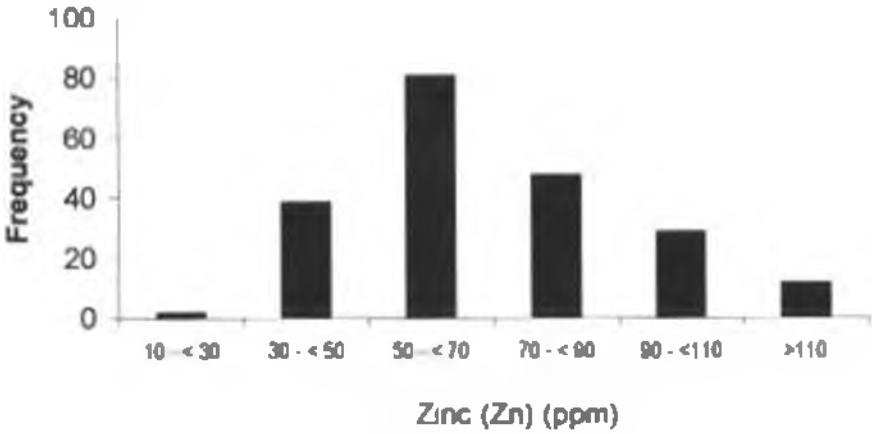


Figure 7: Distribution of Zinc in *S. scabrum*

Most of the hybrids in F2 had K between 40000 and 50000 µg/g, with comparison to GPAs, which had 49490 µg/g and 25070 µg/g of GPA62 and GPA111, respectively (Fig 3) while the highest hybrid had K concentration of 88600 µg/g The amount of calcium in these hybrids showed that most of the plants had between 20000 and 30000 µg/g (Fig 4), with the highest Ca concentration of 41250 µg/g

The iron content for the *S scabrum* hybrids had the highest concentrations of between 600µg/g and 800 µg/g (Fig 5), which was relatively high when compared to GPA62 and GPA111. Figure 6 shows that the high concentration of Mn was skewed to lower levels of the element Zinc concentrations showed a normal distribution (Figure 7) although the values were low thus it would also be recommended to get more Zn from other sources

4.4 Morphological data for GPA111 and GPA62 *Solanum scabrum* accessions

Table 7: Mean heights showing steady growth over time for GPA111 and GPA62

Accession	GPA111		GPA62	
Time (weeks)	Height (cm)	Standard deviation	Height (cm)	Standard deviation
11	12 33	10 05	16 67	8.39
13	21 41	7.51	25 09	6.45
15	56 64	18 63	48 05	7 19
16	62.36	19.15	57 91	10 88
17	67 77	21 88	61 86	10 38
18	68 77	20 47	64 45	9.89

There was a steady increase in height for GPA111 (Table 7) from week 11 to week 18 of *S scabrum* and started showing signs of senescence The increase in height for GPA62 (Table 7) could have been an indication that the numbers of leaves available would also be increased for consumption

4.5 Chi-square (χ^2) test for qualitative traits in F2 generation of *S. scabrum*

The qualitative traits observed for the study were leaf margin, leaf tip shape and stem colour where Mendelian heritability ratios were observed.

4.5.1 One-way Chi-square (χ^2) test for qualitative traits of *Solanum scabrum*

Table 8: Chi-square values for qualitative traits for F2 generation of *Solanum scabrum* showing 1:2:1 Mendelian Inheritance

Trait	Class interval	Observed values	Expected values	Tabulated value	Probability (5% level of significance)
Leaf margins	Smooth	79	52.75	5.99	0.004
	Smooth-serated	148	105.5		
	Serrated	24	52.75		
Stem color	Green	78	52.75	5.99	0.1225
	Purple green	71	105.5		
	Purple	62	52.75		

The values for Chi-square were outlined in Table 8. GPA62 was mainly serated while GPA111 was mainly smooth. These results show that the intermediate (smooth and serrated) leaf margin had the highest number thus none of the two *Solanum scabrum* parents dominated. The Chi-square value at 5% level of significance, showed a tabulated value of 5.99. The calculated value of 0.004 showed that the leaf margin of *S. scabrum* (Table 8) was inherited in Mendelian 1:2:1 ratio.

All the three categories were almost the same in numbers but the colour mainly dominated by GPA111 had the least. The calculated figure of 0.1225 is below the tabulated value of 5.99, showing that the leaf margin (Table 8) of *S. scabrum* was inherited in Mendelian 1:2:1 fashion.

4.5.2 Two-way chi-square ($\chi 2$) tests for leaf margin and stem colour

Chi-square test were done for the three variables, namely leaf margin, tip shape and stem colour to determine if there was any significant relationship. The first two variables to test were stem colour and leaf margin (Table 9).

Table 9: Two-way Chi-square for stem colour and leaf margin in *Solanum scabrum*

Variables		Leaf margin			
		Smooth	Serrated	Smooth serrated	Sum
Stem color	Green	53	0	19	72
	Purple	7	23	39	69
	Purple green	19	2	49	70
	Sum	79	25	107	211
		Leaf tip shape			
		Smooth	Pointed	Sum	
Stem color	Green	57	19	76	
	Purple	4	57	61	
	Purple green	18	56	74	
	Sum	79	132	211	
		Leaf tip shape			
		Smooth	Pointed	Sum	
Leaf margin	Smooth	59	18	77	
	Serrated	0	24	24	
	Smooth serrated	26	84	110	
	Sum	85	126	211	

Leaf margin and stem color had no relationship since the observed value of 85.4 was greater than the expected value 9.4. Therefore, the leaf margin and stem color have no relationship. The observed value of 77.7 was greater than the expected value of 5.99, implying that leaf margin and stem color have no relationship. The tabulated value of 5.99 was greater than the expected value of 71.3 (Table 9), indicating that leaf margin and stem color have no relationship.

4.6 Correlations for Leaf length and Nutrient concentrations for all generations

The correlations for *Solanum scabrum* and the element concentrations were analysed to find out which type of association exists between them in the parents and F2 generations. High negative correlations were observed for all generations

CHAPTER FIVE

5. Discussions

5.1 Variability in concentrations and heritability of mineral elements of nutrient value in *Solanum scabrum*

GPA62 had higher concentrations in the leaves of all the nutrients in this study as compared to GPA111 except zinc. This is a confirmation by preliminary work done by Akundabweni (2004) who found high elemental concentrations in the seed of *S. scabrum* showing that GPA62 has high elemental concentrations. Hybrids of *Solanum scabrum* had the highest concentration of K, Ca, Zn, Mn and Fe and a record of up to three times higher for some micronutrients compared to GPA62 and GPA111. Nonetheless, *S. scabrum* showed higher elemental concentrations of K in GPA62 seeds than in GPA111 but the concentrations were relatively high in the leaves for GPA111.

Heritability of K was low for GPA62 (0.96%) as compared to GPA111 (6.4%) but it was independently inherited. Similarly, independent assortment of K has been reported for rice with up to 50% heritability levels (Garcia *et al*., 2006). All the other nutrients in this study showed independent assortment, although heritability values were very low. It is of importance at this point to say that since GPA62 had higher concentrations of most mineral elements in the study as opposed to GPA111, the former accession had low heritability levels for single nutrients classifications. This could further suggest that although GPA62 had initial high elemental concentrations in the seed and leaf, the mineral elements of nutrient value in this accession could have had the environment play a greater role in inheritance.

The MAE and MIE heritability values were high for GPA111-F2, which implies that GPA111 is a nutrient-dense accession as compared to GPA62-F2. MAE-MIE

criteria seem to be of value since the genotypes had higher heritability levels as compared to single nutrients criterion. This could also be considered a novel criterion in classifying mineral elements of micronutrient value. In contrast, high concentration levels for GPA62 could have been due to environmental influences. This study of heritability of *S. scabrum* shows that the inheritance mechanism of the element factor is variety-dependent showed by the higher heritability values for GPA111-F2 as opposed to GPA62-F2 relationships.

The contrasts in display of nutrient content in seed, leaves and heritabilities could be attributed to the time of sample collection, which was done after flowering and to ploidy level. This can be confirmed by reports made by Grusak and Eduardo (1999), which showed that in the leaves, Zn is known to be mobile throughout plant growth but the other nutrients concentrate in mature leaves due to immobility. In addition, Cu, Zn and Fe are known to be high in the seed bran (White and Broadley, 2005) confirming that there was translocation of the nutrients from the leaves to the flowers for seed formation as it is a storage organ (Hartman *et al.*, 2002). This could explain the reason why there were low concentrations in the young leaves analysed in this study. Polyploids, on the other hand, have been reported to be complex in inheritance (Allard, 1989, Strickberger, 1990), to maximise genetic diversity and heterosis, but differ in adaptive strategies (Carputo *et al.*, 2003, Li *et al.*, 1996; Osborn *et al.*, 2003), and this could have affected the nutrient concentrations, although further studies should be done to verify this. This observation could partly explain the high variations in GPA62, GPA111 and their progeny.

In this study, there was positive correlation between Fe and Zn for GPA62, GPA111 and F2 generation, having values of 0.5, 0.4 and 0.4, respectively, which could indicate the possibility of increasing both nutrients for *S. scabrum*, concurrently

Other studies on cereals (White and Broadley, 2005) and rice and beans (Gregorio, 2000) reported similar results where the relationship between the two nutrients was positive and indicated the possibility of bio-fortifying both nutrients at the same time. The high correlation coefficients also indicated the possibility of pleiotropism (multiple phenotypic effects of single genes) (Strickberger, 1990) for Zn and Fe for *S. scabrum*.

From this study, it was observed that the inheritance mode for K, Ca, Zn, Fe and Cu are monogenic (singly inherited) for *S. scabrum*, since they all showed Mendelian inheritance pattern. Differences in leaf margin data taken before and after flowering could have been due to the presence of modifiers and/or pleiotropism. Modifiers are genes that change the phenotypic effects of other genes in a quantitative manner due to either increased or decreased enzyme activity, while pleiotropism is the multiple phenotypic effects of single genes (Strickberger, 1990).

Falconer (1989) cited that high heritabilities are expected for genotypes grown in uniform conditions. Although this study was undertaken in the greenhouse, the low heritability levels could have been due to the time of sample collection for analysis by EDXRF for nutrient concentration, which was after flowering when translocation of nutrients was in process. Leaf samples were collected after flowering had started because preliminary results by Akundabweni (2004) were elemental concentrations in seed. The study on leaf samples was to find out if the concentrations would be found comparable to the seed in any way.

5.2 Mineral elements of micronutrient value of *Solanum scabrum* in relation to malnutrition

Nutrient-dense accession, GPA111, has the ability to consistently pass on high heritability levels of elements from one generation to another, therefore showing

elemental sustainability However, for GPA62 to confer such abilities, it should be grown on fertile soils to give higher nutrient concentrations This notwithstanding, *S scabrum* will contribute towards sustainable provision of vegetables throughout the year Studies were conducted on improving micronutrient levels for staple foods (Graham and Welch, 1996), although indigenous vegetables have been known to have considerably high levels of micronutrients (Aphane *et al*., 2003; Maundu *et al*., 1997) Analysis on *Solanum scabrum* has shown to have higher Fe and Zn up to ten times and two times (from this study) respectively than beans, wheat, maize and rice (Gregorio, 2000) This suggests that *S. scabrum* vegetable could consequently be alternative to *S nigrum*, which is a commonly consumed vegetable in the Kenyan highlands

5.3 Energy Dispersive X-ray Fluorescence (EDXRF) as a pre-breeding tool for trait analysis

In this study, EDXRF was used to investigate nutrient heritability of *S. scabrum* and was found to be effective Other studies by Akundabweni (2004) and Munene (2005) also found EDXRF as a reliable tool for nutrient analysis for plants and soil This suggests that EDXRF could be used as a cheaper pre-breeding tool as compared to molecular markers for heritability studies EDXRF can be used effectively to analyse many elements in a sample at a given time This method has proved to be cheaper for research organisations than other spectroscopy methods because the latter involves expensive calibration (Sherman, unknown) Wet chemistry techniques require skilled labour, expensive reagents and are time consuming (Miller and Houghton, 1945)

CHAPTER SIX

6. Conclusions

- In general, F2 progeny showed higher mean values than any of the parents for all the nutrients analysed in this study
- GPA62 showed high concentrations for all elements except for zinc as compared to GPA111 even though GPA111 had high heritability values
- The parent-offspring regression and Chi-square results showed that the uptake of single elements showed very low heritability levels (<0.1) and that factors were probably inherited independently
- GPA111 gave h^2 of 0.24 for MAE (Ca and K) while GPA62 gave h^2 = 0.074
- MAE-MIE criterion seems to be of interest since it improved heritability of the genotypes. It increased heritability levels as compared to single nutrient criterion heritability levels, which were very low
- Chi Square values were suggestive of a monogenic inheritance of mineral density in *S. scabrum*
- On the overall, data also suggest that mineral micronutrient density is not entirely genetic and the environmental component in the phenotype may to a large extent be playing a role in leaf concentration
- *Solanum scabrum* as a vegetable prepared with other vegetables or milk and possibly of breeding enhancement to increase the concentration, is an important leafy vegetable contributing to mitigation of Type I and II deficiencies

7. Recommendations

1) Further investigation focussing on (Genotype) x Environment (G-E) interaction and studies on specific and general combining abilities (GCA) on selected individual strains or cultivar/ecotypes are warranted This GCA line of inquiry will yield data on average performance of parental lines as characterized by the average amount of heterosis in all hybrid combinations It would also be useful for determining mineral density of parental lines, among the genetic components and a breeding method for high mineral density

2) In mean time, farmers can expect high mineral micronutrient yields by prudently applying appropriate agronomic husbandry interventions such as

(a) Choosing a strain proven to show high mineral density promise and

(b) Choosing the right site and soil fertility conditions

APPENDICES

Appendix 1: Analysis of variance for GPA62 and GPA111 generation

Source		Ca	K	Mn	Fe	Zn
	d.f.	MS	MS	MS	MS	MS
Generation	1	2.327E+06 ns	2.515E+08 ns	205626 ns	59963 ns	2959.9 *
Residual	220	3.666E+07	1.035E+08	243658	63019	779.8
Total	221	3.65E+07	1.04E+08	243486	63005	789.6
SE		6054.6	10175.2	493.6	251	27.92

ns = not significant
* = Significant
** = Highly significant

Appendix 2: Analysis of variance for GPA111 and F2 generation

Source		Ca	K	Mn	Fe	Zn
	d.f	MS	MS	MS	MS	MS
Generation	1	8.313E+08**	2.552E+09 **	329350 ns	568229*	195.6 ns
Residual	220	3.627E+07	1.032E+08	242796	62714	775.1
Total	221	3.987E+07	1.143E+08	243187	65001	772.5
SE		6022.9	10161.1	492.7	250.4	27.8

Appendix 3: Analysis of variance for F1 and F2 generations

Source		Ca	K	Mn	Fe	Zn
	d.f	MS	MS	MS	MS	MS
Generation	1	1.185E+08 ns	8.509E+08 *	354106 ns	101108 ns	1186.9 ns
Residual	220	3.688E+07	1.038E+08	243593	63195	777.4
Total	221	3.725E+07	1.072E+08	244095	63367	779.2
SE		6072.6	10188.9	493.6	251.4	27.88

Appendix 4: Analysis of variance for Parents and F2 generations

Source		Ca	K	Mn	Fe	Zn
	d.f	MS	MS	MS	MS	MS
Generation	2	4.157E+08**	1.365E+09**	255220 ns	324038*	1543.9 ns
Residual	230	3.514E+07	9.961E+07	233402	60323	747.9
Total	232	3.842E+07	1.105E+08	233590	62596	754.8
SE		5928	9980	483.1	245.6	27.84

Appendix 5: Analysis of variance for Parents, F1 and F2 generations

Source		Ca	K	Mn	Fe	Zn
	d.f	MS	MS	MS	MS	MS
Generation	3	3.074E+08**	1.140E+09**	270942 ns	245161 *	1358.6 ns
Residual	239	3.429E+07	9.649E+07	224653	58272	720.6
Total	242	3.767E+07	1.094E+08	225227	60589	728.5
SE		5855.8	9823.2	474	241.4	26.84

Appendix 6: Analysis of variance for mid parent and F2 generations

Source		Ca	K	Mn	Fe	Zn
	d.f.	MS	MS	MS	MS	MS
Generation	1	4.391E+08**	2.099E+09**	502810ns	123394ns	2228.3*
Residual	231	3.669E+07	1.019E+08	232425	62333	748.4
Total	232	3.842E+07	7.938E+07	233590	62596	754.8
SE		6057	10095.1	482.1	249.7	27.36

Appendix 7: Analysis of variance for MAE and MIE for GPA62 and F2 generation

Source		MAE	MIE
	d.f.	MS	MS
Generation	1	7.557E+07ns	17291 ns
Residual	220	4.882E+07	98060
Total	221	4.891E+07	97694
SE		6986.8	313.1

Appendix 8: Analysis of variance for MAE and MIE for GPA111 and F2 generation

Source		MAE	MIE
	d.f.	MS	MS
Generation	1	1.574E+09**	450031*
Residual	220	4.875E+07	97768
Total	221	5.566E+07	99362
SE		6982.2	312.7

Appendix 9: Analysis of variance for MAE and MIE for parents and F2 generation

Source		MAE	MIE
	d.f.	MS	MS
Generation	1	1.114E+09**	306679*
Residual	231	4.883E+07	94157
Total	232	5.34E+07	95073
SE		6987.9	306.8

Appendix 10: Deduction of Heritability by Parent-offspring regression

(Adopted from Falconer, 1989) From the second method of calculating heritability, h^2 = VA/VP, it shows the regression of breeding value on phenotypic value

Then how come h^2 = bAP,

Where bAP is the regression of the breeding value and phenotypic value. If phenotypic value is split into breeding value and the remainder (R), consisting of environmental, dominance and interaction then,

P = A + R

Since A and R are uncorrelated

Cov AP = VA (Cov is the covariance between breeding value and phenotypic value)

So b AP = VA/VP = h^2

Covariance of offspring with parents is therefore calculated from summation of cross products and degree of resemblance expressed as regression of offspring on parents

Regression given by $bOP = \frac{Cov\ OP}{VP}$ where O = offspring, P = Parents

It is also important to note that covariance of offspring and parent values is equal to the additive genetic variation on condition that the sexes are equal in phenotypic variance more so the parent and offspring values should have the same variance although it is not common (Falconer, 1989). There two ways applied in deduction of covariance and the first method is the offspring-one parent regression while second is offspring-mid parent regression

In offspring-one parent regression, covariance to be deduced is that genotypic values of individuals with genetic values of offspring produced by random mating in a population. If these values are expressed as a deviation from the population mean, then the mean value of offspring is by definition half the breeding value of parent thus having CovOP = ½ VA

The regression of offspring is thereby obtained by dividing covariance of parents, which is the VP of population giving $bOP = \frac{½\ VA}{VP}$

For offspring and mid-parent, it constitutes of the covariance mean of offspring and mean of both parents (mid-parents)

This gives the calculation as P (mid parent) = ½ (X + Y)

So, summation of the cross products of parents and offspring is as follows

Sum OP = 1/2 (Sum OX + Sum OY)

When covariance is included, the formula results in ½ (Cov OX + Cov OY) = Cov OP

If X and Y have same variance then CovOX = Cov Oy

Thus CovOX = Cov OY = ½ VA

Appendix 11: Correlation matrix for GPA62 nutrients

Ca	1					
Cu	-0 566116	1				
Fe	-0 435411	0 160585	1			
K	-0 567974	0 313345	0 219556	1		
Mn	0 190189	-0.610986	-0.00852668	-0 561689	1	
Zn	0.0383922	-0.0581064	0 534833	0.163772	-0 301607	1
	Ca	Cu	Fe	K	Mn	Zn

Appendix 12: Correlation matrix for GPA111 nutrients

Zn	1					
Mn	-0.165456	1				
K	-0 316728	-0 239138	1			
Fe	0 411067	0 590405	-0 793305	1		
Cu	-0 143881	0 0266755	-0 40149	-0 0887241	1	
Ca	0 738693	-0.214405	-0.257917	0.0851484	0 299914	1
	Zn	Mn	K	Fe	Cu	Ca

Appendix 13: Correlation matrix for F2 generation nutrients

Fe	1					
K	0.197308	1				
Mn	0.361726	0 247816	1			
Zn	0 376202	0.178861	0 714701	1		
Ca	0.190292	0 459196	0 352918	0 284763	1	
Cu	0.147521	0 143188	0 269975	0 337373	0 0656622	1
	Fe	K	Mn	Zn	Ca	Cu

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