INFLUENCE OF PRODUCTION PRACTICES ON SEED QUALITY OF SPIDERPLANT (*Cleome.gynandra* L.) ECOTYPES FOUND IN WESTERN KENYA AND EASTERN UGANDA

BY

VERONICA NZILANI NDETU

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DECLARATION

Declaration by Candidate

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Ndetu Veronica Nzilani _____ _____
Reg. No. AGR/PGC/09/05 Signature Date

Declaration by Supervisors

This thesis has been submitted for examination with our Approval as supervisors

Dr. Linnet S. Gohole _____ _____
Signature Date
University of Eldoret, Kenya

Prof. Reuben Muasya _____ _____

Signature Date

South Eastern Kenya University, Kenya

DEDICATION

This thesis is dedicated to my husband Victor Ndetu and my children Reginald, Loretta Mutheu, Joy Mutethya, and my father Joseph Nzangi for their appreciation of my efforts, encouragement and support during this study.

ABSTRACT

Spiderplant is an African leafy vegetable that has gained popularity in the recent past, it is grown in a wide range of environmental conditions and is a rich source of vitamins A (β-carotene) and C and minerals Calcium (Ca), Magnesium (Mg) and Iron (Fe). This study was done in four sites Sigalagala in Kakamega, Kanduyi in Bungoma, Rubongi in Tororo and Nakalama in Iganga representing four AEZs namely Lower midland (LM) Upper Midland (UM), Lake Victoria Basin (LVB) and Mt. Elgon Farmlands (MEEFLS) in western Kenya and eastern Uganda. Its objective was to enhance understanding of ecological requirements for production and utilization of Spiderplant by determining the genetic makeup, nutritional make up in terms of vitamins and minerals levels and the appropriate on-farm seed production techniques in the four AEZs in western Kenya and eastern Uganda. Genetic analysis was by use of Random Amplified polymorphic DNA (RAPDs) analysis for leaf samples of Spiderplant ecotypes collected from each of the four AEZs. For vitamins and minerals analysis, edible parts of the spiderplant ecotypes were analyzed for vitamins A (β-carotene) C (Ascorbic) and for Minerals Ca, Mg and Iron Fe while the seed quality of spiderplant ecotypes was determined by germination % and electrical conductivity of seeds produced in each of the AEZs. Seed quality of spiderplant ecotypes using different management practices, Nitrogen application, leave harvesting and pod drying was also investigated. The study revealed genetic variations of Spiderplant ecotypes grown in the four AEZ. Variations were also found in the levels of vitamins C and β-carotene and the minerals Ca, Mg and Fe in the spiderplant ecotypes. Seed quality of the spiderplant was found to vary across ecotypes while management practices were also found to affect spiderplant seed quality produced in western Kenya and eastern Uganda. Molecular variation was found to relate to the seed quality, with the more polymorphic ecotypes having higher seed quality than the less polymorphic ones. Ecotypes with higher nutritional quality especially the vitamins were also found to have higher seed quality. In conclusion spiderplant ecotypes grown in western Kenya and eastern Uganda were found to vary in genetic makeup and the nutritional quality and these were found to significantly affect the quality of seeds produced by the ecotypes.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAS	atomic absorption spectroscopy
AEZ	Agro ecological zone
ALVs	African Leafy Vegetables
AFLP	Amplified Fragment length polymorphism
AOAC	Association of Official Analytical Chemists
BHT	butyl-hydroxytoluene
Ca	Calcium
CAN	Calcium Ammonium Nitrate
CTAB	Cethyltrimetylammonium bromide
DNA	Di Nucleic acid
dNTPs	Deoxynucleotide triphosphates
EC	Electrical Conductivity
EDTA	Ethylene diaminetetraacetic acid
FAO	Food and Agriculture Organization
Fe	Iron
I.S.I	International Statistical Institute
Ha	Hectare
HCl	Hydrochloric Acid
KOH	Potassium hydroxide
LM	Lower midland
LVB	Lake Victoria Basin
MEFLs	Mount Elgon Farmlands
Mg	Magnesium
$MgCl_2$	Magnesium Chloride
N	Nitrogen
PCR	Polymerase Chain Reaction
ppm	Parts per Million
RAPD -	Random Amplified Polymorphism DNA
RFLP -	Restriction Fragment Length polymorphism),

RNA-	Re-Nucleic Acid
rpm-	Revolutions per minute
SSR-	Simple Sequence Repeats
THF	Tetrahydrofuran
UM-	Upper Midland
UV-	Ultra Violet
WHO-	World Health Organization

LIST OF APPENDICES

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CHAPTER ONE

INTRODUCTION

1.1. Background information

1.1.1. African Leafy Vegetables

African Leafy Vegetables (ALVs) are reported to significantly contribute to the dietary vitamin and mineral intakes of local populations (Smith, and Eyzaguirre, 2007). They have increasingly become known for their importance in providing food and nutritional security in rural and urban areas in sub- Saharan Africa. Quite a large number of these ALVs have long been known and reported to have health protecting properties and uses (Okeno, *et al.,* 2003, Smith and Eyzaguirre, 2007). In Kenya and neighboring countries combination of cultural pride, interest in healthy foods, and a growing taste for diversity has created a favorable opportunity to protect and revitalize ALVs as nutritious resources derived from Africa's biological and cultural diversity (Smith, and Eyzaguirre, 2007).

ALVs are important source of food and income in many western Kenya communities. A survey conducted in some markets in western Kenya indicated that ALVs contributed 10% of the income generated by commodities in the markets (Onyango, 2002). In Uganda consumption of ALVs is reported to go up to an average of 160 g /person/day during the rainy season (Rubaihayo, 2002). The acceptance and utilization of ALVs in the urban areas of Uganda has resulted in some popular varieties being grown as cash crops for the urban markets (Hart *et al.,* 2002). There is need to promote production of these ALVs to meet the likely continual market demand in Kenya and Uganda. Sustainable production of any crop depends on sustainable supply of improved and high

quality seeds (George, 1999). The quantity and quality of African Leafy Vegetables also depend on the harvesting and processing of the seeds (K'opondo *et al.,* 2005). Measures leading to increased production of high quality seeds of ALVs therefore need to be given prominence. Focus on improvement of both field management and post harvest handling practices is likely to result in high quality of ALVs seeds and hence increased production of these crops.

1.1.2. Importance of Spiderplant in Kenya and Uganda

Spiderplant (*Cleome gynandra* L) is an ALV rich in vitamins A and C and minerals calcium, magnesium and iron. Its leaves are reported to contain over and above the normal recommended adult daily allowance of vitamins A and C and the minerals calcium and iron (Arnold *et al.,* 1985). Its leaves and seeds are used in indigenous medicine in many countries (Baruah and Sarma, 1984). It is a source of income and employment mostly to rural women in some African countries including Uganda and Kenya who sell the leaves and young tender shoots in rural and urban markets (Maundu *et al.,* 1995). Its cultivation has increased in low-rainfall areas in Uganda, and other parts of Africa (Schippers, 2002). It is grown in a wide range of environmental conditions and does well in altitude ranges of 0-2400 meters above sea level and is able to tolerate high and low temperatures (Chweya, 1995). It is an important and popular vegetable in western Kenya and eastern Uganda.

1.1.3 Areas of the study

This study was carried out in four areas representing major spiderplant growing AEZs in western Kenya and eastern Uganda the lower midland (LM), upper midland (UM), Lake Victoria Basin (LVB and Mount Elgon Farm Lands (MEFLS). Sigalagala area in Kakamega and Kanduyi in Bungoma represented the UM and LM in western Kenya while Rubongi in Tororo and Nakalama area in southern Iganga and represented Mount Elgon Farmlands (MEFLS) and Lake Victoria Basin (LVB) in eastern Uganda.

The project sought to establish whether there are Molecular and nutritional differences of the Spiderplant ecotypes found in western Kenya and eastern Uganda, as well as increase the insight into the effect of different crop and seed handling methods on quality of seed produced by farmers in the same regions.

1.2 Problem Statement

Low seed quality has resulted to low production and consumption of spiderplant despite its significance as an ALV in western Kenya and eastern Uganda. This low seed quality is likely to result from lack of information on the ecological conditions suitable for production of high quality spiderplant seeds in the region. Variations in ecological conditions likely to causes differences in genetic makeup, nutritional levels and consequently seed quality are also not known. The different farmers' agronomic practices also likely to affect the quality of spiderplant seeds produced in the region are not understood.

This effect of the agro ecological conditions on the, genetic makeup and nutritional quality and the appropriate seed production practices affecting spiderplant seed quality is

not understood.

This research therefore sought to determine whether Spiderplant ecotypes found in different AEZs in western Kenya and eastern Uganda differ in genetic makeup, nutritional levels and seed quality.

1.3 Justification

The study was carried out in western Kenya and eastern Uganda where Spiderplant is a major ALV. The cropping systems across the common border are the same and any research efforts addressing production and consumption of quality spiderplant would benefit the two sides of the countries equally. Owing to the proximity between western Kenya and eastern Uganda, there is plenty of cross border sharing and exchange of food stuffs and seeds between neighbors, relatives and common markets. Information on the suitable ecological conditions and appropriate production practices for high quality seed and vegetables of spiderplant is necessary for farmers and commercial seed producers to know from which AEZ they can produce good quality seeds of spiderplant.

Molecular characterization was necessary to establish whether there are genetic variations of Spiderplant resulting from the different environmental conditions prevailing in the different AEZs. This would help in establishing the genetic potential of spiderplant grown in the different AEZs that could be used for the crop improvement in the region.

Nutritional analysis was done to determine the suitability of different AEZs of western Kenya and eastern Uganda for production of spiderplant of good nutritional quality. The analysis was done for vitamins and minerals which are the most important nutrients in vegetables.

Seed quality of spiderplant produced in different AEZs was studied to find out the ecological conditions as well as best farmers' production practices suitable for production of good quality seeds of spiderplant in the in western Kenya and eastern Uganda. Good quality seed is important for improvement of the production of spiderplant in the region. The information on the suitable AEZs for production of best quality seeds of spiderplant can then be used for commercial seed production of the crop.

1.4 Objectives

1.4.1 Overall Objective

To enhance production and utilization of spiderplant in western Kenya and eastern Uganda by determining areas suitable for production of highly nutritious and high quality seed of the crop.

1.4.2 Specific Objectives

1. Determine the molecular characteristics of Spiderplant grown in different Agro-ecological Zones of western Kenya and eastern Uganda.
2. Determine the nutritional (vitamins and minerals) levels of Spiderplant found in different Agro-ecological Zones in western Kenya and eastern Uganda.
3. Determine the effects of farmers production practices on quality of Spiderplant seeds produced in different Agro-Ecological Zones in western Kenya and eastern Uganda.

1.5 Hypothesis

1. Spiderplant grown in different AEZ in western Kenya and eastern Uganda do not differ in genetic makeup**.**
2. Nutritional levels of Spiderplant grown in different AEZs in western Kenya and eastern Uganda are the same.
3. Quality of Spiderplant seeds produced in western Kenya and eastern Uganda is not affected by the farmers' production practices

CHAPTER TWO

LITERATURE REVIEW

2.1 Genetic variations due to ecological differences

Genetic variation or genetic diversity can be defined as the genetic differences among organisms within the same species or population (Conner and Hart, 2004). Genetic variation within populations means that more than one allele or genotype is present in the population while variation among populations is caused by spatially-varying selection processes such as genetic drift and past bottlenecks. These processes transform variation among individuals within populations to differences in genotype or genetic differentiation (Futuyma, 1998). For populations to survive they have to poses genetic traits that make them adapt to the prevailing ecological conditions (Frankham, 2005). Environmentally induced genetic changes may occur as a result of these environmental adaptation processes (Cullis, 1977)). Ecological and climatic conditions are known to determine the level of genetic variation of plants (Guleray *et al.*, 2010).

Information concerning the extent and nature of genetic diversity within a crop species is useful for selection of parents for hybridization (Rabbani *et al.*, 1998). This is because crops perform well when they grow within the environmental conditions to which they are suited. Cool climate legumes, peas, broad beans, chickpeas and lentils show the best developments in temperatures of around 18-20^{o} C while the hot-climate legumes like beans and cowpeas show a better development within temperatures of 20-24^{o} C (Ceyhan *et al.*, 2011).

2.2 Effects of ecological factors on Nutrient levels of plants

Plant nutrients are influenced primarily by a plant's genetic makeup and environmental conditions. Findings by Kramer *et al.* (2005), confirm that the nutrient content of plant parts depends upon the agro ecological zones in which they are grown. Temperature influences the composition of plant tissue, the uptake and metabolism of mineral nutrients by plants (Lee and Kader, 2000), while rainfall affects water supply to the plant, which may influence the composition of the harvested plant parts. Variations of micronutrients contents within same species have also been found to exist between samples of ALVs collected from areas with different soil characteristics (Gadgil *et al.*, 1993).

2.3 Effects of ecological factors on seed quality

Seed quality is one of the most important factors affecting the performance and productivity of most agricultural crops. Environmental factors acting during plant growth and seed development play a big role in determining seed characteristics produced (Kigel, 1999). The germination of a seed lot can be affected by environmental conditions in the field during seed growth and development particularly temperature, rainfall and relative humidity as well as by the conditions the seeds are exposed to during harvesting, drying, cleaning and storage (Egli *et al.*, 2005). Most factors that affect the physiological quality of seed are ecological conditions during filling and maturation stages (Lysiak, 2007). Electrical conductivity is also a test to determine the physiological potential of seeds. Seeds with lower physiological potential liberate a greater amount of electrolytes as a consequence of lower cellular membrane selectivity (Vieira *et al.*, 2001.

2.4 Ecological variation of the spiderplant growing areas in western Kenya and eastern Uganda

Agro Ecological Zones (AEZs) are land units within which soil, landform and climatic conditions are quantified (Amien, 1998). They have varied environmental conditions including temperature, rainfall and soils. The UM in western Kenya lies in an altitude range from 1300 to 1900 m with a mean annual temperature range of 18^{o} C to 21^{o} C and mean annual bimodal rainfall of 1800 mm per annum. LM which lies in an altitude from 800 to 1500 m has warmer temperatures ranging from 21^{o} C to 24^{o} C and a bimodal rainfall averaging 1500 mm per annum (Jaetzold *et al.,* 2007). The soils in UM are volcanic, well drained and deep while those in LM are described as having good physical properties but low in nutrient levels (Jaetzold *et al.,* 2007). In eastern Uganda the Lake Victoria Basin (LVB) which is mainly a banana-coffee system has a rainfall regime of 1 000 - 1 500 mm evenly distributed throughout the year in a bimodal pattern (Mwebaze, 2002). The LVB has generally sandy soils, with low soil organic matter levels, highly susceptible to leaching and consequently low in base saturation and rather acidic while MEFLs has mainly rich volcanic soils (Wortmann and Eledu, 1999). Owing to these variations, spiderplant grown in each of the AEZ is likely to have different genetic characteristics from the others.

2.5 Spiderplant ecotypes

An ecotype describes a genetically distinct geographic variety, population or race within species which is adapted to specific environmental conditions **(**Turesson, 1992). It is also described as a group of organisms, normally a subdivision of a species that is adapted to a

specific environment (Steven *et al.*, 1997). Typically, ecotypes exhibit phenotypic differences such as in morphology or physiology stemming from environmental heterogeneity and are capable of interbreeding with other geographically adjacent ecotypes without loss of fertility or vigor (Molles, 2005). Habitat heterogeneity, combined with natural selection, often result in multiple genetically distinct ecotypes within a single species (Linhart and Grant, 1996). Spiderplant ecotypes in this study were considered to be the spiderplant populations growing in different AEZs likely to vary from each other in genetic makeup, nutrient levels and seed quality.

2.6 Top dressing effects on seed quality

Nitrogen (N) is a very important nutrient element especially for green leafy vegetables (Dursun *et al.*, 2006). It promotes rapid growth of plants, increasing seed and fruit production and improving the quality of leaf and forage crops. The prudent application of nitrogen is essential to get an even maturing crop with full grain size. In seed production, it is added to ensure maximum growth of the side branches that enhances seed yield. Studies conducted by AVRDC (2003), established that, top-dressing spiderplant with 100 Kg/Ha of calcium ammonium nitrate (26% N at thinning stage, 3 weeks after seedling emergence) led to increase in the number of pods per plant in Spiderplant. The studies do not clarify how the quality of the Spiderplant seed produced is affected by this application of Nitrogen. Increase in plant nitrogen concentration has been found to show a significant increase of the nutrient in the seed (Bressani *et al.*, 1987). Given that nutrient supply to the maternal plant may affect not only seed chemistry but also seed coat structure and hormone content (Gray and Thomas, 1982) and given that nitrogen in

seeds is stored mainly in the form of protein, the nutrient treatments applied to the maternal plant can influence the seed quality. There is some evidence that nutrient treatments applied to the maternal plant can influence the nutritional quality of seeds.

2.7 Effects of defoliation on Seed quality

Studies have shown that defoliation of the maternal plant may lead to affects mean seed weight (Maun and Cavers, 1971). Leaf removal affects carbohydrate supply influencing hormonal and nutrient supply as well as translocation patterns (Salleo *et al.*, 2002)**.** This change in nutrient supply and consequent change in seed weight is likely to have an effect on the quality of the seed produced.

2.8 Seed drying methods and seed quality

Seeds are generally harvested at high moisture content and need to be dried before storage. The rationale of drying seeds is to reduce their moisture content to a level, which encourages longevity and consequently increase the regeneration intervals. Sun and shade drying which are common farmers' practices of drying seeds have been recommended by Ellis *et a*l., (1990) as some of the low cost methods of seed drying. Further drying seeds under ambient relative humidity and temperature is a common practice in many countries in Africa for small-scale seed drying (Probert *et al.*, 2009), but the results obtained depend mainly on the season, location and species (Hong and Ellis, 1996). Ravi *et al.*, (2007) also observed that all kinds of drying methods do not suit equally well under given set of conditions in retaining vigor and vigor of seeds.

CHAPTER THREE

MOLECULAR CHARACTERISTICS OF SPIDERPLANT ECOTYPES GROWN IN DIFFERENT AGRO-ECOLOGICAL ZONES IN WESTERN KENYA AND EASTERN UGANDA

3.1 Introduction

Spiderplant is grown in a wide range of climatic conditions and its morphological traits are reported to be significantly influenced by environmental conditions (Omondi, 1990; Chweya and Mnzava, 1997). A species with a broad distribution rarely has the same genetic makeup over its entire range (Hamrick and Godt, 1996). Spiderplant is therefore likely to poses genetic variations which make it adaptable to the varied ecological conditions in which it grows.

As spiderplant continues to gain popularity as an agricultural crop in Kenya and Uganda, understanding of ecological conditions to which it is best adapted becomes essential for the crop's improvement. Characterization of diversity is a necessary requirement for the improvement, use and conservation of plant genetic resources (Archak *et al.,* 2003). Understanding spiderplant ecotypes genetic variability and adaptability can be used as a stepping stone in identifying the ecotypes with novel genes which can be used in the improvement of the crop.

Molecular markers have proved to be powerful tools in the characterization and evaluation of genetic diversity within and between species and populations (Raoudha *et al.,* 2010). Random Amplified polymorphic (RAPD) DNA markers described by (Williams *et al.,* 1990) have been widely used for a number of applications in plant and genetic studies (Kumar *et al.,* 2001). Moreover, RAPD uses arbitrary primers that provide

a large number of multilocus markers, and can be applied to analyze organisms, even those for which no previous genetic or molecular information is available (Guleray, *et al.*, 2010). This study therefore sought to establish the genetic variation of Spiderplant found in different agro-ecological zones of western Kenya and eastern Uganda using RAPD molecular analysis.

3.2 Materials and Methods

3.2.1 Sample Collection

Tender leaf samples of Spiderplant were collected from each of the four sites Sigalagala in Kakamega, Kanduyi in Bungoma, Rubongi in Tororo and Nakalama in Iganga representing the four western Kenya and eastern Uganda AEZs UM, LM, MEFLs and LVB respectively. A total of 72 leaf samples were picked, 18 from each site with 6 representing each of the three spiderplant morphotypes, green, purple-green and purple. The leaf samples were kept in a cool box and transported by road to KARI Njoro for the samples picked in Kenya or to the Molecular Laboratories of Makerere University for those picked in Uganda

3.2.2 DNA Extraction

DNA was extracted from each of the collected samples using the protocol by Saghai-Maroof *et al.*, (1984) whereby about 850 g of leaf tissue was put in a mortar and liquid nitrogen poured halfway the mortar. Using a pestle the leaf was pressed gently to break it into smaller particles, followed by gentle grinding done by pressing the pestle on the

mortar in a circular motion and applying more pressure and more speed, as the nitrogen evaporated to crush the leaves into fine powder. Micro tubes were put in a rack placed in a container having liquid nitrogen to keep the crushed leaf tissue cool. The powder was put into the micro tubes using a spatula, which was cleaned after every sample to avoid contamination. The micro tubes were labeled with the sample number on the lid and on the side of the tube. After grinding DNA was extracted by the following procedure:

1 ml of CTAB incubated in a water bath at 65 ^{0}C to resolve for 35 minutes was added to the ground plant tissue. The sample was incubated for 60 min at 35 ^{0}C in a water bath with periodic shaking; tubes were removed and let to cool to room temperature. 1 ml of chloro; isoamyl alcohol (24:1) was added and mixed by periodically inverting the tubes for 10 min. The phases were separated by centrifuging for 10 min at 3600 rpm at room temperature. 600 µl of the top aqueous phase was recovered and put into a new tube using a sterile wide bored plastic pipette and avoiding the interface layer. 700 µl of cold isopropanol was added into each of new tubes and the tubes inverted 10-15 times and allowed to precipitate for 30 min at room temperature. The mixture was centrifuged for 10 min at 12000 rpm at room temperature and the supernatant removed by decanting. 1.5 ml of washing buffer was added and the tubes put on a vertical shaker for 30-60 min. after which they were centrifuged for 10 min at 12000 rpm at room temperature and the supernatant removed. The pellet was air-dried for 30 min and the DNA re-suspend in 100-200 µl of TE buffer (storage buffer) pH 8.0 and left to dissolve overnight in a cold room. The DNA solution was then transferred into a 1.5-ml eppendorf tube using a wide-bore pipette (blue). The second extraction was done to remove RNA by treating each of the samples with 8 µl of RNAase and incubating for 1 hour at 37 ^{0}C. DNA quality was

tested, using 0.8 % agarose gel electrophoresis, and its concentration was determined with a UV spectrophotometer. Part of the DNA was then diluted to 20 ng/µl for PCR amplification.

3.2.3 RAPDs Analysis

DNA analysis was done by Random Amplified Polymorphic DNA (RAPDs), Preliminary PCR amplification trials were performed with four samples one representing each AEZ and 20 RAPD primers were screened (Appendix I). Optimization was done using different concentrations of $MgCl_2$, DNA, dNTPs, and Taq DNA polymerase to obtain the most reproducible and reliable DNA amplification profiles. Optimal conditions, which revealed clear and reproducible amplification fragments, were, a total reaction mix of 10 µl containing 0.8 µl of 20 ng DNA 1 µl primer, 1 µl of 1x reaction buffer , 0.5 µl of DNTPs, 0.8 µl of 25 mM $MgCl_2$ 0.1 µl of Taq DNA polymerase and 5.8 µl of sterile distilled water. Amplification was performed in a thermal cycler with initial denaturation temperature of 93 ^{0}C for 1 minute followed by 45 cycles of 92 ^{0}C for 1 minute, annealing temperature of 36 ^{0}C for 1 minute, and extension temperature of 71 ^{0}C for 1 minute, followed by a final extension of 72 ^{0}C for 5 minutes. Amplification products were separated on 1.5 % agarose gel by electrophoresis at 150 Volts for three hours and detected by staining with ethidium bromide. The gels were photographed under UV light.

Primers that gave polymorphism (OP-07, OPV-06, OPU-08, and OPW-02 and OPD-20) were used to analyze all the 72 samples.

3.2.4 Data Analysis

Photographs from Ethidium bromide stained agarose gel were used to score RAPD data

for analysis. The presence of a particular band was scored as 1 and absence as 0. The positions of PCR bands were compared with molecular weight standards.

Data analysis was performed using the software Popgene version 1.31 for Population Genetic Analysis and subjected to diploid data analysis for genetic variation presented bygenetic distance and polymorphism which measures the proportion of genetic diversity within populations, (Nei, 1978). The levels of diversity and polymorphism determined the genetic diversity of the spiderplant ecotypes.

3.3 Results

The PCR bands separation of the by 1.5 % Agarose gel revealed polymorphic bands for the samples as in Figure 1

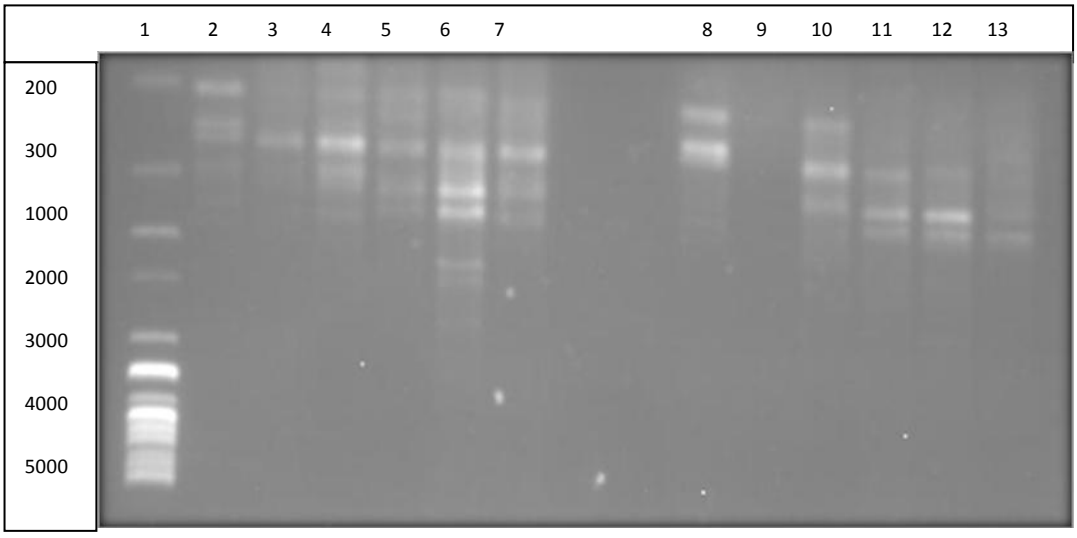

Figure 1: PCR RAPDs products bands where sample 1 was the ladder, samples 2, 3 and 4 represent samples from UM in Sigalagala- Kakamagea 5, 6 and 7 represented samples from LM in Kanduyi-Bungoma, 8,9 and 10 represent samples from MEFLs in Rubongi-Tororo while 11, 12 and 13 represented LVB in Nakalama in Igaga.

(Source: Author, 2010)

Molecular characteristics of Spiderplant grown in different Agro-ecological Zones of western Kenya and eastern Uganda were found to vary. Genetic variation was demonstrated by genetic distance determined by pair wise comparisons of the spiderplant ecotypes which ranged from 0.1352 to 0.4114 (Table 1.1). A large value comparing two ecotypes indicates large genetic variation between the two ecotypes while a small value shows less genetic variation. The least genetically varied ecotypes were therefore those between UM and MEFLs, followed by LVB and MEFLs, UM and LM, UM and LVB, LM and MEFLs and LM and LVB in that order (Table 1.1). Generally the spiderplant ecotypes from AEZs in one country were more genetically closer to each than from the other country AEZs except those from UM and MEFLs that were the genetically closest to each other (Table 1.1). The ecotypes from western Kenya were genetically more distant from each other compared to the eastern Uganda ones (Table 1.1)

Table 1.1: Genetic distance between Spiderplant ecotypes grown in AEZs in western Kenya and eastern Uganda

AEZ	UM	LM	MEFLs	LVB
UM	****			
LM	0.1960	****		
MEFLs	0.0944	0.3471	****	
LVB	0.2935	0.4114	0.1352	****

There was high polymorphism within spiderplant ecotypes grown in western Kenya and eastern Uganda with percentage of polymorphism being highest for UM ecotypes followed by LM, MEFLs and LVB in that order (Table 1.2). High polymorphism was an indication of high genetic diversity within the genotypes and so spiderplant ecotypes grown in UM had the highest genetic diversity within them while LVB had the lowest. The Western Kenya ecotypes also had more within genetic diversity compared to the eastern Uganda ones which tended to have lower polymorphism (Table 1.2).

Table 1.2: Genetic diversity within spiderplant ecotypes from different AEZs of western Kenyan and eastern Uganda

AEZ	No. of polymorphic Loci	% of polymorphic Loci
UM	13	43.33
LM	15	50.00
MEFLs	10	33.33
LVB	4	13.33

The general genetic diversity for both among and within spider plant ecotypes were also revealed by both the average gene diversity within populations Hs and the diversity for the entire population shown by Ht (Table 1.3). When the two values for Hs and Ht are different, both within and among genetic variations exist. The genetic variation among the spiderplant ecotypes is however more significant than the within ecotypes variation as revealed by the proportion of genetic diversity among populations Gst (Table 1.3). When Gst is low (approaching 0), the majority of variation is found within populations and

when it is high (approaching 1) the variation among a populations is significant than within them. In this study, the Gst is above 0.5 and hence an indication that genetic variation of spiderplant ecotypes from western Kenya and eastern Uganda results more from among than within the ecotypes (Table 1.3).

Table 1.3: Genetic diversity for Subdivided Popu0olations of spiderplant ecotypes grown in western Kenya and eastern Uganda.

Locus	Sample Size	Ht	Hs	Gst
Mean	12	0.2839	0.1284	0.5476
St. Dev		0.0309	0.0092	

3.4 Discussion

Results obtained in this study revealed high genetic diversity in spiderplant ecotypes grown in western Kenya and eastern Uganda. Populations of plants growing in a particular AEZ have to enhance their fitness to the environmental conditions encountered in that area. This leads to natural selection where the most favorable allele(s) increase in frequency as the unfavorable alleles become rare and eventually disappear from the gene pool. As this happens in each of the AEZs with time, the populations from different AEZs (ecotypes) become more genetically distinct from the others. This kind of natural selection where each ecotype became more adapted to its growing environment and more genetically different from the other ecotypes contributed to the genetic difference among the spiderplant ecotypes grown in western Kenya and eastern Uganda.

Rates of mutation and recombination also are dependent on environmental conditions and have the potential to generate new genetic diversity in the population (Hoffmann and

Harshman (1999). This may have contributed to genetic diversity between the spiderplant ecotypes grown in western Kenya and eastern Uganda. Habitat heterogeneity, combined with natural selection results in multiple, genetically distinct ecotypes within a single species (Linhart and Grant, 1996).

Ecotypes growing in neighboring AEZs could also be more closely related genetically compared to those growing in AEZs far off from each other. This is because there is more likelihood of seed exchange between farmers and traders across closely neighbouring AEZs as opposed to those that are far off from each other. This could explain why in this study there was less genetic diversity between UM and LM and also between MEFLs and LVB which are in the same country and physically closer to each other. MEFLs though on the other side of the border, immediately neighbours the western Kenya AEZs and due to its proximity seed exchange between farmers in this AEZ and those in the western Kenya AEZs is more likely between than with LVB which is further off from the border. This explains why the spiderplant grown in MEFLs are more closely genetically related the ones from the western Kenya side. Proximal distance has been found to affect genetic variation in other plants. McCauley, (1995) examined spatial patterns of chloroplast DNA in *Silene alba*, and found the most similar genotypes were those in close proximity to each other. Zhang *et al.,* (2009) found significant influence in the genetic diversity by environmental conditions and the proximal distance between the populations of forage grass *Leymus chinensis.*

The high within ecotypes genetic diversity of spiderplant ecotypes were as a result of gene flow which is the transfer of alleles or genes between different populations of the same species. Gene flow in plants takes place through seeds and pollen dispersal.

Spiderplant (*Cleome gynandra*) is both self- and cross-pollinated with more likely high rate of out crossing (Omondi, 1990). The pollinators are ants and flying insects and its seeds are wind-dispersed (Chweya and Mnzava, 1997). This nature of seed and pollen dispersal of spiderplant is therefore likely to be the cause of gene flow which is responsible for the high genetic diversity within the spiderplant ecotypes. Wind pollination and wind dispersal have been found to foster high genetic diversity. Ruiz *et al.,* (2006) observed that high gene flow through seed dispersal and/or pollen dissemination is correlated with high levels of genetic diversity within populations. Sombroek *et al.,* (1982) did molecular characterization of mango species and also found high genetic differences within populations which he attributed to gene flow since mangoes are cross pollinated. Spiderplant ecotypes grown in western Kenya and eastern Uganda therefore exhibited genetic variation as a result of factors causing variation across different ecotypes and also and in plants within the same ecotypes.

CHAPTER FOUR

NUTRITION STATUS OF SPIDERPLANT ECOTYPES GROWN IN DIFFERENT AEZS OF WESTERN KENYA AND EASTERN UGANDA

4.1 Introduction

4.1.1 Nutritional quality of Spiderplant

Spiderplant (*Cleome gynandra* L.), has high nutritional value and is a good source of vitamins and minerals. Its interest and popularity as a source of food and income, in many parts of the world has been on the increase. In western Kenya and eastern Uganda Spiderplant grows in different AEZs. The variation ecological conditions found in the different AEZs are likely to cause differences in nutrition status of the Spiderplant. In this study, the variations in vitamins A and C and minerals Ca, Mg and Fe levels in Spiderplant grown in four AEZs of western Kenya and eastern Uganda were investigated.

4.1.2 Vitamins and Minerals

Micronutrients such as calcium, iron, vitamins A, and vitamin C have a great role in boosting the human diet and in boosting immune system. Vitamin C, one of the most important vitamins in the human body cannot be produced in the body and it has to be entirely obtained through one's diet (Kramer *et al.,* 2005). About 90% of this vitamin comes from mainly fruits and vegetables (Combs, 1992). Vitamin A which in plants is found in carotenoid form is mainly obtained from dark green, leafy vegetables (Smith *et al.,* 1996). The carotenoid concentrations in vegetable crops are influenced by

environmental factors such as light, temperature, and soil fertility (Kopsell and Kopsell, 2007). Spiderplant contains a considerable amount of the vitamins and its raw edible parts have been found to contain a range of Vitamin C (127 – 484 mg), β-carotene (6.7 - 18.9 mg), Calcium (213 – 434 mg), Magnesium (86 mg) and Iron (1 – 11 mg in 100 g) (Mathooko and Imungi, 1994; Chweya and Mnzava, 1997). It is one of the ALVs whose local production can provide households with access to foods rich in these micronutrients. However spiderpant growing in different AEZs could vary in the vitamins concentrations due to environmental variations in the AEZs.

Minerals are organic elements that originate in the soil and cannot be created by living things, such as plants and animals (Barminas *et al.,* 1998). Rainfall and temperatures variations in the sub-Saharan Africa and the soil types determine the level and availability of plant nutrients (Blair, 1999). The amount of minerals content in plants is influenced a great deal by environment (Ceyhan, 2006) and therefore varies depending on the environment where they are grown. This study investigated how these important micronutrients, vitamins A and C and minerals Ca, Mg and Fe are affected by the AEZs in western Kenya and eastern Uganda in which Spiderplant is grown.

4.2 Materials and Methods

4.2.1 Samples collection

Seeds planted in this experiment were obtained from the plants from which leaves for molecular analysis in chapter two had been harvested. Plots one square metre were planted each with seeds harvested from the same site AEZ UM and LM in western Kenya and MEFLs and LVB in eastern Uganda. The resulting spiderplant crop from theses plots

contained the three spiderplant morphotypes, green, purple and purple-green. Four weeks after emergence, three bunches of plant samples representing each of the three spiderpant morphotypes were harvested by uprooting and tying together ten plants of the same plant colour. For vitamins analysis, the uprooted plant samples were put in a cool box and transported by road to Makerere University Food Science Department laboratories where the vitamins analysis was done. All the samples were delivered to the laboratories the same day they were harvested. For minerals analysis, uprooted plants were packed in brown paper bags and transported to Kericho Tea Research Foundation Chemistry laboratories where the analysis was done. In the laboratories, the samples were washed under tap water and excessive water dripped off. The edible parts were cut into small pieces and homogenised using a blender for 2 minutes.

4.2.2 Vitamin C analysis

Standard Indophenol Solution was prepared by dissolving 0.05 g of 2, 6 dichlorophenol Indophenol in water, diluted to 100 ml and filtered. The dye solution was freshly prepared and standardized before use. Standardization of dye was done by pipetting 10 ml of standard Ascorbic acid solution in a small flask and titrating with the standard Indophenol solution until a faint pink colour persisted for 15 seconds.

To Determine Vitamin C, ten grams of each collected spiderplant sample was mechanically macerated in 100 ml of 1% metaphosphoric acid. The slurry was adjusted to 250 ml with 1% metaphosphoric acid and filtered through Whatman filter paper. 50 ml of the filtrate was pipetted into 100 ml volumetric flask and 25 ml of 20%

metaphosphoric acid added then made up to volume. 10 ml of the mixture was pipetted into a small flask and titrated with Indophenol solution per the method described by AOAC (1984) until a faint pink colour persisted for 15 seconds. This was repeated three times.

The concentration of Ascorbic acid (vitamin C) content was then calculated using the formula:

Vitamin C (mg / 100 ml) = 20 (V) (C).

Where V is volume in ml of Indophenol solution,

C is Vitamin C per ml Indophenol solution expressed as milligrams of Ascorbic acid equivalent to 1 ml of dye solution.

4.2.3 Vitamin A (β-Carotene) Analysis

Beta-Carotene was extracted and analyzed according to the procedure published by Kurilich *et al.*, (1999). 100 mg of sample were put into a test tube and 10 ml of ethanol containing 0.1 g of BHT added. The test tube with the sample was placed in a water bath at 70 ^{0}C for 15 min. After removing the tubes from the water bath, the tissue was completely homogenized in a mortar and filtered through Whatman no. 42 filter paper. Then 180 ml of 80% KOH was added to each tube. The sample was then saponified at 70 ^{0}C for 30 min to remove chlorophyll and other lipids. The sample tubes were placed directly in an ice bath and 2.5 ml of de-ionized water and 2.5 ml hexane: toluene mixture (10:8) was added. Then the tubes were vortexed and centrifuged at 2100 rpm for five minutes. The upper layer hexane: toluene fraction was then transferred to a separate test

tube. The hexane: toluene extraction was repeated two more times. The combined hexane: toluene fractions were dried using a Speed-vac concentrator. The residue was reconstituted in 200–400 ml Tetrahydrofuran (THF). The solution was filtered on a 0.2 mm nylon filter and 20 ml of the filtered solution was injected in the Shimadzu High performance liquid chromatography. The mobile phase consisted of acetonitrile: methanol: THF (52:40:8) (v/v/v) at a flow rate of 0.7 ml/min. The absorbance was recorded at 450 nm for β-carotene. The retention time for the standard β -carotene was recorded as 6.192. The β-carotene analysis was done by open-column chromatography using a magnesium oxide column to separate the carotenoids from xanthophylls on the basis of polarity and this was followed by visible absorption spectrophotometry according to AOAC (1984). The first fraction eluted was β-carotene. This was replicated three times.

4.2.4 Minerals analysis

In the laboratory leaves and shoots (edible parts) were plucked from the plants, washed and oven-dried at 100 ^{0}C in about 24 hours. The material was then milled into a fine homogenous powder. 0.25 gm. of each of the milled samples was weighed into dry specimen tubes and ashed for 4.5 hours in a muffle furnace set at temperature of 450 ^{0}C for analysis of major elements i.e. Ca and Mg. To analyse Fe, 1.0 gm of the samples was weighed and ashed for 8 hours.

The digestion mixture was freshly prepared by measuring 20 ml of the mixed acid and 30 ml of 20-volume hydrogen peroxide into a labeled dry clean digestion tube, thoroughly shaking the mixture then pouring into a 25-ml burette.

To each specimen tube containing the ashed samples 0.5ml of acid/peroxide mixture was added. The tubes were put into a metal tray placed on electric hotplate and evaporated to near dryness. The tubes were then removed and into each 25 ml of 0.05M Hydrochloric (Hcl) solution added. The tubes were stoppered and shaken thoroughly. 2 ml of wet-digested sample solution was pipetted into a 50 ml volumetric flask and made to mark with distilled water. 15 ml of 1M Hcl was added to each flask and made up to 500 ml with distilled water and kept in the reagent bottles.

Working solutions of the elements Ca and Mg were made by adding 50 ml of 1000 ppm (stock solution) to 100 ml of distilled water to make 500 ppm. Standard solutions were made by pippeting stock solution of the elements to make 0, 5, 10, 15, 20 and 30 ppm.

Determination of mineral elements done by atomic absorption spectroscopy (AAS) by spraying sample solutions directly into the flame of the atomic absorption spectrophotometer (wavelength 7665 A, slit 0.07 mm) starting with the blank. The amount of the element was then read off from the solutions from the calibration curve. This was repeated three times to obtain three separate readings for each element.

Iron standard solutions of 1000 ppm was prepared by dissolving 1.0 g of Iron metal wire (99.9%) in 20 ml of 1:1 hydrochloric acid and diluted to 1 litre to give 100 ug/ml of Fe. This standard solution was stored in a reagent bottle. Calibration Standards of 1, 2, 3, 4 and 8 ppm were made by pipetting 1 ml, 2 ml, 3 ml, 4 ms and 8 ml of 1000 ppm and sprayed starting with the blank, Fe standard series and sample solutions in to the flame of the atomic absorption Spectrophotometer at a wavelength of 248.3. Fe absorbance was measured for the Fe standard series and a calibration curve of absorbance against

concentration constructed. The sample solutions were then sprayed on the AAS and the concentrations read off based on the curve in ppm. This was done three more times to achieve three readings (replications).

4.2.5 Moisture Content Determination

Moisture percentage was calculated as outlined in the AOAC (1984). Five grams of well mixed dried sample was placed in tarred moisture dish (about 75 mm wide and 25 mm deep). The dish was placed in an air oven maintained at a temperature of 1052 ^{0}C and dried for 2 hours. The contents were **c**ooled in a desiccator and weighed. This was repeated until the difference between two successive weighing was less than 1 mg and the lowest weight recorded.

Moisture Percent by weight = 100 $(M_1 - M_2)/ (M_1 - M)$,

Where M_1 is weight in gm. of dish with material before drying,

M_2 is weight in gm. of dish with the dried material

M is weight in gm. of empty dish.

4.3 Data analysis

The nutrients' data was analyzed using the procedure of analysis of variance (ANOVA) using Genstat computer package (2000). Separation of means was done by Tukey's 95% confidence intervals.

4.4 Results

4.4.1 Levels of Vitamins C and A (β-carotene) and Minerals Ca, Mg and Fe in spiderplant ecotypes of western Kenya and eastern Uganda.

Levels of vitamin C and β–carotene in Spiderplant ecotypes grown in western Kenya and eastern Uganda were found to differ (Table 2.1). The ecotypes grown in the western Kenya AEZs had higher levels of both vitamins C and β-carotene compared to those grown in eastern Uganda (Table 2.1). Spiderplant ecotype grown in UM had the highest level of vitamin C and β-carotene followed by those from LM, MEFLs and LVB respectively (Table 2.1). The levels of vitamin C differed significantly ($P \leq 0.05$) between the spiderplant ecotype grown in the western Kenya but the difference in β-carotene was not significant. The differences in vitamin C and β-carotene levels were not significant for the eastern Uganda ecotypes (Table 2.1).

Levels of minerals Ca and Mg in Spiderplant ecotypes grown in western Kenya and eastern Uganda were found differ across the ecotypes but that of Fe did not (Table 2.1). The spiderplant ecotypes grown in eastern Uganda had higher Ca and Mg minerals content compared to those grown in western Kenya. The ecotypes from LVB had the highest levels of both Ca and Mg levels followed MEFLs, UM and LM in that order (Table 2.1). These differences were significant ($P \leq 0.05$) across all the four ecotypes.

Table 2.1: Amounts of Vitamins C and β- Carotene, Calcium, Magnesium and Iron (mg/100g) in spiderplant ecotypes grown in different AEZs of western Kenya and eastern Uganda

AEZ	Vit C	β-Carotene	Ca	Mg	Fe
UM	445.6 a	103.02 a	1.902c	0.152c	1.383a
LM	346.2b	100.38a	1.650d	0.112d	1.000a
MEFLs	216.2c	73.10ab	2.167b	0.376b	1.224a
LVB	172.0c	61.99b	2.380a	0.577a	1.602a

Means with a common letter within a column are not significantly different compared by a Tukey's test at 5% confidence level

4.4.2 Levels of Vitamins C, β-carotene, Ca, Mg and Fe levels in morphotypes of spiderplant grown in western Kenya and eastern Uganda

The level of vitamin C and β-carotene varied widely across the morphotypes of spiderplant grown in western AEZs, with the green having the highest levels of both vitamins (Table 2.2). In Vitamin C were highest in the green Morphotype followed by the purple/green and then purple while β-carotene was highest in green but purple had higher level than purple/green (Table 2.2). The levels of both vitamins however did not differ significantly between the three morphotypes.

The levels of the three analyzed minerals, Ca, Mg and Fe also did not significanty differ across the three morphotypes.

Table 2.2. Amounts of Vitamins C and β- Carotene, Calcium, Magnesium and Iron (mg/100g) in spiderplant morphotypes grown in different AEZs of western Kenya and eastern Uganda

Morphotype	Vit C	β-Carotene	Ca	Mg	Fe
Green	307.64	98.73	2.01	0.30	1.53
Purple	243.63	87.13	2.02	0.25	1.28
P/G	272.11	56.41	1.99	0.35	1.09
Mean	274.46	80.76	2.01	0.30	1.30
Sig *(P=0.05)*	0.40 ^{ns}	0.31^{ns}	0.78 ^{ns}	0.13 ^{ns}	0.50 ^{ns}

Means at 5% confidence level of significance according to LSD test

4.5 Discussion

Results from this study revealed that there is a large variation in nutrient levels of the different spiderplant ecotypes grown in western Kenya and eastern Uganda.. Ecotypes grown from the Western Kenya AEZs were found to be richer in the Vitamins C and β-carotene than those from eastern Uganda while the opposite was true for the minerals Ca and Mg. The environmental factors like soil types, rainfall amounts and temperatures regimes are different for each AEZ in which spiderplant was grown and could have been the cause of variations on the nutrient levels of spiderplant ecotype. No manure or fertilizer was added to the experimental plots and so plants nutrients were purely dependent on the status and availability of the soil nutrients to the plants in the growing area. Acidic soils are known to have a binding effect to minerals like Mg and Ca making

them unavailable to plants. This may have contributed to the lower levels of these minerals in spiderplant ecotypes grown in western Kenya compared to those grown in eastern Uganda. In western Kenya AEZs the relatively more acidic soils may have led to leaching of nitrogen making it less available to the plant. Low nitrogen supply to the plant is likely to lead to enhanced carbohydrate production resulting to increased vitamin C which is synthesized from carbohydrates (Worthington, 2001). Soils types in western Kenya and eastern Uganda have been reported to vary, with western Kenya ones being mainly humic nitisols while the eastern Uganda soils vary from lowland ferralsols to highland nitisols (Delve and Ramisch, 2002). The largely nitisols rich soils in western Kenya region are said to be much more Phosphorus deficient and more acidic than the eastern Uganda ones (Delve and Ramisch, 2002). This could explain why Vitamin C levels of spiderplant ecotypes grown in western Kenya were higher than in the eastern Uganda ecotypes.

In addition to the soil types, high rainfall amounts too contribute to low pH and high levels of aluminum which binds the basic minerals like Ca and Mg making them unavailable to plants (Buol *et al*., 2011). In this study the spiderplant ecotypes grown in western Kenya had lower levels of the minerals Ca and Mg compared to the lower rainfall AEZs of eastern Uganda which could have been due to the unavailability of the minerals to the plants in the wetter western Kenya AEZs. Studies by Yu *et al*. (2007) also found that there were differences in Calcium and Magnesium concentration in leave tissues due to different water supply amounts whereby more water supplies resulted to lower minerals levels and low supply to higher minerals levels. Vitamins C levels in

vegetable amaranth were also found to vary with rainfall conditions as well (Yauri *et al.*, 2011).

The variation in temperatures may too have contributed to the variations in the vitamins C and β-carotene and mineral Ca, and Mg levels of spiderplant ecotypes grown in western Kenya and eastern Uganda. Temperatures affect differently the absorption and subsequent assimilation of nutrients leading to different levels the nutrients in the plant. Since each of the four AEZs in western Kenya and eastern Uganda where the study was conducted have different temperatures regimes, the rate of absorption and assimilation of the various nutrients in each of the spiderplant ecotypes was different. This could have influenced the levels of the minerals Ca and Mg and Fe in the spiderplant ecotypes. This variation of vitamins and minerals to changing environmental temperatures may be species specific. Other studies have revealed effect of temperature on various plant nutrients in different crops. According to Lee and Kader, (2000), temperature has been found to influence the composition of plant tissues during growth and development. In rocket (*Eruca vesicaria* subsp. *sativa)* higher temperatures were found to result to higher vitamin C content but reduced levels of Ca and Fe (Funda, 2011). β-carotene concentrations in the leaves of kale (*Brassica oleracea* L. variety. *acephala* D.C) was found to increase with temperature increase from 15 to 30 ℃, while increase of temperature from 10 to 25℃ resulted to decrease in β-carotene concentrations in spinach (Lefsrud and Kopsell, 2005). In this study spiderplant ecotypes grown in western Kenya AEZs which have relatively lower temperatures had higher vitamins C and β-carotene and lower minerals Ca and Mg levels while that grown in the warmer eastern Uganda AEZs had lower levels of the same vitamins and higher levels of the minerals. A study by

Kopsell and Kopsell, (2006) found accumulation of β -carotene in spinach and kales to be influenced by environmental temperature.

Though spiderplant grows well in a wide range of AEZs, the levels of vitamins and minerals content varies from one AEZ to another depending on soil types, rainfall and temperature variations. Consumers of spiderplant grown in the different AEZs in western Kenya and eastern Uganda benefit differently from the plant vitamins and minerals with those in western Kenya benefitting more from the high vitamins levels while those from eastern Uganda AEZs benefit more from the minerals in spiderplant.

CHAPTER FIVE

QUALITY OF SPIDERPLANT SEEDS PRODUCED IN DIFFERENT AGRO-ECOLOGICAL ZONES OF WESTERN KENYA AND EASTERN UGANDA

5.1 Introduction

ALVs are rarely cultivated deliberately to produce seed (Adebooye *et al.,* 2005).but seeds are harvested from remnants of crops after vegetables harvesting period is over. Seeds allowed to mature naturally in the field can be subjected to potentially unfavorable field conditions (TeKrony *et al*., 1980). Different farmers use their own practices to produce ALVs seeds and such seed is of unknown quality. Some farmers normally apply nitrogen fertilizers to boost vegetative growth of the ALVs for leave harvesting. Defoliation is done as farmers harvest the tender shoots and leaves of ALVS for their vegetables consumption. It is recommended that spiderplant seeds be harvested at yellow pod stage (Kamotho, 2004). The seeds are normally harvested and dried while still in the pods before threshing them. Such seeds still have high moisture content and drying them is therefore a critical step in maintaining its quality. In this study effects of various farmers' practices of top dressing with nitrogen fertilizer, defoliation, and seed drying practices on seed quality of Spiderplant were investigated.

5.2 Materials and Methods

5.2.1 On- Farm Trials

Seeds harvested from tagged plants from which leave samples for the molecular characterization in chapter three had been harvested in the previous season were used to

plant these trials. Four on-farm trials were planted one in each of the four sites Sigalagala-kakamega, Kanduyi Bungoma, Rubongi-Tororo and Nakalama-Iganga representing the four AEZs, Upper midland (UM) and Lower Midland (LM) in western Kenya and Lake Victoria basin (LVB) and Mt Elgon Farmlands (MEFLs) in eastern Uganda. The plots were planted in 2x2 factorial experiments laid in a RCBD (Appendix VIII) with topdressing with nitrogen fertilizer and defoliation of the plants by nipping off of the terminal branch as the factors then replicated four times Each plot measured 1.2 m x 1m with an 80 cm path between plots and blocks. No planting fertilizer was used. The seed was drilled within rows of 70 cm spacing. After 2 weeks the plots were thinned to a spacing of 25 cm inter row.

5.2.2 Nitrogen application

To investigate the effects of topdressing spiderplant with Nitrogen on seed quality, Nitrogen was applied in form of CAN three weeks after germination in two treatments: the first treatment T0- no top dressing was done while in the second treatment T1- 31.2 g of CAN was applied to each of the 1.2 m2 plot representing CAN (26% N) application rate of 100Kg/Ha.

5.2.3 Defoliation

To investigate the effects of defoliation on seed quality, plants were subjected to defoliation treatments. The spiderplant was defoliated 4 weeks after planting. In the first treatment (D_0) no defoliation was done and in the second (D_1) all the plants were

defoliated by nipping off the terminal branch.

5.2.4 Seed drying

From each plot pods were harvested separately from the four selected plants representing the three plants representing the three different of spiderplant morphotypes. Each of the harvested pods samples was then subdivided into three more samples, which were dried using different drying methods; open sun, shade and the standard recommended drying condition using a dryer in the laboratory as control.

Open sun drying was done according to the way farmers do to create uniformity in all the sites, samples were spread on canvas placed on wooden structure 1 metre high from the ground under direct sunlight. The pods were dried every day as from 9 a.m. to 4 p.m and kept inside a room in the farmers' houses overnight. The temperature and relative humidity were dependent on the environmental conditions prevailing in the experimental sites. When the bad weather conditions during the experiment period made it not possible to display pods for drying every day they remained inside in the house.

Drying under shade was done by creating shading conditions by using grass thatched wooden structures which ensured there was controlled drying to avoid direct sun. Inside the structure, the pods were laid on a linen sheet placed on wooden tables raised to one metre high. All the pods were dried in the same ecological zones they were produced. These conditions were done to simulate the conditions under which farmers dry their seeds under farm conditions.

Drying using a Seed drier was done at the University of Eldoret Seed Technology laboratories. The pods were placed in the drier and dried at 39 ^{0}C and 24 % relative

humidity (RH) for four days. This was a control where by the seeds were dried under ideal conditions.

5.2.5 Germination test

Pods of spiderplant produced using the two field treatment of topdressing and defoliation and dried in the three different drying methods were separately threshed, and cleaned, wrapped in aluminum foil paper and taken for seed quality testing at University of Eldoret Seed technology laboratories. After cleaning, 50 seeds were counted from each sample, arranged inside a Petri dishes on-top of paper number (ISTA, 1993). This was replicated four times for each seed sample. Seeds in each replica was moistened, labeled and placed in a growth chamber set at 30 ^{0}C in darkness. Starting from the 4th day the seeds were observed for germination, germinated seeds counted, recorded and removed from the Petri dish. This was repeated daily up to the 10th day until no more germination was taking place and it was concluded that the remaining seeds that had not germinated were non-viable. The total number of germinated seeds for each replication were summed up, divided by 50 and multiplied by 100 to get the germination percent.

5.2.6 Bulk electrical conductivity

To test for seed vigor of spiderplant seeds produced from the four AEZS and in the different production methods, fifty seeds from each seed sample were weighed (0.01 g precision) and soaked in 500 ml plastic cups containing 250 ml distilled water, for 24 h at 20 ^{0}C (Hampton & TeKrony, 1995). This was replicated four times for each sample. Afterwards, the electrical conductivity of the solution was determined using a EC meter

and the average values obtained for each seed lot were expressed in µS cm^{-1} g^{-1}.

5.3 Data analysis

Statistical analysis of the spiderplant seed quality was done with the statistical package SPSS. Analysis of variance was done to test the significance of means and the separation of the means done using Turkey's 95% confidence intervals and LSD tests. Variation of the seed quality for the different AEZs, seed production and drying methods were determined by separated means of seed germination percent and the electrical conductivity.

5.4 Results

5.4.1 The quality of seed from the four AEZs

The seed quality of spiderplant ecotypes grown in wastern Kenya and eastern Uganda was different (Table 3.1). Spiderplant seeds grown in MEFLs had the highest germination % while those grown in LVB had the lowest but seeds of spiderplant ecotypes grown in UM and LM in western Kenya did not differ significantly in germination % (Table 3.1). On average quality of spiderplant ecotypes expressed in germination % for spiderplant ecotypes grown in western Kenya was found to be higher than of the ones grown in eastern Uganda ones. Difference in germination % for spiderplant seeds of the two eastern Uganda ecotypes was significant ($p \leq 0.05$) from each other (Table 3.1). Spiderplant seed vigor expressed in EC was highest for the spiderplant ecotypes grown in UM followed by that from MEFLs, LVB and LM respectively (Table 3.1). The difference in seed vigor was significant ($p \leq 0.05$) between seeds of spiderplant ecotypes grown in

UM and MEFLs. Seeds from LVB and LM did not significantly (p=0.05) differ in seed vigor as expressed by EC (Table 3.1).

Table 3.1 Quality (Germination % and EC) of spiderplant seeds produced in different AEZs in western Kenya and eastern Uganda

AEZ	Germination %	EC (µs cm^{-1} g^{-1})
UM	66.78^{b}	32.6^{a}
LM	67.63^{b}	44.7^{c}
MEFLs	71.55^{c}	37.7^{b}
LVB	50.47^{a}	41.8^{bc}

Means with a common letter within a column are not significantly different compared by a Tukey's test at 5% confidence level,

5.4.2 Seed quality of spiderplant top-dressed with Nitrogen

The quality of spiderplant seeds was higher for the plants that were top-dressed with Nitrogen at the rate of 100 Kg/Ha of CAN. The seed quality from the top- dressed plants was significantly ($p \leq 0.05$) higher than that from non-top dressed plants (Table 3.2.). The vigor of the top dressed plants was also higher as their EC was lower compared to that of those not top-dressed although the difference was not significant (Table 3.2). The effect of top dressing spiderplant was found to vary across the AEZs as the interaction between AEZ and topdressing was found to be significant for spiderplant seed quality (Appendix VIII)

Table 3. 2. Quality (Germination % and EC) spiderplant top-dressed with Nitrogen fertilizer (CAN)

Nitrogen Treatment	Germination %	EC(µs cm^{-1} g^{-1})
0.0	57.95	38.4
31.2	65.33	36.6
Mean	66.40	37.5
LSD *(P=0.05)*	0.02*	0.8 ns

Means at 5% confidence level of significance according to LSD test

5.4.3 Effects of defoliation of spiderplant on seed quality

Defoliation by nipping of the terminal branch just before flowering had some effect on quality of spiderplant quality. Seeds from the defoliated plants had slightly higher quality than those that were not defoliated though the difference was not significant as expressed by both germination % and electrical conductivity (Table 3.3). However the effect of defoliation affected seed quality differently across the AEZ as the interaction between AEZs and defoliation was found to be significant for spiderplant seed quality (Appendix VIII).

Table 3.3 Effects of spiderplant defoliation on seed quality (Germination % and EC)

Defoliation	Germination %	EC (µs cm^{-1} g^{-1})
Defoliated	63.30	38.0
Not defoliated	59.98	37.1
Mean	61.64	37.5
LSD *(P=0.05)*	0.145 ns	0.09 ns

Means at 5% confidence level of significance according to LSD test

5.4.4 Effects of seed drying method on the seed quality of spiderplant

The drying method used was found to have an effect on the seed quality of spiderplant (Table 3.4). Laboratory dried seeds had the highest germination percent followed by those dried under shade and then the seeds dried in the sun. Electrical conductivity was highest for the sundried seeds followed by shade dried and then laboratory dried.

The differences in spiderplant seed quality in terms of germination percent and vigor expressed in EC were significantly ($p \leq 0.05$) different for the three drying methods with the laboratory dried seeds having the highest seed quality followed by shade drying and then sun drying in that order (Table 3.4). Drying seeds in the different AEZs also had an effect on seed quality as the interaction between the AEZs and spiderplant seed drying was found to be significant (Appendix VIII).

Table 3. 4. Quality (Germination% and EC) of spiderplant seeds dried using different methods

Drying method	Germination %	EC ($\mu s\ cm^{-1}\ g^{-1}$)
Sun	60.30a	39.3c
Shade	63.67b	37.2b
Lab	68.35c	36.4a

Means with a common letter within a column are not significantly different compared by a Tukey's test at 5% confidence level.

5.4.5 Seed quality of different spiderplant morphotypes

Seed germination percent was significantly ($p \leq 0.05$) lower for seeds from the purple morphotype compared to the other two whose germination percent did not significantly differ (Table 3.5). The vigor expressed by EC was significantly ($p \leq 0.05$) different for the

three morphotypes being highest for purple, green and purple/green in that order (Table 3.5). Interaction between AEZ and spiderplant morphotypes was significant meaning that Seed quality of Spiderplant morphotypes was differently affected by AEZ s in which the spiderplant was grown (Appendix VIII)

Table 3.5: Seed quality (Germination % and Ec) of spiderplant morphotypes

Morphotype	Germination %	EC (µs cm^{-1} g^{-1})
Green	66.23a	37.3b
Purple-green	66.21a	40.6c
Purple	59.99b	35.0a

Means with a common letter within a column are not significantly different compared by a Tukey's test at 5% confidence level.

5.5 Discussion

5.5.1 Ecological effects on seed quality

In this study the AEZs in which spiderplant seeds were produced were found to affect the seed quality of the spiderplant ecotypes. The variable environmental factors across the AEZs being temperature, rainfall/moisture, and soils are likely to have played a role in determining the variation in spiderplant ecotypes seed quality. MEFLs and UM AEZs which generally are on higher altitudes and experience lower temperatures produced higher quality spiderplant seeds compared to LM and LVB which are on lower altitudes and experience higher temperatures. Exposure of the parent plant to different temperatures affects the quality of seeds in several species (Egli and Wardlaw, 1980).

Environmental temperatures during seed plant growth have been attributed to seed quality of other crops with high temperatures being associated with low quality while lower temperature lead to higher quality seeds. Low environmental temperatures increases seed vigor by improving the quality of the seed coat which mediates seed germination. Gibson and Mullen (1996) stated that high temperature during the first 10 to 30 days of seed development lowered germination and vigour. Egli *et al.* (2005) found that high temperatures during the seed filling period lowered the germination and vigour. This explains why generally the seeds harvested from the high altitude AEZs in western Kenya and eastern Uganda that experience lower temperatures produced higher quality spiderplant seeds compared to the warmer lower altitude AEZs.

Defoliation by nipping of the main branch of Spiderplants, just before flowering is done by farmers to delay flowering in Spiderplant and other indigenous vegetables that flower early in order to increase productivity. In this study it was also found to contribute to improvement of spiderplant seed quality in terms of germination % and also seed vigor. Defoliation could have acted to increase the light intensity and temperature thus reducing competition for sunlight and air thus improving the photosynthesis process within the plant canopy. This may have increased the mean seed weight by increasing the carbohydrate and nutrient supply and on translocation patterns to the plant reproductive parts. Also defoliation which leads to increased light intensity and canopy temperature during seed development and maturation reduces seed infection by pests and diseases and this may lead to production of health seeds free from infestation hence improving seed quality. Results obtained by Gutema (2006) revealed that defoliation improved seed germination percent of soybeans. Defoliation of maize plants has also been found to

increase seed weight and quality of maize grains (Thomison and Allen, 2006). Removal of leaves or flowers has been found to increase seed quality probably by removing reproductive sinks.

In seed production nitrogen is added to ensure maximum growth of the side branches that enhances seed yield (Browning and George, 1981). Results of this study revealed that adding of Nitrogen by topdressing with CAN at the rate of 100 Kg/Ha resulted to increased Spiderplant seed quality. The increase in seed quality due to addition of Nitrogen can be attributed to improvement of nutritional status of the plant which is a means of increasing the seed nutrient content. The seed nutrients like the proteins and lipids in the seed provide valuable reserves for use during the early seed germination and their increase therefore leads to increased seed quality. Increase in nutrient supply has been found to increase seed quality. Seed germination and requires proteins at increasing quantities. Higher soil nitrogen levels have been found to lead to increased protein reserves in the seeds which are used during seed germination (Wang and Daun, 2006). Prakash (2008) found that there was higher germination % and lower EC of Capsicum seeds with more availability of nitrogen in the soil. Bodamwad *et al.* (2006) also found that more availability of nitrogen in okra resulted in higher quality seeds.

In this study while drying spiderplant pods in a drier in the laboratory produced spiderplant seeds with the highest quality, of the two spiderplants famers' drying methods shade drying was found to produce seeds with higher quality than sun drying. The spiderplant seed quality expressed both in germination percent and seed vigor as measured by EC was highest for seeds from the laboratory dried spiderplant pods. This could be due to the fact that laboratory drying was done in controlled optimum conditions

which resulted in maintaining the integrity of seed coat, intact embryo and non-breaking of cell membrane in the seed. Sun and Shade drying of spiderplant pods was done at the areas of production which were in different AEZs with varied environmental conditions and these therefore affected differently the spiderplant seed quality. The lower quality these farmers' drying methods of pods of spiderplant as opposed to the laboratory drying could have resulted from various causes including seed infection by fungi, rupturing of seed coat, damaging embryo and endosperm depending on the prevailing environmental factors. These findings are in agreement with other findings by Babiker *et al. (*2010) who observed highest germination percentage for seeds dried in the seed dryer is opposed to those dried in open sun under shade. Shade drying of the pods produced seeds of slightly higher quality compared to the sun drying. This may have been due to the fact that the areas in which the study was carried out could be rainy sometimes during the drying operations during which the seeds dried under sun would have to be moved in and out of houses every day and this may have affected the quality. Under shade the pods remained in one place until when they were fully dry. Hong and Ellis (1996) also observed that the result obtained from drying seeds under ambient environmental conditions depends mainly on the season, location and species. Many times farmers prefer to keep the seeds of spiderplant in pod and only thresh them about planting time. Farmers may therefore prefer to dry their spiderplant seeds under shade for convenience if the quality is not compromised. In a study on wild shrub, *Millettia leucantha Vatke*, endemic to east Africa, Muthoka *et al.* (2003) found out that neither sun nor shade drying were detrimental to seed quality.

The seed quality for the three morphotypes did not differ in this study. (K'opondo *et al.*,

2011) found slight differences with the green morphotype giving a better germination performance compared to the other two.

CHAPTER SIX

GENEREAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1. General Discussion

Seed quality depends on the genetic makeup of the hybrid and the environmental conditions during the development stages of plant. In this study the genetic makeup and seed quality of spiderplant ecotypes grown western Kenya and eastern Uganda were found to have some relationship. The spiderplant ecotypes with higher % of polymorphism had higher seed quality compared to those with low % of polymorphism. The variation in seed quality for the genetically different ecotypes can be explained by the fact that seed quality of seed is a trait characteristic of each individual genotype. Ecotypes varying in genetic makeup are therefore likely to produce seeds with varied quality. Genetic makeup influences plant traits and affect the amount of resources that a maternal parent packs into its seeds and are used for seed germination and seedling establishment (Wulff, 1995). Also the environmental alteration on gene structure in either the endosperm or embryo of the ecotypes seeds, would affect the seed quality thus causing differences in seed quality of different ecotypes.

The ecotypes grown in the western Kenya AEZs were found to vary in vitamins C and β-carotene and minerals Ca and Mg levels. The spiderplant ecotypes with high levels of the Vitamins C and β-carotene generally had higher seed quality compared to the others. There is therefore positive correlation between Vitamins C and B-carotene with seed quality of spiderplant ecotypes grown in western Kenya and eastern Uganda. Variation in nutrient levels of the ecotypes is likely to result to seeds with varied chemical

composition and hence varied seed quality. Nutrient availability to the maternal plant could potentially affect seed production and seed traits (Roach and Wulff, 1987). Maternal plants of *Senecio vulgaris* with lower nutrient levels were found to produce seeds with lower quality than those from plants with higher nutrient levels (Aarssen and Burton, 1990). Germination of *S. aralocaspica* seeds was also found to be significantly affected by maternal nutrient levels (Gutterman, 2000).

6.2 Study findings

i. Molecular characterization of spiderplant ecotypes grown in western Kenya and eastern Uganda established that there is genetic diversity existing in spiderplant grown in the different AEZs in western Kenya and eastern Uganda.

ii. The level of genetic variation was found to be positively correlated to the seed quality with those ecotypes with higher % of polymorphism having higher seed quality compared to those with low percentage of polymorphism.

iii. Vitamins A and C (beta-carotene) levels were found to be higher in the western Kenya ecotypes than in the eastern Uganda while the minerals (Ca, Mg, and Fe) levels were higher in the ecotypes from eastern Uganda.

iv. The nutrients levels were found to have also a positive correlation with seed quality, with those spiderplant ecotypes with high nutrient levels having higher seed quality compared to the ones with low nutrient levels.

v. Defoliation by nipping of the main branch of Spiderplants just before flowering was found to improve the seed quality.

vi. Topdressing spiderplant with 100 Kg of CAN per Ha four weeks after planting resulted to higher quality of spiderplant seeds produced

vii. Drying spiderplant pods under shade produced higher quality seeds than drying them under direct sun.

6.3 Recommendations

There is need for farmers in western Kenya and eastern Uganda to add nitrogen to their spiderplant seed crop in order to enhance quality seeds. However the level of nitrogen to be added to achieve best spiderplant seed quality need to be further investigated.

Defoliation of spiderplant by just removing the terminal branch of the spiderplant will slightly improve seed quality. This study however did not investigate repeated harvesting of leaves on seed quality. Farmers can therefore only nip off the terminal branches of some of plants seeds and leave them to produce seed.

Drying spiderplant pods in the sun and under shade does not affect seed of spiderplant so farmers can dry either way for as long as the seeds are adequately dry and to the farmers' satisfaction.

6.4 Areas for future Research

Further research may seek to establish the following

There is need to confirm the ecological effects of spiderplant genetic makeup of spiderplant by use of other genetic markers. The variation of spiderplant nutrients in different ecological conditions and their interaction need to be established.

The optimal amount of nitrogen for production of good quality of spiderplant seeds in different AEZs need to be established by investigating different levels of Nitrogen treatments.

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APPENDICES

Appendix I: RAPDS primers screened.

OPA-03 5’-AGTCAGCCAC-3’, OPA-09 5’-GGGTAACGCC-3’ OPB-02 5’-GGTGACGCAG-3’OPB-07 5’-GGTGACGCAG-3’, OPB-10 5’-CTGCTGGGAC-3’, OPB-20 5’-GGACCCTTAC-3’, OPD-19 5’-GAGTCAGCAG-3’, OPM-17 5’-TCAGTCCGGG-3’, OPR-04 5’-CCCGTAGCAC-3’, OPR-08 5’-CCCGTTGCCT-3’, OPR-13 5’-GGACGACAAG-3’, OPR-17 5’-CCGTACGTAG-3’, OPS-04 5’-CACCCCCTTG-3’, OPS-14 5’- AAAGGGGTCC-3’, OPS-19 5’-GAGTCAGCAG-3’, OPU-08 5’-GGCGAAGGTT-3’, OPU-19 5’-GAGTCAGCAG-3’, OPW-02 5’-ACCCCGCCAA-3’, OPV-03 5’-CTCCCTGCAA-3’, OPV-06 5’-ACGCCCAGGT.

Appendix II: Molecular Diploid RAPD Data Set

Number of populations = 4

Number of loci = 30

Locus name:

0PB07-1 0PB07-2 0PB07-3 0PB07-4

OPV06-1 OPV6-2 OPV06-3 OPV06-4 OPV06-5 OPV06-6

OPU08-1 OPU08-2 OPU08-3 OPU08-4 OPU08-5 OPU08-6

OPW02-1 OPW02-2 OPW02-3 OPW02-4 OPW02-5 OPW02-6 OPW02-7 OPW02-8 OPW02-9

OPD20-1 OPD20-2 OPD20-3 OPD20-4 OPD20-5

Name = UM

0011 010011 010100 000001100 10010

0011 000000 010100 000001110 00000

0010 000111 000000 000011111 00100

Name = LM

0111 001110 000000 000011100 01110

1111 001111 101100 111111111 00110

0111 001110 101001 000111100 00000

Name = MEFLs

0000 010110 000000 000000100 01011

0000 000100 000000 000000000 00000

0000 010001 000000 000001100 00100

Name =LVB

0000 001001 000000 000101000 01100

0000 001001 000000 000100001 01100

0000 001100 000000 000100000 01100

Appendix III: ANOVA for Vitamin C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	14195.	7098.	2.66	
AEZ	3	419536.	139845.	52.43	<.001
Morphotype	2	198307.	99153.	37.18	<.001
AEZ.Morphotype	6	303361.	50560.	18.96	<.001
Residual	22	58678.	2667.		
Total	35	994077			

Appendix IV: ANOVA for β-carotene

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1425.8	12.9	0.97	
AEZ	3	11088.8	3696.3	5.04	0.008
Morphotype	2	4371.7	2185.9	2.98	0.072
AEZ.Morphotype	6	11589.6	1931.6	2.63	0.044
Residual	22	16136.5	733.5		
Total	35	44612.4			

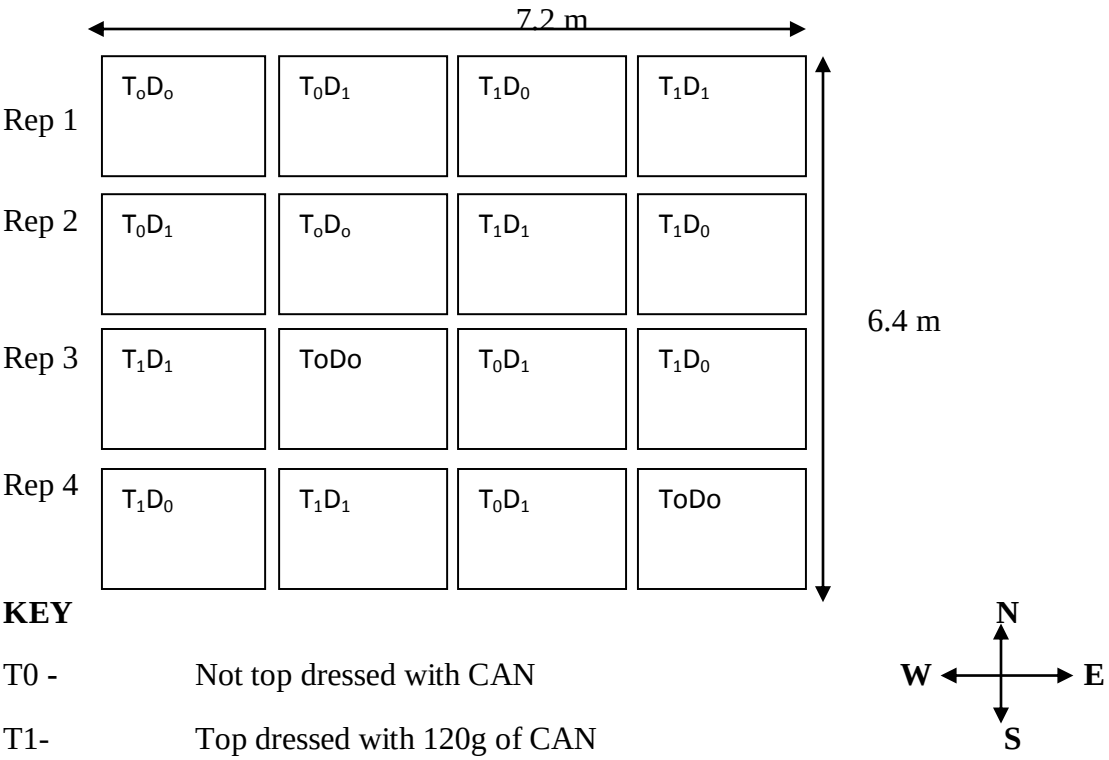
Appendix V: ANOVA for Ca

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.19984	0.19984	8.92	
AEZ	3	1.81158	0.60386	26.97	<.001
Stem_coulour	2	0.02351	0.01175	0.52	0.606
AEZ.Stem_coulour	6	0.10136	0.01689	0.75	0.619
Residual	11	0.24631	0.02239		
Total	23	2.38260			

Appendix VI: ANOVA for Fe

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	4791841.	4791841.	5.00	
AEZ	3	1162770.	387590.	0.40	0.753
Stem_coulour	2	765084.	382542.	0.40	0.680
AEZ.Stem_coulour	6	3438021.	573004.	0.60	0.727
Residual	11	10532613.	957510.		
Total	23	20690329.			

Appendix VII: Seed production experiment, Plot layout



KEY

T0 - Not top dressed with CAN

T1- Top dressed with 120g of CAN

D0- Not defoliated

D1- Defoliated

Appendix VIII: Tests of Between-Subjects Effects
Dependent Variable: Germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	165168.326(a)	143	1155.023	42.578	.000
Intercept	2367238.674	1	2367238.674	87264.025	.000
AEZ	37561.396	3	12520.465	461.545	.000
Morp	5134.264	2	2567.132	94.633	.000
N	7084.028	1	7084.028	261.140	.000
Defol	70.840	1	70.840	2.611	.107
Dry	6270.899	2	3135.450	115.583	.000
AEZ * Morp	6318.000	6	1053.000	38.817	.000
AEZ * N	3603.514	3	1201.171	44.279	.000
AEZ * Defol	798.396	3	266.132	9.810	.000
AEZ * Dry	8201.156	6	1366.859	50.387	.000
AEZ * Morp * N	16647.028	6	2774.505	102.277	.000
AEZ * N * Defol	607.819	3	202.606	7.469	.000
AEZ * N * Dry	6073.163	6	1012.194	37.313	.000
AEZ * morp * Defol	6246.083	6	1041.014	38.375	.000
AEZ * Morp * Dry	7495.542	12	624.628	23.026	.000
AEZ * Morp * N * Defol	1797.056	6	299.509	11.041	.000
AEZ * Morp * N * Dry	13754.514	12	1146.209	42.253	.000
AEZ * N * Defol * Dry	1873.566	6	312.261	11.511	.000
AEZ * Morp * Defol * Dry	11339.667	12	944.972	34.835	.000
AEZ * Morp * N * Defol * Dry	7944.778	12	662.065	24.406	.000
Error	11719.000	432	27.127		
Total	2544126.000	576			
Corrected Total	176887.326	575			

a R Squared = .934 (Adjusted R Squared = .912)
Based on observed means
*The mean difference is significant at the .05 level.